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Diagnostic performance of Mac-2 binding protein glycosylation isomer (M2BPGi) in screening liver fibrosis in health checkups

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Abstract

Background: Mild-to-moderate fibrosis is rarely diagnosed because the disease is asymptomatic in the early stage. The serum level of Mac-2 binding protein glycosylation isomer (M2BPGi) has been found to increase with the severity of liver fibrosis. The aim of this study was to determine the diagnostic performance of M2BPGi in screening liver fibrosis using magnetic resonance elastography (MRE) as a reference standard and to compare it with using the aspartate aminotransferase-to-platelet ratio (APRI) and the Fibrosis-4 index (FIB-4) in health checkups.

Methods: This cross-sectional study consecutively selected subjects at health examinations who underwent MRE and M2BPGi testing at eight health promotion centers in Korea between January and September 2019. The serum M2BPGi level was measured using the chemiluminescence enzyme immunoassay method. The measured levels were indexed using the cutoff index (COI). COI values of M2BPGi were compared with the MRE results.

Results: The median (interquartile) values of COI for fibrosis stages F0 (normal liver stiffness), F1 (mild fibrosis), F2 (significant fibrosis), and ≥F3 (advanced fibrosis) were 0.49 (0.34-0.61), 0.48 (0.38-0.68), 0.64 (0.43-1.03), and 1.01 (0.75-1.77), respectively ($P < .0001$). The AUCs of the COI for the screening of fibrosis stage ≥F1, ≥F2, and ≥F3 were 0.591, 0.698, and 0.853, respectively. Using a threshold of 0.75 for COI to exclude advanced fibrosis had a sensitivity, specificity, and negative predictive value of 80.0%, 77.9%, and 98.9%, respectively. The AUC for excluding advanced fibrosis was better for M2BPGi than for FIB-4 and APRI.

Conclusion: Serum M2BPGi was useful for screening significant and advanced fibrosis in health checkups.

KEYWORDS
aspartate aminotransferase-to-platelet ratio, fibrosis-4 index, liver fibrosis, M2BPGi, Mac-2 binding protein glycosylation isomer, magnetic resonance elastography

1 | INTRODUCTION

Chronic liver diseases are very common worldwide and have become a major global public health issue. These trends are expected to increase due to aging populations, the increasing obesity epidemic, and the continuing emergence of clinical manifestations among individuals with long-standing chronic infection with hepatitis B (HBV) or hepatitis C virus (HCV).[1,2] The main histological consequence of chronic liver diseases is the continuous deposition of collagen fibers, which causes progressive liver fibrosis, and eventually cirrhosis.[3] Mild-to-moderate fibrosis is rarely diagnosed because the disease is asymptomatic in the early stage. An early diagnosis would allow identification of the causal factors responsible for liver inflammation and the subsequent application of specific targeted interventions, such as antiviral therapy for HBV or HCV infection, psychosocial interventions for alcoholic liver disease,

