Gut and Liver
https://doi.org/10.5009/gnl230128
pISSN 1976-2283 eISSN 2005-1212

Original Article

Proposal of a Novel Serological Algorithm Combining FIB-4 and Serum M2BPGi for Advanced Fibrosis in Nonalcoholic Fatty Liver Disease

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Article Info
Received April 7, 2023
Revised June 12, 2023
Accepted June 13, 2023
Published online August 14, 2023

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Background/Aims: Noninvasive methods have become increasingly critical in the diagnosis of fibrosis in chronic liver diseases. Herein, we compared the diagnostic performance of serum Mac2 binding protein glycosylation isomer (M2BPGi) and other serological panels for fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) and proposed an improved two-step diagnostic algorithm for advanced fibrosis.

Methods: We enrolled 231 patients diagnosed with NAFLD who underwent a liver biopsy. We subsequently evaluated the diagnostic performance of serological panels, including serum M2BPGi, a fibrosis index based on four factors (FIB-4), aspartate aminotransferase-to-platelet ratio index (APRI), and NAFLD fibrosis score (NFS), in predicting the stage of liver fibrosis. We then constructed a two-step algorithm to better differentiate advanced fibrosis.

Results: The areas under the receiver operating characteristic curves of serum M2BPGi, FIB-4, APRI, and NFS for advanced fibrosis (≥F3) were 0.823, 0.858, 0.779, and 0.827, respectively. To reduce the performance of unnecessary liver biopsy, we propose a two-step algorithm using FIB-4 as an initial diagnostic tool and serum M2BPGi (≥0.6) as an additional diagnostic method for patients classified as intermediate (23%). Using the proposed algorithm, the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were 0.812, 0.814, 0.814, 0.600, and 0.927, respectively.

Conclusions: Serum M2BPGi is a simple and effective test for advanced fibrosis in patients with NAFLD. Application of the two-step algorithm based on FIB-4 and M2BPGi proposed here can improve diagnostic performance and reduce unnecessary tests, making diagnosis easily accessible, especially in primary medical centers. **(Gut Liver 2024;18:283-293)**

Key Words: Non-alcoholic fatty liver disease; Mac-2 binding protein glycosylation isomer; Liver fibrosis

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an increasingly critical cause of chronic liver disease.[1] A meta-analysis conducted on studies published from 1999 to 2019 revealed that the prevalence of NAFLD in Korea was 32.9% and that the incidence of NAFLD in Asia has increased significantly over time.[2] Consequently, NAFLD has emerged as a major contributor to chronic liver disease, and its incidence and prevalence are growing at an alarm-

ing rate, adding to the already high socioeconomic burden of the disease.[1,3-5]

Predicting the progression of liver fibrosis in chronic liver disease is important as fibrosis is strongly associated with long-term prognosis, including the development of liver cirrhosis, hepatocellular carcinoma, and liver-related death.[6,7] Although liver biopsy is the gold standard for evaluating liver fibrosis, it has several clinical limitations, including invasiveness, sampling error, relatively high cost, and variations in histological interpretations.[8] In addition, it is clinically impossible to perform a repeated biopsy to identify histological improvement or deterioration. As such, there is an increasing emphasis on the demand for noninvasive serum and imaging biomarkers for liver fibrosis grade.[9,10]

Serum Mac2 binding protein glycosylation isomer (M2BPGi) is a promising biomarker for detecting liver fibrosis in patients with chronic hepatitis. Several prior studies have shown that M2BPGi levels increase with the severity of liver fibrosis.[11-13] Liver fibrosis causes specific modifications to the glycosylation and sugar chain structure of Mac-2 binding protein (M2BP), resulting in modified M2BP proteins that have a significant correlation with fibrosis progression.[14] Quantification assays of serum M2BP using a lectin called *Wisteria floribunda* agglutinin allow for the detection of M2BPGi, which comprises fibrosis-specific modified sugar chain structures.[14] This test can be completed in 17 min using a fully automated machine and requires only a small amount of blood, making the test simple and efficient.[14]

Herein, we compared the diagnostic performance of serum M2BPGi and other serological panels for advanced fibrosis in patients with NAFLD and proposed a novel diagnosis method for advanced fibrosis through an algorithm that applies a fibrosis index based on four factors (FIB-4) and serum M2BPGi consecutively in primary medical centers.

