INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 228
2023 July; 29(3): 228 - 233
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Diagnostic Performance of Mac 2–Binding Protein Glycosylation Isomer in Chronic Hepatitis B

Wita Prominensa, B. Rina A. Sidharta, Lusi Oka Wardhani, JB. Suparyatmo, MI. Diah Pramudianti

Department of Clinical Pathology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi General Hospital, Surakarta, Indonesia.
E-mail: wprominensa@gmail.com

ABSTRACT

Chronic Hepatitis B (CHB) is a concern for Chronic Liver Disease (CLD) and causes a 74% mortality rate in Asia Pacific. World Health Organization (WHO) showed Indonesia is the highest second country of Hepatitis B (HB) in the South East Asian Region, Central Java is the highest in Java and Dr. Moewardi Hospital (RSDM) Surakarta in 2019 increased to 15%. Liver biopsy is fibrosis gold standard staging. It has limitations and requires invasive procedure pain in 40% of patients. This study aimed to determine M2BPGi diagnostic test against to transient elastography (TE) Fibroscan® (sensitivity 85.7%, specificity 81.6%) as a predictor of significant liver fibrosis of CHB in RSDM. Fibroscan® examination was performed on patients diagnosed with CHB by a clinician performed at the endoscopy department of RSDM, whereas laboratory tests were carried out from December 2020 to January 2021. Plasma M2BPG-I cut-off value was determined using Receiving Operating Characteristic (ROC) curve, M2BPGi levels were measured sandwich ELISA using spectrophotometry at a wavelength of 450 ± 2 nm. A total of 70 subjects was divided into 35 subjects with significant and 35 subjects with non-significant fibrosis. The results of the statistical calculation showed that plasma M2BPGi levels had a cut-off of 12.939 ng/mL (mean value of 17.841 ng/mL with significant fibrosis at 16.74 ng/mL and non-significant fibrosis at 10.14 ng/mL) had a moderate performance as a marker of liver fibrosis in CHB (71.4% sensitivity; 68.6% specificity; 69.4% PPV; 70.6% NPV and PLR 2.273), NR 0.417 with AUC of 0.727, CI 96% (0.681-0.0906).M2BPGi plasma levels at a cut-off of 12.939 ng/mL had a moderate performance as a predictor of significant liver fibrosis in chronic hepatitis B patients.

Keywords: Mac-2 binding protein glycosilation isomer, transient elastography, Fibroscan®, significant liver fibrosis, CHB

INTRODUCTION

Chronic Liver Disease (CLD) is a global issue worldwide because it is associated with an elderly population; mild-to-moderate stages are rarely diagnosed because it is asymptomatic at an early stage. One of the CLDs, which remains a current concern is Chronic Hepatitis B (CHB) with a prevalence of 6% of the population worldwide. Data from the World Health Organization (WHO) shows that 74% of global deaths caused by complications of CHB are found in the Asian region. Indonesia is the second country with the highest incidence of HB in the South East Asian Region, with 18 million cases of hepatitis B, 37-76% risk of fibrosis and cirrhosis, and 37-68% risk of HCC.[1] Mortality rate, which reaches 30% places CHB complications as the seventh main cause of death in Indonesia. Based on data from the 2019 Directorate General of P2P of the Indonesian Ministry of Health, Central Java is the region with the highest number of hepatitis B cases in Java. Prevalence of CHB in Dr. Moewardi Hospital (RSDM) Surakarta increased from 10% in 2017 to 15% in 2019.

Chronic hepatitis B begins with an inflammatory process in the liver, which can then develop into fibrosis (scar tissue). Liver biopsy is currently the gold standard for detecting fibrosis; however, this procedure is invasive, expensive, a painful in 40% of patients.[2,3] Therefore, a more practical test is needed, such as the M2BPGi marker using transient elastography (TE) Fibroscan® reference with a sensitivity of 85.7% and specificity of 81.6%.[4,3] Selection of M2BPGi is considered fast, efficient, effective, and non-invasive for predicting liver fibrosis. Therefore, it is expected that treatment will be carried out immediately and cirrhosis can be avoided.[5] This study aimed to determine the performance of M2BPGi against TE in predicting significant liver fibrosis in CHB patients at RSDM.