TABLE 1 Demographic and clinical characteristics of the study subjects

	Liver fibrosis according to MRE (kPa)				
	<1.94	1.94-2.90	2.91-3.59	3.60≤	
Characteristic	(N = 43)	(N = 159)	(N = 24)	(N = 10)	P
Age, y	53.0 (27-68)	50.0 (27-78)	48.5 (39-67)	56.0 (41-81)	.295
Male sex, N (%)	23 (53.5)	128 (80.5)	16 (66.7)	9 (90.0)	.002
BMI, kg/m^2	24.2 (18.2-31.0)	24.9 (17.1-34.1)	26.2 (19.3-32.4)	27.8 (21.6-32.4)	.078
Platelet count, 10^3/uL	240.0 (150-417)	234.5 (128-455)	261.0 (111-334)	198.0 (63-455)	.016
AST, IU/L	25.0 (12-93)	26.0 (11-145)	29.0 (18-108)	38.5 (17-148)	.009
ALT, IU/L	22.0 (9-99)	26.0 (11-184)	36.0 (11-104)	33.5 (14-109)	.017
AST/ALT ratio, %	1.15 (0.59-2.03)	1.00 (0.35-4.14)	0.97 (0.64-1.82)	1.17 (0.79-3.43)	.043
ALP, IU/L	198.0 (99-414)	207.0 (95-357)	230.0 (108-389)	193.5 (155-2,945)	.280
GGT, IU/L	23.0 (13-464)	33.5 (9-365)	41.0 (10-643)	55.0 (20-1,337)	.005
Total. bilirubin, mg/dL	0.88 (0.39-2.11)	0.95 (0.36-1.99)	1.00 (0.47-2.36)	0.80 (0.46-3.39)	.366
Total. protein, g/dL	7.2 (6.6-8.1)	7.3 (6.2-8.6)	7.4 (6.8-8.1)	7.2 (6.0-8.2)	.179
Albumin, g/dL	4.5 (3.9-5.0)	4.5 (3.5-5.2)	4.5 (4.2-4.9)	4.5 (3.7-4.6)	.333
A/G ratio, %	1.7 (1.3-2.4)	1.6 (1.0-2.5)	1.6 (1.3-2.0)	1.6 (1.1-2.0)	.627
APRI	0.25 (0.11-1.39)	0.28 (0.12-1.92)	0.36 (0.15-1.18)	0.34 (0.21-1.98)	.005
FIB-4	1.10 (0.3-3.33)	1.04 (0.37-4.70)	1.18 (0.67-3.00)	1.61 (0.81-11.02)	.030
TC, mg/dL	196.5 (113-280)	195 (110-318)	187 (138-449)	182 (116-237)	.484
TG, mg/dL	80.0 (37-485)	121.0 (30-783)	101.0 (40-699)	123.5 (68-242)	.006
LDL-C, mg/dL	117.5 (58-193)	114.0 (39-224)	109.0 (69-171)	104.0 (48-161)	.349
HDL-C, mg/dL	54.5 (34-84)	48.0 (32-97)	57.0 (35-80)	40.0 (32-77)	.002
AFP, ng/mL	2.58 (1.05-8.78)	2.79 (0.84-7.60)	3.08 (1.62-6.26)	4.45 (1.57-359.5)	.070
FBS, mg/dL	96.5 (71-145)	97.0 (70-237)	98.0 (74-203)	109.5 (88-207)	.046
HbA1$_C$, %	5.7 (4.9-6.9)	5.6 (4.6-9.1)	5.7 (5.0-9.6)	6.0 (5.6-10.5)	.578
Insulin, mIU/L	3.89 (0.83-11.32)	4.56 (0.62-9.54)	10.64 (6.67-15.30)	–	.044
M2BPGi COI	0.49 (0.17-0.96)	0.48 (0.15-1.77)	0.64 (0.23-1.40)	1.01 (0.47-5.16)	.000
Fatty liver grade, N (%)					
Mild	11 (27.5)	44 (30.3)	6 (28.6)	2 (22.2)	.599
Moderate	5 (12.5)	20 (13.8)	7 (33.3)	1 (11.1)	
Severe	–	4 (2.8)	–	–	
HBsAg or anti-HCV positivity, N (%)	4 (12.9)	12 (10.8)	4 (20.0)	3 (42.9)	.080

Note: Data are median (range) or frequency (percentage) values.

P values are from Kruskal-Wallis test or Fisher's exact test.

Abbreviations: AFP, alpha-fetoprotein; A/G ratio; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; COI, cutoff index; FBS, fasting blood sugar; FIB-4, Fibrosis-4 index; GGT, gamma-glutamyl transpeptidase; HCV, hepatitis C virus; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; MRE, magnetic resonance elastography; M2BPGi, Mac-2 binding protein glycosylation isomer; TC, total cholesterol; TG, triglyceride.