MATERIALS AND METHODS

1. Study population

In this retrospective study, we enrolled 231 patients aged 18 years or older diagnosed with NAFLD who had undergone liver biopsy at Dong-A University Hospital and Kyungpook National University Hospital between March 2015 and September 2022. The diagnosis of NAFLD was based on ultrasonography or computed tomography findings of fatty liver without chronic liver disease due to secondary causes, such as significant alcohol consumption (weekly alcohol consumption ≥210 g in men and ≥140 g in women), drug-induced liver injury, or viral hepatitis. Clinical information, medical history, and laboratory analyses, such as complete blood count and routine biochemical analyses, were obtained within a week of liver biopsy. This study was approved by the Institutional Review Board of the Dong-A University College of Medicine (IRB number: DAUHIRB-22-245). The informed consent was waived because this design is a retrospective study.

2. Clinical measurement and laboratory assessment

Clinical data, including age, sex, body weight, height, and body mass index (BMI), were collected for each patient. BMI was calculated by dividing the weight in kilograms by the square of the height in meters. The definition of obesity in the Asian population is a BMI ≥25 kg/m^2.[15] Patients' medical history was reviewed to determine whether they were being treated for diabetes mellitus (DM), and impaired glucose tolerance (IGT) was defined as fasting blood glucose levels between 100 and 125 mg/dL. Laboratory tests were performed to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase, albumin, platelet count, total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglyceride levels. Noninvasive serological panels, such as FIB-4, AST-to-platelet ratio index (APRI), and NAFLD fibrosis score (NFS), were applied to assess fibrosis in addition to serum M2BPGi. These parameters were calculated using the following formulas:

$$\text{FIB-4} = [\text{age (yr)} \times \text{AST (U/L)}] / [\text{platelet count } (\times 10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}];$$

$$\text{APRI} = [(\text{AST (U/L)} / \text{upper normal limit} \times 100)] / \text{platelet count } (\times 10^9/\text{L});$$

$$\text{NFS} = -1.675 + 0.037 \times \text{age (yr)} + 0.094 \times \text{BMI (kg/m}^2) + 1.13 \times [\text{IGT or DM (yes=1; no=0)}] + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet count } (\times 10^9/\text{L}) - 0.66 \times \text{albumin (g/dL)}.$$ [16-18]

These serological panels were primarily developed and validated in patients aged 35 to 65 years, and subgroup analyses of diagnostic performance in patients aged <35 years were also performed in this study.

3. Histology assessment

Of the 231 patients analyzed, 202 underwent ultrasonography-guided liver biopsy, and the remaining 29 underwent surgical liver biopsy during abdominal surgery. The liver samples were fixed in formalin and embedded in paraffin. Liver biopsy samples were examined by a single experienced liver pathologist at each hospital where the tissue examination was performed. Liver fibrosis stage (F)

was assessed in accordance with a system devised by the Pathology Committee of the Nonalcoholic Steatohepatitis (NASH) Clinical Research Network. The NASH Clinical Research Network system describes NAFLD activity score, which is a composite score of steatosis, lobular inflammation, cytological ballooning, and fibrosis.[19] NASH was defined as a NAFLD activity score of ≥5. Steatosis was scored using the following criteria: <5%, 0; 5%–33%, 1; 33%–66%, 2; and >66%, 3. Fibrosis was scored using the following criteria: no fibrosis, F0; mild and moderate zone 3 periarticular fibrosis or portal/periportal fibrosis, F1; zone 3 perisinusoidal and portal/periportal fibrosis, F2; bridging fibrosis, F3; and cirrhosis, F4. F2–F4 were defined as significant fibrosis. F3–F4 were defined as advanced fibrosis, and F4 was defined as cirrhosis.[20]

4. Vibration-controlled transient elastography and enhanced liver fibrosis test

Our study included 231 patients, of whom 90 underwent vibration-controlled transient elastography (VCTE), and 71 underwent an enhanced liver fibrosis (ELF) test. Subgroup analyses were performed using the two-step algorithm recommended by existing guidelines for the VCTE and ELF groups, respectively.[21-23] We assessed the diagnostic performance of the two-step algorithms for the VCTE and ELF groups using subgroup analyses and compared them with the algorithm proposed in our study, which uses serum M2BPGi.