METHODS

This study was an observational analytic study with a cross-sectional approach, which aimed to determine the cut-off of M2BPGi to TE (Fibroscan®) to distinguish between significant and non-significant liver fibrosis in CHB patients. The normality of data

distribution was analyzed using Shapiro-Wilk. The cut-off value of plasma M2BPGi was determined by the receiver Operating Characteristic Curve (ROC).

The study was performed at the Clinical Pathology Installation of RSDM Surakarta from December 2020 to January 2021. The inclusion criteria were CHB patients with evidence of reactive HBsAg and detectable HBV on HBV-DNA test, male and female, aged 18–75 years, and agreed to participate in the study by signing informed consent. Exclusion criteria were hepatitis C, obesity (BMI ≥ 30 kg/m^2), ascites, history of diagnosis of inflammation and malignancy, CKD, cardiac disease, diabetes mellitus, and history of alcohol consumption based on medical records.

Based on the formula for the sample size of the diagnostic test study design with an accuracy of 0.7–0.8 and a precision of 0.02%. Subjects were selected consecutively, and identity and anamnesis were collected while signing informed consent at the Clinical Pathology Installation of RSDM Surakarta. A total of 3 mL EDTA blood was used for the measurement of plasma M2BPGi. M2BPGi levels were measured using the sandwich ELISA principle. WFA-M2BP-specific antibodies and HRP conjugates will produce a red color, then the enzyme-substrate reaction is terminated by the addition of a stop solution until the color changes to yellow. Optical Density (OD) was measured spectrophotometrically at a wavelength of 450±2 nm.[6]

This study has received approval from the Biomedical Research Ethics Commission of Dr. Moewardi General Hospital of Surakarta number 1.324/XII/HREC/2020.

RESULTS AND DISCUSSIONS

This study involved 70 subjects who were divided into significant TE fibrosis ≥ 7.8 kPa (35 subjects) and non-significant TE fibrosis < 7.8 kPa (35 subjects). Fibroscan® examination was performed on patients who were diagnosed with CHB by a clinician performed at the endoscopy department of RSDM Surakarta, whereas laboratory tests were carried out at the Laboratory of Clinical Pathology of RSDM Surakarta. The general characteristics of the research subjects are described in Table 1.

Based on Table 1, it was known that the significant fibrosis group had a mean age of 46.14+11.53 years, and the non-significant fibrosis group had a mean age of 38.66+10.78 years. The independent T-test showed a p-value of 0.007 (p <0.05), which indicated that there were significant differences in patient characteristics based on age between the significant fibrosis group and the non-significant fibrosis group. Based on the results of this study, there was a significant incidence of fibrosis in CHB patients in males as much as 54.3% with an age of 46.14+11.53. This was in accordance with previous research in Japan in 2017, which found 58% CHB population in males aged 50.2+11.3 years.[7] Molecular mechanisms via sex hormones such as androgens and estrogens and receptors play an important role in the progression of HBV infection and the development of fibrosis to cirrhosis and HCC.[8]

There was no significant difference in patient characteristics based on BMI between the significant fibrosis and non-significant fibrosis groups, in accordance with previous studies (mean BMI of

Table 1. General characteristics of research subjects

Variable	TE		p-value
	Significant Fibrosis (n=35)	Non-Significant Fibrosis (n=35)	
Age	46.14±11.53	38.66±10.78	0.007*
Gender[c]			0.028*
Male	19 (54.3%)	10 (28.6%)	
Female	16 (45.7%)	25 (71.4%)	
BMI (kg/m^2)[a]	24.20±1.92	23.06±2.92	0.059
SGOT (u/L)[b]	25.00 (12.00-75.00)	23.00 (12.00-49.00)	0.019*
SGPT (u/L)[b]	24.00 (12.00-73.00)	21.00 (10.00-73.00)	0.026*
Creatinine (mg/dL)[b]	0.70 (0.40-1.80)	0.70 (0.40-1.90)	0.425
Hemoglobin (g/dL)[a]	13.88±1.66	14.14±1.40	0.477
Leukocyte (/uL)[a]	7.26±2.10	8.06±1.96	0.103
Platelet (10^3/uL)[a]	230.77±80.67	285.89±84.55	0.007*