FIGURE 1 Box plots of Mac-2 binding protein glycosylation isomer (M2BPGi) cutoff index (COI) according to liver fibrosis
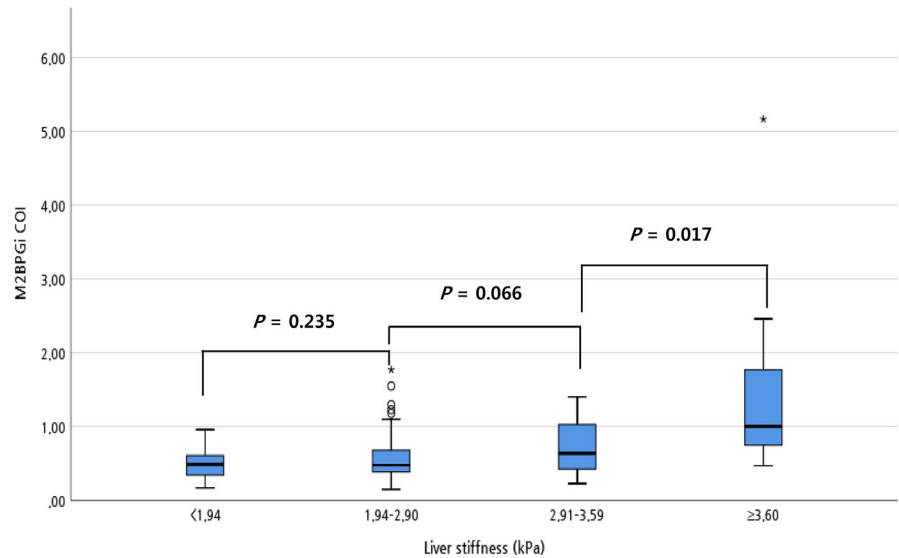

TABLE 2 Diagnostic performance of the M2BPGi test for identifying different stages of liver fibrosis

Liver fibrosis	AUC	(95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
From mild fibrosis (≥F1)	0.591	(0.501, 0.680)	0.42	72.5	46.5	85.9	27.4
From significant fibrosis (≥F2)	0.698	(0.591, 0.805)	0.59	70.6	63.9	24.7	92.8
From advanced fibrosis (≥F3)	0.853	(0.734, 0.972)	0.75	80.0	77.9	13.8	98.9

Abbreviations: AUC, area under the receiver operating characteristic curve; CI, confidence interval.

and lifestyle changes and the treatment of diabetes, obesity, and non-alcoholic fatty liver disease (NAFLD).[4] The elimination of the causative factor stops the hepatic inflammation and leads to fibrosis regression.[3,5]

While liver biopsy is the current gold standard for determining the fibrosis stage, it not only has its own limitations, such as the risk of sampling error, high rates of interobserver differences, risk of complications, and the high cost but is also not well accepted by patients.[6,7] Various elastographic techniques such as magnetic resonance elastography (MRE) have been demonstrated to show high diagnostic accuracy for liver fibrosis.[8,9] However, considering its cost and need of MRE, it is inappropriate to apply these techniques to screen fibrosis in an asymptomatic general population in primary health care, and hence simple, reliable, and noninvasive biomarkers need to be developed for detecting liver fibrosis. *Wisteria floribunda* agglutinin-positive (WFA+) Mac-2 binding protein glycosylation isomer (M2BPGi), which is the isoform of the glycan structure of Mac-2-binding protein (M2BP) was recently discovered to be a novel serum glycomarker for liver fibrosis. The serum M2BPGi level reportedly increases with the severity of liver fibrosis in patients with chronic hepatitis, and also those with NAFLD.[10-12]

The aim of this study was to determine the utility of M2BPGi, and to compare it with routine biochemical markers such as the aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and the Fibrosis-4 index (FIB-4) in screening liver fibrosis diagnosed by MRE as a reference standard in health checkups.

2 | MATERIALS AND METHODS

2.1 | Study subjects

This cross-sectional retrospective study consecutively selected subjects from health examinations who underwent MRE and M2BPGi testing at eight health promotion centers in Korea between January and September 2019. The 236 examinees comprised 103 with normal subjects, 100 with fatty liver, five with chronic liver disease, five with liver cirrhosis, and 23 with HBsAg (+) based on ultrasonography or abdominal computed tomography. All MRE examinations were performed on MRE hardware (GE Healthcare) with a 1.5-T imaging system using a two-dimensional MRE protocol.[13] The cutoff values for liver fibrosis using MRE were 1.94, 2.90, 3.59, and 3.6 kPa for fibrosis stage F0 (normal liver stiffness), F1 (mild fibrosis), F2 (significant fibrosis), and ≥F3 (advanced fibrosis), respectively.[9] This study protocol was reviewed and approved by the institutional review board.