5. Statistical analysis

All data analyses were performed using R version 4.2.2. (R Foundation for Statistical Computing, Vienna, Austria). Continuous data were analyzed using the Student t-test and are presented as the mean±standard deviation. Categorical data were analyzed using the chi-square or Fisher exact tests. The diagnostic performance of all serological panels was expressed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curve (AUROC). We selected the optimal cutoff value that maximized the sum of the sensitivity and specificity for the presence of advanced fibrosis (≥F3) and liver cirrhosis (F4) based on histological findings. A subgroup analysis of 64 patients aged <35 years analyzed the diagnostic performance for significant fibrosis (≥F2) and advanced fibrosis (≥F3). A logistic regression analysis was applied to identify factors associated with advanced fibrosis in NAFLD, and the odds ratios and 95% confidence intervals (CIs) were calculated. Classification tree analysis was performed to obtain better sensitivity using a combination of serum M2BPGi and other noninvasive serological panels. Statistical significance was set at $p<0.05$.

RESULTS

1. Baseline characteristics of study population with and without advanced fibrosis

The baseline characteristics of the study population (n=231) and the comparison groups according to the presence of advanced fibrosis (≥F3) are summarized in Table 1. The mean age of the enrolled patients was 45.68 years, and there were 124 (53.68%) males. The associated metabolic diseases were obesity (BMI ≥25 kg/m^2) in 195 (84.42%), DM in 75 (32.47%), and IGT in 67 (29.00%) patients. The fibrosis grade on liver biopsy was F0 in 61 (26.41%), F1 in 70 (30.30%), F2 in 41 (17.75%), F3 in 41 (17.75%), and F4 in 18 (7.79%) patients. Significant fibrosis (≥F2) was observed in 100 (43.3%), and advanced fibrosis (≥F3) was observed in 59 (25.5%) patients. When comparing groups according to the presence of advanced fibrosis, the group with advanced fibrosis was significantly older and had a higher diagnosis of DM and NASH. The BMI and steatosis grade showed no significant difference between the two groups.

2. Correlation of serological panels with liver fibrosis stage

Fig. 1 shows the serum M2BPGi levels and other serological panel values according to the liver fibrosis stage. Spearman's rank correlation analysis showed that serum M2BPGi ($\rho=0.487$, $p<0.001$), FIB-4 ($\rho=0.536$, $p<0.001$), APRI ($\rho=0.577$, $p<0.001$), and NFS ($\rho=0.427$, $p<0.001$) levels increased as fibrosis worsened. Serum M2BPGi, FIB-4, and NFS levels showed a moderate correlation. The correlation coefficient (ρ) measures the strength and direction of the relationship between serological panels and the liver fibrosis stage, but it may decrease in value if the relationship is nonlinear or if outliers are present.

3. Diagnostic performance of serological panels for liver fibrosis grade

The AUROCs of serum M2BPGi, FIB-4, APRI, and NFS for advanced fibrosis (≥F3) were 0.823 (95% CI, 0.761 to 0.885), 0.858 (95% CI, 0.795 to 0.921), 0.779 (95% CI, 0.712 to 0.846), and 0.827 (95% CI, 0.759 to 0.894), respectively (Fig. 2). The AUROCs of serum M2BPGi, FIB-4, APRI, and NFS for cirrhosis (F4) were 0.913 (95% CI, 0.856 to 0.970), 0.920 (95% CI, 0.855 to 0.985), 0.772 (95% CI, 0.674 to 0.870), and 0.946 (95% CI, 0.895 to 0.998), respectively. All noninvasive tests, except for APRI, were comparable. The results of the optimal cutoff value, sensitivity, specific-

Table 1. Baseline Characteristics of the Study Population with and without Advanced Fibrosis (≥F3)