Abbrev: BMI = Body Mass Index; SGOT = Serum Glutamic Oxaloacetic Transaminase; SGPT = Serum Glutamic Pyruvic Transaminase; u/L = micro per liter; g/dl = gram per desiliter; mg/dL = milligram per desiliter; ng/mL: nanoogram per milliliter; [a] independent T-test; [b] Mann-Whitney test; [c] Chi-Square/Fisher exact test; *Significant at α =5%

23.20 kg/m^2) to avoid the limitations of transient elastography interpretation as a gold standard.[2,9]

There was a significant difference in the AST levels between the significant fibrosis group and the non-significant fibrosis group, in accordance with previous studies showing normal or insignificantly increased SGOT levels because these patients were in the replicative or immune tolerant phase. However, SGOT and SGPT levels decreased along with the progression of fibrosis.[10]

There was a significant difference in SGPT levels between the significant fibrosis group and the non-significant fibrosis group, in accordance with previous studies in the CHB population with a slight increase in SGPT levels, which found liver fibrosis with liver stiffness of 8.34+2.37 kPa (significant fibrosis) compared to CHB accompanied with normal SGPT levels with liver stiffness of 7.22+1.41 kPa.[11]

There was a significant difference in the platelet count between the significant fibrosis group and the non-significant fibrosis group. This was consistent with previous studies, which found a tendency of decreased platelet count in CHB with normal liver fibrosis.[12] Platelets act as VEGF transporters; excessive VEGF production will interfere with the production of thrombopoietin (TPO) in the liver. As the liver has the ability to compensate for progressive fibrosis, platelet levels can return to normal.[11,12]

There were no significant differences in leukocytes and hemoglobin between the significant fibrosis and non-significant fibrosis groups. This confirms previous research showing that blood counts tend to be normal and leukocyte count in CHB with liver fibrosis is accompanied by normal or slightly increased transaminase enzymes. Erythropoietin (EPO) is also present in the cytoplasm of liver cells; therefore, its excessive production triggered by liver injury such as liver fibrosis can initially increase production, EPO affects the formation of hemoglobin and returns to normal when the liver is able to compensate with fibrosis.[13,14]

There was no significant difference in creatinine levels between the significant fibrosis and non-significant fibrosis groups. The mean creatinine level in the significant fibrosis and non-significant fibrosis was 0.70 (0.40–1.80) mg/dL and 0.70 (0.40–1.90) mg/dL, respectively. The renin-angiotensin-aldosterone system in the acute phase triggers cytokines, which leads to a risk of fibrosis formation; the acute phase of chronic inflammation includes an increase in TNFα, intercellular adhesion molecule-1 may trigger glomerular damage in the kidney by stimulating cell apoptosis.[10,13] However, this study was following other studies which found that creatinine did not increase by more than 10% and there were no significant differences in tubular changes in the kidneys between patients who received or did not receive therapy for CHB.[15,16]

Table 2 shows a comparison of the M2BPGi results in the median (min-max) in the significant fibrosis group and the non-significant fibrosis group. The mean of M2BPGi levels in the significant fibrosis group was significantly higher [16.74 (1.46 - 24.93) ng/mL] compared to that of the non-significant fibrosis group [10.14 (1.80-19.89) ng/mL] with a p-value of 0.001.

The cut-off value of the M2BPGi cut-off value was determined using the ROC curve (Figure 1).