2.2 | Laboratory measurements

Venous blood was drawn after an overnight fast for health checkups that included complete blood count (CBC), biochemical measurements, and M2BPGi. CBC and biochemical parameters were measured using the Sysmex XE-2100D analyzer (Sysmex) and the Hitachi 7600 analyzer (Hitach). The serum M2BPGi level was measured
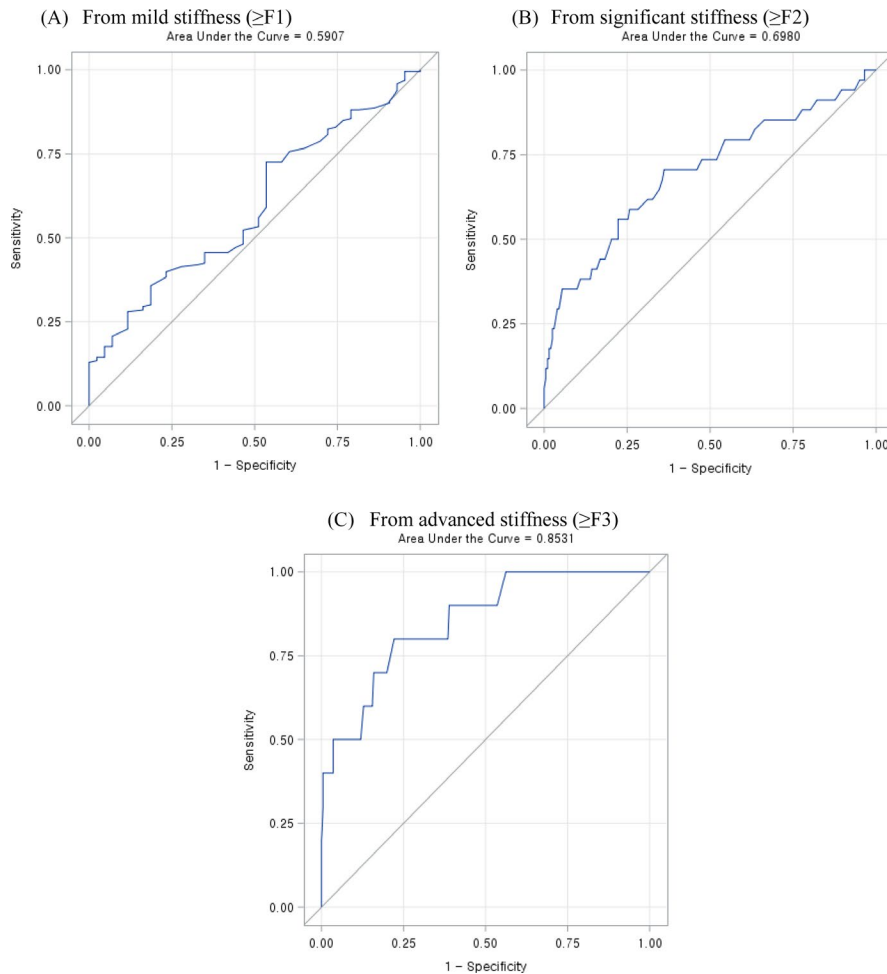
(A) From mild stiffness (≥F1)
Area Under the Curve = 0.5907

(B) From significant stiffness (≥F2)
Area Under the Curve = 0.6980

FIGURE 2 Analysis of areas under the receiver operating characteristic curves (AUCs) for the diagnostic ability of the M2BPGi test for liver fibrosis