Characteristic	Overall (n=231)	Advanced fibrosis (≥F3)		p-value*
		No (n=172)	Yes (n=59)	
Age, yr	45.68±15.71	42.01±14.79	56.37±13.34	<0.001
Sex				<0.001
Male	124 (53.68)	105 (61.05)	19 (32.20)	
Female	107 (46.32)	67 (38.95)	40 (67.80)	
Height, cm	165.01±9.88	167.02±9.22	159.14±9.46	<0.001
Body weight, kg	81.09±18.01	83.32±18.19	74.61±15.90	<0.001
BMI, kg/m^2	29.58±4.84	29.68±4.98	29.28±4.43	0.7
Obesity (BMI ≥25 kg/m^2)	195 (84.42)	158 (84.30)	50 (84.75)	>0.9
Diabetes mellitus	75 (32.47)	43 (25.00)	32 (54.24)	<0.001
IGT	67 (29.00)	57 (33.14)	10 (16.95)	0.009
AST, U/L	69.27±48.00	63.47±46.91	86.19±47.54	<0.001
ALT, U/L	93.25±74.50	98.67±80.18	77.46±52.11	0.2
Albumin, g/dL	4.52±0.38	4.59±0.36	4.33±0.40	<0.001
GGT, U/L	83.74±81.82	77.53±76.63	101.85±93.74	0.002
Platelet, ×10^9/L	245.73±73.14	261.16±68.36	200.75±68.36	<0.001
Total cholesterol, mg/dL	192.69±42.77	197.98±43.13	177.25±38.02	0.003
Triglyceride, mg/dL (n=93)†	192.84±143.23	189.74±105.20	202.54±225.40	0.2
LDL, mg/dL (n=88)†	122.82±39.17	126.74±38.07	110.06±40.38	0.012
HDL, mg/dL (n=88)†	47.97±16.56	48.31±16.81	46.85±15.86	0.6
Glucose, mg/dL (n=77)†	121.62±40.12	117.21±35.51	135.58±50.05	0.021
M2BPGi (COI)	0.81±0.71	0.61±0.39	1.40±1.04	<0.001
FIB-4	1.80±1.92	1.18±1.00	3.61±2.70	<0.001
APRI	0.85±0.76	0.70±0.68	1.31±0.79	<0.001
NFS	−1.71±1.94	−2.28±1.59	−0.05±1.91	<0.001
Histologic finding				
Steatosis				0.2
None	2 (0.87)	2 (1.16)	0	
Mild	74 (32.03)	49 (28.49)	25 (42.37)	
Moderate	82 (35.50)	62 (36.05)	20 (33.90)	
Severe	73 (31.60)	59 (34.30)	14 (23.73)	
Lobular inflammation				0.009
None	23 (9.96)	23 (13.37)	0	
Mild	98 (42.42)	74 (43.02)	24 (40.68)	
Moderate	80 (34.63)	57 (33.14)	23 (38.98)	
Severe	30 (12.99)	18 (10.47)	12 (20.34)	
Ballooning				<0.001
None	67 (29.00)	66 (38.37)	1 (1.69)	
Few	86 (37.23)	68 (39.53)	18 (30.51)	
Many	78 (33.77)	38 (22.09)	40 (67.80)	
Fibrosis				<0.001
F0	61 (26.41)	61 (35.47)	0	
F1	70 (30.30)	70 (40.70)	0	
F2	41 (17.75)	41 (23.84)	0	
F3	41 (17.75)	0	41 (69.49)	
F4	18 (7.79)	0	18 (30.51)	
NAS				<0.001
Not NASH (NAS ≤2)	33 (14.29)	32 (18.60)	1 (1.69)	
Borderline NASH	75 (32.47)	59 (34.30)	16 (27.12)	
Definite NASH (NAS ≥5)	123 (53.25)	81 (47.09)	42 (71.19)	

Data are presented as mean±SD or number (%).

BMI, body mass index; IGT, impaired glucose tolerance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; M2BPGi, Mac2 binding protein glycosylation isomer; COI, cutoff index; FIB-4, fibrosis index based on four factors; APRI, AST-to-platelet ratio index; NFS, nonalcoholic fatty liver disease fibrosis score; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; F, fibrosis.

*Pearson chi-square test (categorical data), Wilcoxon rank sum test (continuous data); Fisher exact test (categorical data); †If any of the measurements are missing, the percentage of subjects with available data for the variable is reported as the number in brackets after the characteristic.