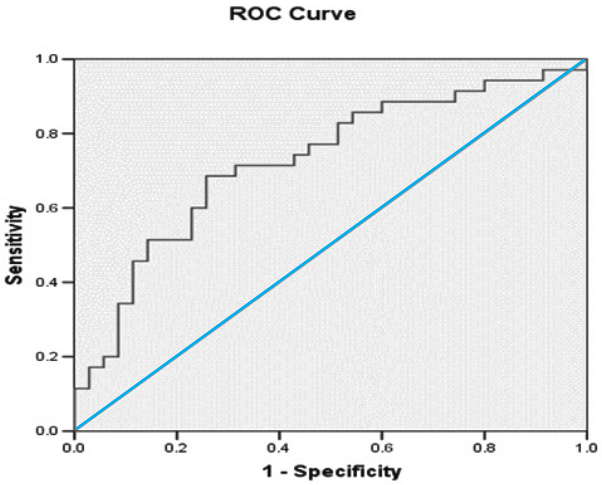

Figure 1. ROC curve of M2BPGi to liver fibrosis in CHB

The AUC value was determined for the M2BPGi cut-off, and the optimal cut-off point was then selected. Based on the ROC curve, the cut-off value for M2BPGi was 12.939 ng/mL with an AUC of 0.727 (95% CI). The graph of the intersection of the sensitivity and specificity values is shown in Figure 2.

Table 2. The difference in M2BPGI levels to TE

Variable	TE		p-value
	Significant Fibrosis (n=35)	Non-Significant Fibrosis (n=35)	
M2BPGi	16.74 (1.46- 24.93)	10.14 (1.80- 19.89)	0.001*

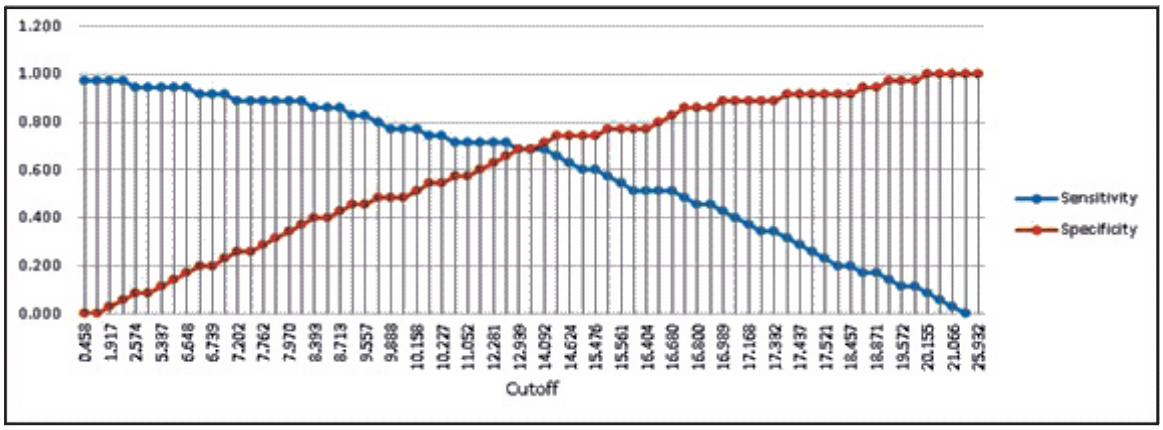

Figure 2. The intersection of a cut-off value of M2BPGi with sensitivity and specificity

Table 3. Cross-tabulation of t-diagnostic test of M2BPGi to transient elastography

M2BPGi	TE		Total	p-value
	≥ 7.8 (Significant Fibrosis)	<7.8 (Non-Significant Fibrosis)		
≥12.939	25	11	36	0.001*
<12.939	10	24	34	
Total	35	35	70	

Based on the cut-off on the ROC curve, the M2BPGi was divided into 2 groups, namely a group with the M2BPGi cut-off value > 12.939 ng/mL and a group with the M2BPGi cut-off value <12.939 ng/mL. The diagnostic test table is shown in Table 3.

Based on Table 3, it was known that of the 35 patients with TE ≥ 7.8 kPa, 25 subjects had M2BPGi levels of > 12.939 ng/mL. In addition, of the 35 patients with TE <7.8, 24 subjects had M2BPGi levels of <12.939. Based on the cross-tabulation above, a diagnostic test for significant fibrosis events (TE ≥ 7.8 kPa) based on the results of the M2BPGi levels > 12.939 ng/mL for liver fibrosis events was carried out to determine the sensitivity, specificity, PPV, NPV, PLR, and NLR. The results of the calculation of the diagnostic test using a cut-off of 12.939 ng/mL are presented in Table 4.