(C) From advanced stiffness (≥F3)
Area Under the Curve = 0.8531

using the chemiluminescence enzyme immunoassay method (HISCL-5000; Sysmex, Kobe, Japan). The measured levels of M2BPGi were indexed with obtained values using the following equation: cutoff index (COI) = ([M2BPGi] sample – [M2BPGi] negative control)/([M2BPGi] positive control–[M2BPGi] negative control).[14] The APRI was calculated as AST (IU/L)/ULN (upper limit of normal)/platelet count (10^9/L) × 100.[15] FIB-4 was calculated using the following formula: FIB-4 = age × AST (IU/L)/platelet count (10^9/L) x √ALT (IU/L).[16]

2.3 | Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute). Data are presented as median (range) or frequency (percentage) values. A univariate analysis was performed to assess differences between the four liver fibrosis groups using the nonparametric Kruskal-Wallis test or Fisher's exact test. The diagnostic performance of the M2BPGi for screening liver fibrosis was assessed using the area under the receiver operating characteristic curves (AUC). The cutoffs for M2BPGi, APRI, and FIB-4 were established based on the optimal combination of sensitivity and specificity. The AUC and 95% confidence interval values were determined using the Wilcoxon rank-sum test. A P value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Demographic and clinical characteristics of the study subjects

The characteristics of the study subjects are presented in Table 1. MRE revealed that there were 43, 159, 24, and 10 subjects in fibrosis stages F0, F1, F2, and ≥F3, respectively. The median age of the subjects was 53 years (range 27-81 years). Those with significant or advanced fibrosis (stage ≥ F2) were predominantly male and had higher AST, alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), blood glucose, and insulin levels, and higher COI of M2BPGi, APRI, and FIB-4 values compared to subjects with normal liver stiffness (stage F0) (P < .01).

3.2 | COI of M2BPGi according to liver fibrosis stage by MRE

The median (interquartile) values of COI for fibrosis stages F0, F1, F2, and ≥F3 were 0.49 (0.34-0.61), 0.48 (0.38-0.68), 0.64 (0.43-1.03), and 1.01 (0.75-1.77), respectively (P < .001). The COI was significantly higher for fibrosis stage ≥F3 than F2 (P = .017), but it did not differ significantly between stages F0 and F1 (P = .235) or between stages F1 and F2 (P = .066) (Figure 1).

TABLE 3 Diagnostic performances of M2BPGi, FIB-4, and APRI for diagnosing liver fibrosis

Liver fibrosis stage	AUC (95% CI)	Cutoff	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value
From mild fibrosis (≥F1)						
M2BPGi	0.604 (0.512, 0.695)	0.42	72.5	46.5	85.9	27.4
FIB-4	0.492 (0.396, 0.589)	1.82	14.5	92.7	89.3	20.5
APRI	0.608 (0.511, 0.705)	0.23	74.4	43.9	84.8	29.0
From significant fibrosis (≥F2)						
M2BPGi	0.680 (0.570, 0.791)	0.59	70.6	63.9	24.7	92.8
FIB-4	0.628 (0.530, 0.727)	0.79	96.9	26.0	18.8	97.9
APRI	0.672 (0.563, 0.780)	0.33	62.5	68.5	26.0	91.2
From advanced fibrosis (≥F3)						
M2BPGi	0.836 (0.709, 0.964)	0.75	80.0	77.9	13.8	98.9
FIB-4	0.766 (0.602, 0.931)	1.29	66.7	65.2	7.8	97.8
APRI	0.735 (0.558, 0.912)	0.29	77.8	54.9	7.1	98.3

3.3 | Diagnostic performance of M2BPGi for identifying different stages of liver fibrosis

The optimal threshold values of COI for the screening of liver fibrosis are presented along with the sensitivity, specificity, and AUC values in Table 2 and Figure 2. The AUC of COI was good for stages F0-F2 vs stage ≥F3 and fair for stages F0 and F1 vs stage ≥F2, but poor for stage F0 vs stage ≥F1. The AUCs of the COI for the diagnosis of fibrosis stages ≥F1, ≥F2, and ≥F3 were 0.591, 0.698, and 0.853, respectively. Using a threshold of 0.75 for COI to exclude advanced fibrosis had a sensitivity of 80.0%, a specificity of 77.9%, and a negative predictive value (NPV) of 98.9%.