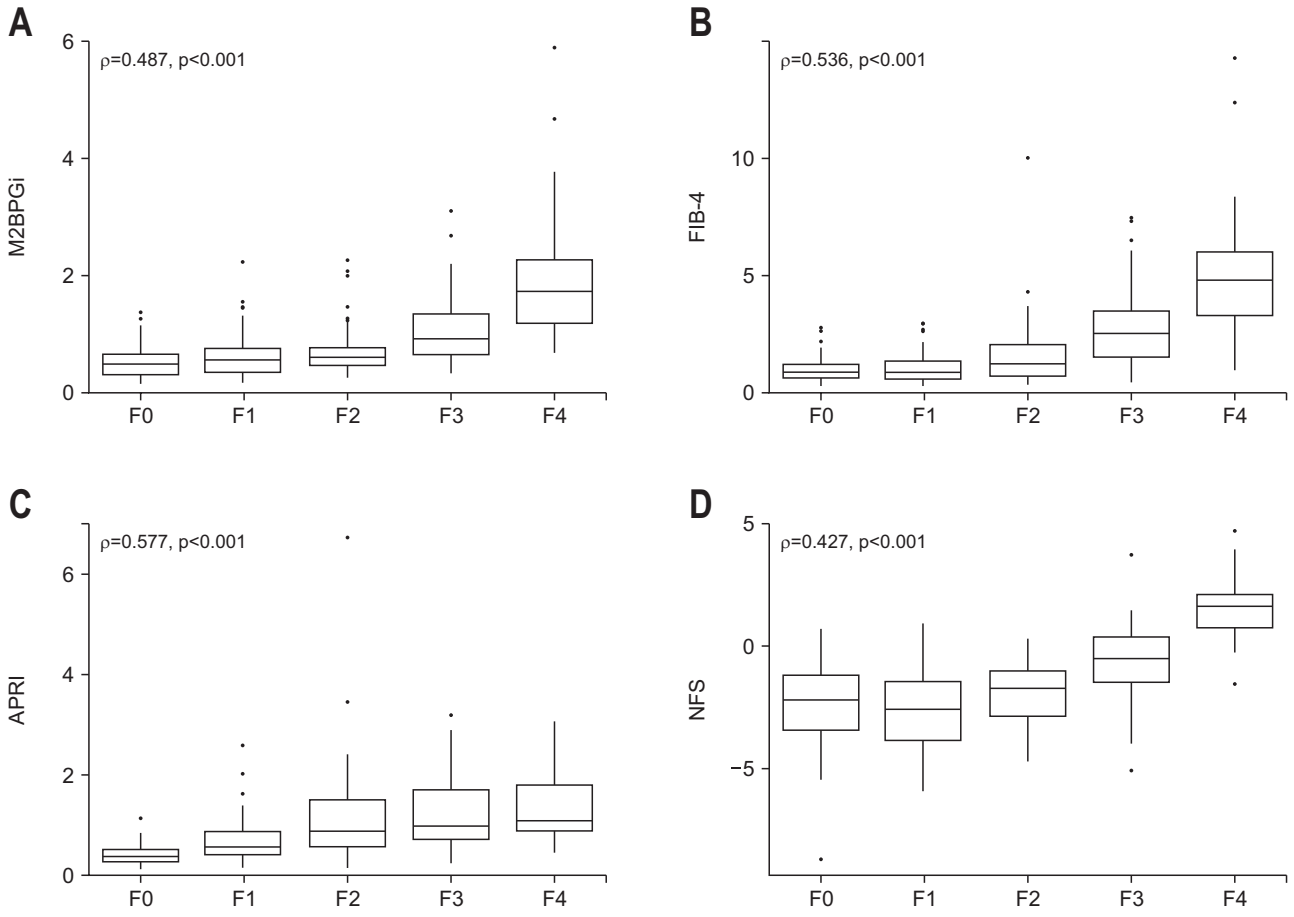

Fig. 1. Liver fibrosis marker values at each liver fibrosis stage in patients with nonalcoholic fatty liver disease (NAFLD). The Spearman rank correlation analysis showed that the (A) serum M2BPGi level, (B) FIB-4, (C) APRI, and (D) NFS levels increased as fibrosis stage. M2BPGi, Mac-2 binding protein glycosylation isomer; FIB-4, fibrosis index based on four factors; APRI, aspartate transaminase to platelet ratio index; NFS, NAFLD fibrosis score.

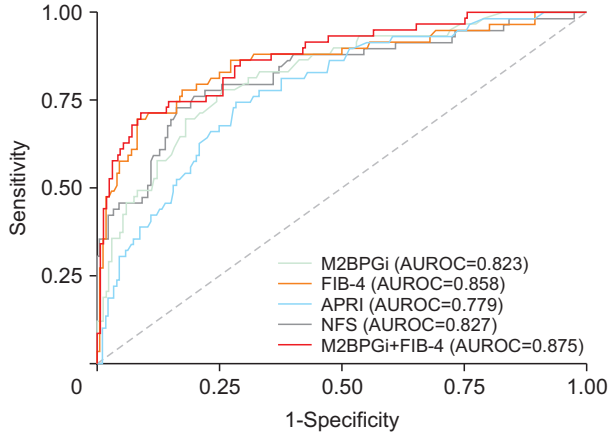

Fig. 2. The AUROCs of serum M2BPGi, FIB-4, APRI, NFS, and combination of serum M2BPGi and FIB-4 for advanced fibrosis (≥F3) in patients with nonalcoholic fatty liver disease (NAFLD). AUROC, areas under the receiver operating characteristic curve; M2BPGi, Mac2 binding protein glycosylation isomer; FIB-4, fibrosis index based on four factors; APRI, aspartate transaminase to platelet ratio index; NFS, NAFLD fibrosis score.