Table 4. Accuracy of M2BPGi with a cut-off of 12.939 ng/mL for diagnosis of liver fibrosis

	Confidence Interval of 95%
Sensitivity (%)	0.714
Specificity (%)	0.686
PPV (%)	0.694
NPV (%)	0.706
NLR	0.417
PLR	2.273

Abbrev: Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, NLR: Negative Likelihood Ratio, PLR: Positive Likelihood Ratio

Diagnostic test results showed that plasma M2BPGi had a sensitivity of 71.4%; specificity of 68.6%; PPV of 69.4%; NPV of 70.6%; PLR of 2.273 and NLR of 0.417 for liver fibrosis in patients with CHB with an AUC of 0.727 and CI 95%. The AUC of M2BPGi levels was 0.727, indicating that M2BPGi would be able to distinguish between significant and non-significant liver fibrosis in 72 cases out of 100 CHB cases with liver fibrosis. These statistical results were not similar to previous studies, which obtained an AUC of 0.698, a sensitivity of 80% and a specificity of 77.9%, and an AUC of 0.763 (p <0.001).[5,15] The PPV value of 69.4% indicated that M2BPGi levels > 12.939 ng/mL would lead to a 69.4% probability of liver fibrosis. The NPV value of 70.6% indicated that M2BPGi levels < 12.939 ng/mL would lead to a 69.4% probability of non-significant fibrosis. The PPV value indicated that the ability of M2BPGi in predicting significant fibrosis or a positive score was 69.4%, while the NPV of 70.9% indicated that the ability of M2BPGi to predict non-significant fibrosis or a negative value was 70.9%. These results were slightly different from previous studies, which obtained a PPV of 79% and NPV of 58.5%.[16] The PLR value obtained in this study was 2.273, indicating that patients with M2BPGi > 12.939 ng/mL had a 2.273 times higher risk of significant liver fibrosis compared to patients with M2BPGi < 12.939 ng/mL. The NLR value was 0.417, indicating that patients with M2BPGi < 12.939 ng/mL had 0.417 times higher risk of significant liver fibrosis compared to those

with M2BPGi > 12.939 ng/mL.

The clinical improvement in patients may affect the faster repair of scar tissue. Increased M2BPGi levels along with the activity of stellate cells in the liver bind to WFA-M2BP, which functions as a carrier of fibrosis signals released at week – 8 but will decrease at week 13 and can increase again at week 23 if there is a worsening condition due to unresponsiveness to therapy. During the event of liver fibrosis, liver dysfunction will affect circulation disorders and liver function from the start of inflammation/injury; therefore, an increase in M2BPGi levels can be affected by acute inflammatory events, e.g. the increase in other inflammatory markers such as IL-17 and TGF-b. This study supports a previous study, which reported a cut-off of 9±3.8 ng/mL (AUC = 0.763; p<0.001) in the CHB population treated after 12 weeks.[15] M2BPGi levels indicate the secretion of glycosylation protein, which basically mediates cell proliferation and angiogenesis. However, several studies have found that M2BPGi is also able to indicate cell adhesion to the extracellular matrix in patients with malignancy, thereby suggesting no association with liver fibrosis.[17,18]

The selection of Fibroscan® as a standard also causes disadvantages compared to other elastography methods such as MRE, which possesses better sensitivity and specificity. The limited number of references regarding the M2BPGi cut-off standard using the sandwich ELISA method remained the limitation of this study. In addition, several previous studies have identified that M2BPGi can be found in several organs other than the liver and plays a role in the development of different fibrosis organs.

CONCLUSIONS AND SUGGESTIONS

Plasma M2BPGi at a cut-off of 12.939 ng/mL had a sensitivity of 71.4%; specificity of 68.6%; PPV of 69.4%; NPV of 70.6%; PLR of 2.273 and NLR of 0.417 with AUC of 0.727 CI 95% (0.681-0.906) to diagnose liver fibrosis in CHB patients.

Further study is needed with a larger number of samples to obtain a cut-off value with better performance (higher levels of sensitivity and specificity). The M2BPGi levels can be considered as a diagnostic test; however, it is advisable to perform a combined test to support the predictors of liver fibrosis by increasing the sensitivity and specificity for better diagnostic performance.