3.4 | Comparison of M2BPGi with FIB-4 and APRI for screening liver fibrosis

The optimal threshold values of COI for M2BPGi, FIB-4, and APRI in screening liver fibrosis are presented along with their sensitivity, specificity, and AUC values in Table 3 and Figure 3. Using a cutoff of 1.29 for FIB-4 to exclude advanced fibrosis had a sensitivity of 66.7%, a specificity of 65.2%, an NPV of 97.8%; the corresponding values when using a cutoff of 0.29 for APRI were 77.8%, 54.9%, and 98.3%, respectively. The AUCs for COI, FIB-4, and APRI for excluding advanced liver fibrosis were 0.836, 0766, and 0.735, respectively. The AUC of COI was good for the excluding of advanced liver fibrosis, while those of FIB-4 and APRI were only fair. The AUC was better for M2BPGi than for FIB-4 and APRI in excluding advanced liver fibrosis.

4 | DISCUSSION

This study found M2BPGi to be a useful biomaker for screening significant liver fibrosis (stage F2) and advanced liver fibrosis (stage ≥ F3) in health examinees. The diagnostic accuracy of M2BPGi

was better than those of FIB-4 and APRI for excluding advanced liver fibrosis.

Chronic liver diseases are characterized by an array of histopathological changes that include the infiltration of liver tissue by inflammatory cells, hepatocyte alterations, necrosis, the proliferation of myofibroblasts, and fibrosis.[3] Fibrosis is the most important of these changes in terms of determining progression of the disease to cirrhosis and clinically relevant outcomes. Among the methods available to assess liver fibrosis, noninvasive biomarkers have been developed for ease of application in routine care. Research on glycan sugar chain-based immunoassays identified a new glycol marker for liver fibrosis.[17] Human endogenous M2BP consists of 10-16 monomers with 70-112 N-glycans attached to each macromolecule.[18] Alterations in M2BP during the progression of liver disease and fibrosis are attributable to changes in N-glycosylation and this serum hyperglycosylated WFA+-M2BP was measured using a glycan-based immunoassay.

The present study found that the serum COI value of M2BPGi was higher in the presence of significant and advanced fibrosis than for normal liver stiffness. The AUC of COI was good for stages F0-F2 vs stage ≥F3 and fair for stages F0 and F1 vs stage ≥F2. It had high NPV values of 92.8% and 98.9% for significant, and advanced fibrosis, respectively. These findings suggest M2BPGi could be used clinically to exclude significant and advanced fibrosis in health examinees. The COI threshold of 0.75 for M2BPGi to exclude advanced fibrosis was lower than the COI value of 1.0 that the manufacturer proposed. Ogawa et al proposed that patients with a COI value of WFA+-M2BP >0.83 should be strongly recommended to undergo elastography for diagnosing significant fibrosis.[19] Moreover, Lai et al showed that WFA+-M2BP was poor for diagnosing steatosis, lobular inflammation, and hepatocyte ballooning non-alcoholic steatohepatitis in NAFLD patients, but that the performance of the test was good for detecting significant fibrosis, advanced fibrosis, and cirrhosis.[20] These results are somewhat consistent with our results, although the characteristics of the subjects in those studies differed from ours.

(A) From mild stiffness (≥F1)