ity, PPV, and NPV obtained using serum M2BPGi, FIB-4, APRI, and NFS for the advanced fibrosis and cirrhosis groups are shown in Table 2. The results of the subgroup analysis of 64 patients aged <35 years showing the optimal cutoff value, sensitivity, specificity, PPV, and NPV obtained using serum M2BPGi, FIB-4, APRI, and NFS for significant fibrosis (≥F2) and advanced fibrosis are presented in Supplementary Table 1.

4. Logistic regression analysis of factors associated with advanced fibrosis

Of the 231 patients enrolled in the analysis, 59 (25.54%) were diagnosed with advanced fibrosis (≥F3). Univariate logistic regression analysis of clinical data revealed that sex, age, DM, IGT, AST, albumin, platelet, total cholesterol, and serum M2BPGi were all significant predictive factors (Table 3). However, of these variables, only AST, platelet, and serum M2BPGi levels remained independently associated with advanced fibrosis in the multivariate analysis.

Table 2. Diagnostic Performance of Serum M2BPGi, FIB-4, APRI, and NFS for Advanced Fibrosis (≥F3) and Cirrhosis (F4)

	AUROC (95% CI)	Cutoff	Sensitivity	Specificity	PPV	NPV	p-value
≥F3 (n=59)							
M2BPGi	0.823 (0.761–0.885)	0.71	0.780	0.744	0.511	0.908	<0.001
FIB-4	0.858 (0.795–0.921)	2.29	0.695	0.919	0.745	0.898	<0.001
APRI	0.779 (0.712–0.846)	0.79	0.746	0.715	0.473	0.891	<0.001
NFS	0.827 (0.759–0.894)	–0.71	0.729	0.837	0.606	0.900	<0.001
F4 (n=18)							
M2BPGi	0.913 (0.856–0.970)	1.47	0.722	0.939	0.500	0.976	<0.001
FIB-4	0.920 (0.855–0.985)	2.19	0.944	0.798	0.283	0.994	<0.001
APRI	0.772 (0.674–0.870)	0.87	0.778	0.704	0.182	0.974	<0.001
NFS	0.946 (0.895–0.998)	–0.27	0.944	0.840	0.333	0.994	<0.001

M2BPGi, Mac2 binding protein glycosylation isomer; FIB-4, fibrosis index based on four factors; APRI, aspartate aminotransferase-to-platelet ratio index; NFS, nonalcoholic fatty liver disease fibrosis score; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; F, fibrosis.

Table 3. Univariate and Multivariate Analyses for Advanced Fibrosis (n=231)

Characteristic	Univariate analysis		Multivariable analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex	3.30 (1.78–6.28)	<0.001		
Age	1.07 (1.05–1.10)	<0.001		
Diabetes mellitus	3.56 (1.92–6.64)	<0.001		
IGT	0.41 (0.19–0.84)	0.020		
AST	1.01 (1.00–1.02)	0.003	1.02 (1.00–1.03)	0.028
Albumin	0.16 (0.07–0.37)	<0.001		
Platelet	0.98 (0.98–0.99)	<0.001	0.99 (0.99–1.00)	0.030
Total cholesterol	0.99 (0.98–1.00)	0.002		
M2BPGi	9.31 (4.71–20.30)	<0.001	4.50 (2.05–10.70)	<0.001

OR, odds ratio; CI, confidence interval; IGT, impaired fasting glucose; AST, aspartate aminotransferase; M2BPGi, Mac2 binding protein glycosylation isomer.