REFERENCES

1. Muljono DH. Epidemiology of hepatitis B and C in republic of Indonesia. Euroasian J Hepatogastroenterol, 2017; 7(1): 55-59.
2. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, *et al.* Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. Gastroenterology, 2016; 150(3): 626-37.
3. Xiao H, Shi M, Xie Y, Chi X. Comparison of diagnostic accuracy of magnetic resonance elastography and Fibroscan® for detecting liver fibrosis in chronic hepatitis B patients: A systemic review and meta-analysis. PLoS One, 2017; 12(11): e0186660.
4. Moon HW, Park M, Hur M, Kim H, Choe W, Yun Y. Usefulness of enhanced liver fibrosis, glycosylation isomer of Mac-2 binding protein, galectin-3 and soluble suppression of tumorigenicity 2 for assessing liver fibrosis in chronic liver disease. Ann Lab Med, 2018; 38: 331-37.
5. Nah E, Cho S, Kim S, Kim H, Cho H. Diagnostic performance of Mac-2 binding protein glycosylation (M2BPGi) in screening liver fibrosis in health checkups. J Clin Lab Annal, 2020; 00: e23316.
6. MyBioSource. Human Mac-2 binding protein glycosylation isomer (M2BPGi) ELISA kit. MyBioSource antibody-protein-ELISA kit. 2019. Cat No: MBS1080990.
7. Shinkai N, Nojima M, Lio E, Matsunami K, Toyoda H, *et al.* High levels of serum Mac-2 binding protein glycosylation isomer (M2BPGi) predict the development of hepatocellular carcinoma in hepatitis B patients treated with nucleotide analogues. J of Gastroenterology, 2017; 53(7): 883-889.
8. Montella M, Arena GD, Crispo A, Capunzo M, Nocerino F, *et al.* Role of sex hormones in the development and progression of hepatitis B virus-associated hepatocellular carcinoma. J of Endocrinology, 2015; 2015: ID 854530.
9. Sasaki R, Yamasaki K, Abiru S, Komori A, Nagaoka S, *et al.* Serum wisteria floribunda agglutinin-positive Mac-2 binding protein values predict the development of hepatocellular carcinoma among patients with chronic hepatitis after sustained virological response. PLos One, 2015; 0129053.
10. Wang H, Wang Q, Yuan Q, Shan X, Fu G. Alanine aminotransferase is more sensitive to the decrease in hepatitis B virus-DNA load than other liver marker in chronic hepatitis B patients. J Clin Lab Anal, 2016; e22141.
11. Wang D, Zhang P, Zhang M. Predictors for advanced liver fibrosis in chronic hepatitis B virus infection with persistenly normal or mildly elevated alanine aminotransferase. Experimental and Therapeutic Medicine, 2017; 14: 5363-5370.
12. Wei B, Feng S, Chen E, Li D, Wang T, Gou Y, Yang T. M2BPGi as a potential diagnostic tool of chirrosis in chinese patients with hepatitis B virus infection. J Clin Lab Anal, 2016; 32e22261.
13. Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol, 2014; 14(3): 181-94.
14. Deray G, Buti M, Gane E, Jia DJ, Chan HLY, *et al.*

Hepatitis B virus infection and the kidney: Renal abnormalities in HBV patients, antiviral drugs handling and specific follow-up. Advances in Hepatology Hindawi, 2015; ID: 596829.
15. Saleh SA, Salama MM, Alhusseini MM, Mohamed GA. M2BPGi for assessing liver fibrosis in patients with hepatitis treated with direct-acting antivirals. World J Gastroenterol, 2020; 7: 26(21): 2682-2888.
16. Zhu MY, Chen PZ, Li J, Yu DM, Huang D, Zhu XJ, Han Y. Serum M2BPGi level is a novel predictive biomarker for the responses to pegylated interferon-α treatment in HbeAg positive chronic hepatitis B patients. J of Medical Virology, 2017; 25010.
17. Maezawa K, Shimogawa RF, Yasukawa A, Ohta N, Iwanaga S. Real-time observation of pathophsiological processes during murine experimental infection using high-resolution ultrasound imaging. Tropical Medicine and Health, 2018; 46: 1.
18. Ichikawa Y, Joshita S, Umemura T, Shobugawa Y, Usama Y, *et al.* 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. Hepatology Research, 2016; 12712.