(B) From significant stiffness (≥F2)
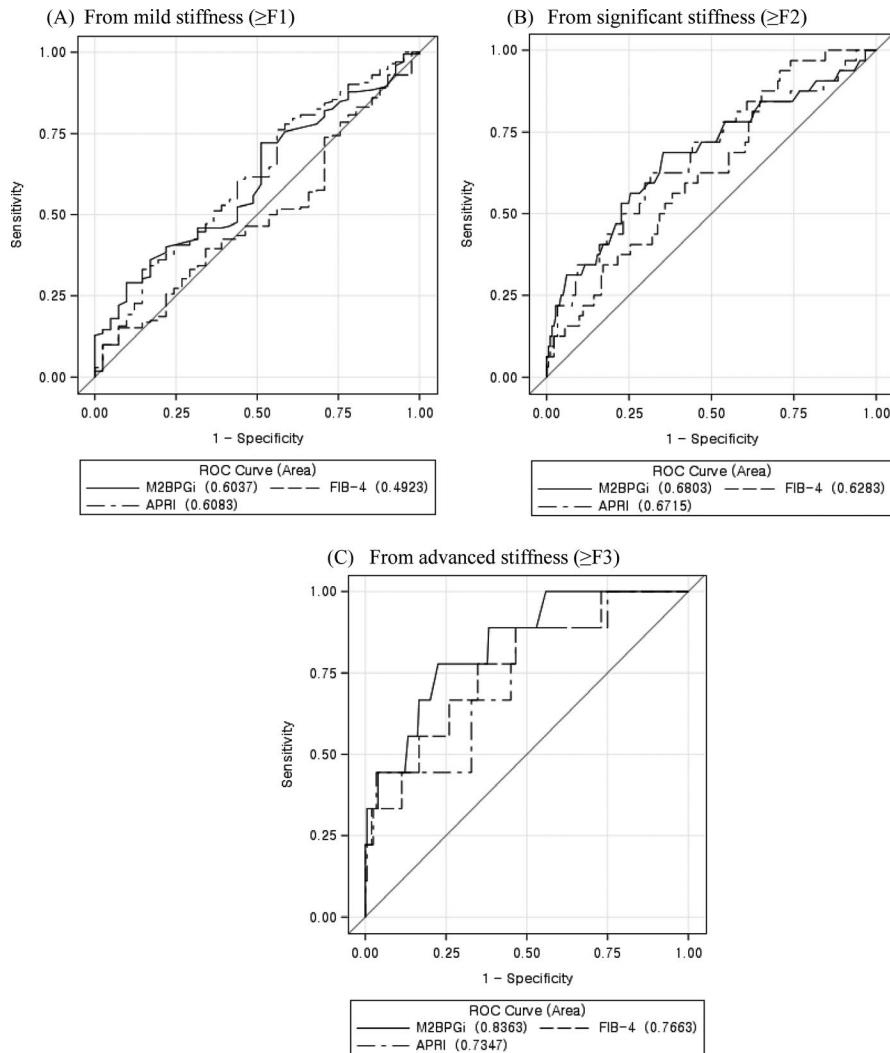
(C) From advanced stiffness (≥F3)

FIGURE 3 Comparison of M2BPGi with Fibrosis-4 index (FIB-4) and aspartate aminotransferase-to-platelet ratio index (APRI) for diagnosing liver fibrosis

FIB-4 and APRI have been validated in NAFLD.[21,22] These biomarkers are known to have advantages of high applicability, wide availability, and moderately low cost. In particular, FIB-4 has been developed as a marker for excluding advanced fibrosis in patients with NAFLD.[21] FIB-4 and APRI are fairly useful for excluding advanced fibrosis, while the performance of COI of M2BPGi was better in the present study.

This study had some limitations. First, it employed a cross-sectional design to investigate the usefulness of the serum M2BPGi, and so future prospective studies are necessary to support the present findings. Second, MRE findings were used as a standard for liver fibrosis, rather than definitive results from liver biopsy. Third, the proportion of subjects with advanced liver fibrosis was low since the subjects were selected consecutively from health examinees who underwent MRE and M2BPGi testing at eight health promotion centers. The limited number of subjects with advanced liver fibrosis in this study might have made it not to be fair to compare the serum biomarkers among them.

In conclusion, COI of M2BPGi was useful for the screening of significant and advanced fibrosis in health checkups. Screening for liver fibrosis could identify examinees with presymptomatic chronic

liver disease susceptible to interventions. M2BPGi could also be integrated into a clinical algorithm to help primary care units to identify patients who should undergo MRE for diagnosing significant and advanced fibrosis. Further studies are needed to explore the usefulness of this biomarker in determining the long-term outcomes in the general population.

AUTHOR CONTRIBUTIONS

All of the authors participated in designing this study. SC and HS performed data collection. SK undertook the statistical analyses. EN, SK, and HC analyzed and interpreted the data. EN wrote the first draft of the manuscript, which was reviewed by all of the other authors, who also provided further contributions and suggestions.

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