5. Proposal for a diagnostic algorithm combining FIB-4 and serum M2BPGi for advanced fibrosis

Recently, many clinical practice guidelines have suggested a two-step algorithm that utilizes noninvasive test for risk stratification. We tested various combinations of the four serological panels (serum M2BPGi, FIB-4, APRI, and NFS) to improve the accuracy of advanced fibrosis discrimination. And we propose a two-step model-based algorithm, which could reduce unnecessary liver biopsies by applying an additional diagnostic method (serum M2BPGi) to the intermediate group by FIB-4 (Fig. 3). We calculated the sensitivity, specificity, accuracy, PPV, and NPV of our proposed algorithm by applying serum M2BPGi to the intermediate FIB-4 group as 0.812, 0.814, 0.814, 0.600, and 0.927, respectively (Fig. 3A). When we applied the first step of our algorithm, FIB-4, the intermediate group accounted for 22.9% (n=53) of all participants. By administering serum M2BPGi to the intermediate group, unnecessary testing was avoided in 20 of 53 patients (37.7 %).

For subgroup analysis, we applied the algorithm to 90 of 231 patients who underwent VCTE. The algorithm followed the previously suggested algorithm applying VCTE (≥8 kPa) to the intermediate group by FIB-4 (Fig. 3B). This two-step algorithm showed a sensitivity, specificity, accuracy, PPV, and NPV of 0.897, 0.771, 0.811, 0.650, and 0.940, respectively. These results demonstrated similar outcomes to our proposed algorithm by using FIB4 with M2BPGi. Similarly, for subgroup analysis, we applied an algorithm to 71 of 231 patients who underwent ELF testing. The resulting algorithm showed a sensitivity, specificity, accuracy, PPV, and NPV of 0.429, 0.875, 0.801, 0.500, and 0.864, respectively. It is estimated that subgroup analysis using ELF may result in a decreased diagnostic performance compared with a previous study owing to the small number of patients and the limited number of cases with advanced fibrosis.[24] In order to compare the performance of our proposed algorithm with the previously suggested algorithm, we conducted the analysis using our proposed algorithm in each subgroup (VCTE group and ELF group) and included the results in Supplementary Fig. 1.

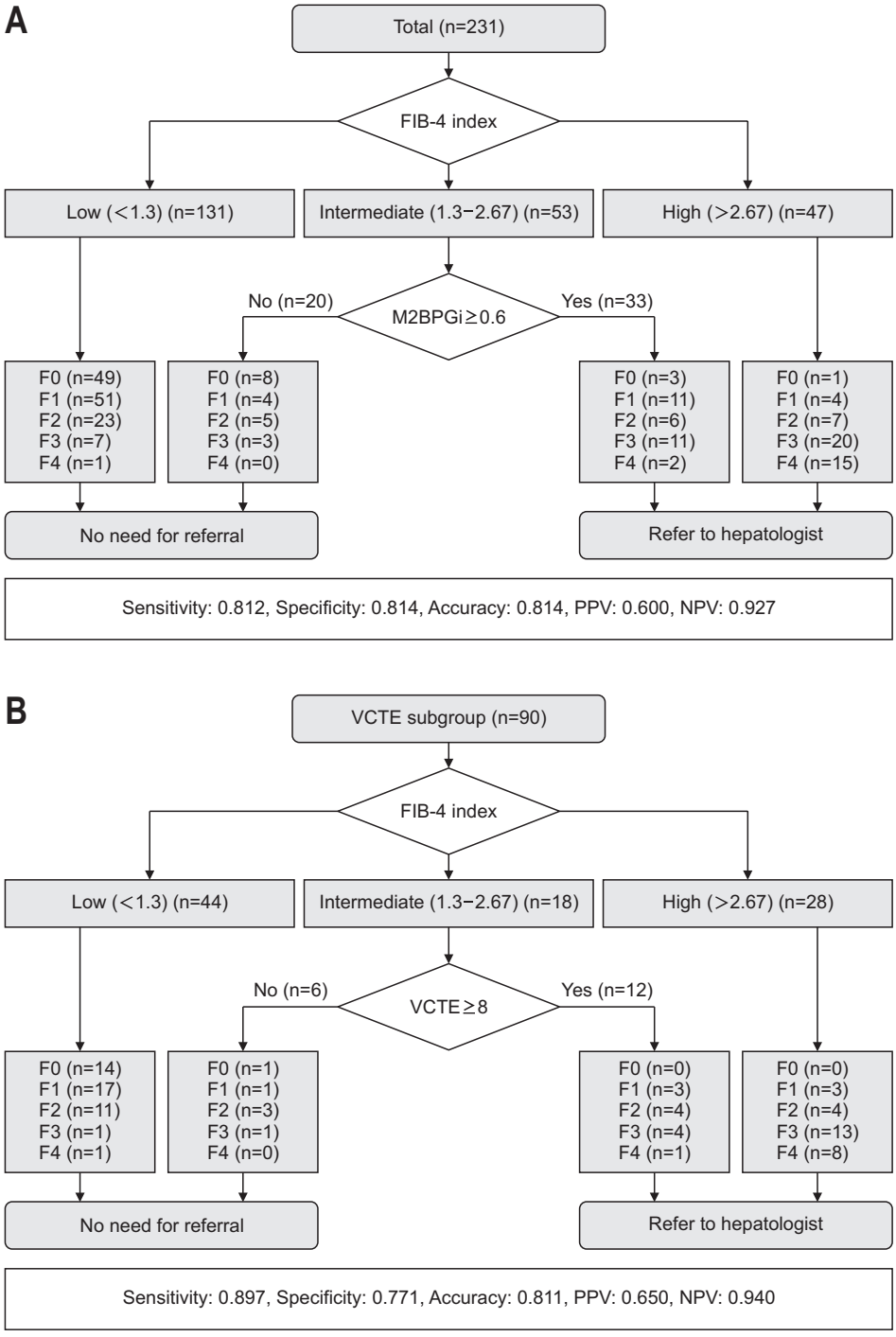

Fig. 3. Proposal of a two-step model-based algorithm for advanced liver fibrosis (≥F3) in patients with nonalcoholic fatty liver disease, and results of subgroup analysis applied with algorithms from existing guidelines. (A) Our proposed algorithm is a two-step model-based algorithm that applies serum M2BPGi to the intermediate group after applying FIB-4. (B) Subgroup analysis applied the previously suggested algorithm, using FIB-4 and VCTE (≥8 kPa) for the intermediate group in 90 patients who underwent VCTE. F, fibrosis; FIB-4, fibrosis index based on four factors; M2BPGi, Mac2 binding protein glycosylation isomer; PPV, positive predictive value; NPV, negative predictive value; VCTE, vibration-controlled transient elastography.

DISCUSSION

Patients with NAFLD commonly exhibit metabolic abnormalities such as diabetes, dyslipidemia, and obesity.[25-27] In addition, a sedentary lifestyle and unhealthy diet are also known contributing factors to NAFLD development.[25,28] The increasing prevalence of diabetes, obesity, and changes in lifestyle habits are associated with the rising incidence of

NAFLD.[25-27] Numerous research studies have highlighted the significance of fibrosis in the prognosis of NAFLD.[6,7,29] According to Dulai *et al.*,[29] as the stage of fibrosis progresses in NAFLD patients, the risk of all-cause and liver-related mortality also increases.[29] The risk of all-cause mortality rises with each increase in the stage of fibrosis: stage 1 (mortality rate ratio [MRR]=1.58), stage 2 (MRR=2.52), stage 3 (MRR=3.48), and stage 4 (MRR=6.40).[29] Similarly, the risk of liver-related mortality also increases with each increase in the stage of fibrosis: stage 1 (MRR=1.41), stage 2 (MRR=9.57), stage 3 (MRR=16.69), and stage 4 (MRR=42.30).[29]

Therefore, the identification of patients with clinically significant and advanced fibrosis is of utmost importance in order to implement intensive interventions and prevent disease progression in individuals with NAFLD. According to the American Association for the Study of Liver Diseases guidance, targeted screening of high-risk populations and further risk stratification in specialized care settings are recommended to identify patients with "at risk" NASH (biopsy-proven NASH with significant or higher fibrosis) or advanced fibrosis.[30] Recently, the identification of "at risk" NASH patients have emerged as an intriguing area, and it is crucial to assess the likelihood of NASH and significant fibrosis. However, the development of biomarkers or imaging techniques for the identification of "at risk" NASH is still under investigation, and performing these tests in primary medical centers can be challenging. The primary aim of our study was to exclude advanced fibrosis using noninvasive tests in primary medical centers, focusing on risk stratification rather than the precise diagnosis of significant or advanced fibrosis.

Various noninvasive methods have been developed as alternatives to liver biopsy for examining the degree of liver fibrosis. Notable noninvasive parameters for assessing liver fibrosis using basic patient clinical and laboratory data include FIB-4, APRI, and NFS. Consequently, they have been widely used in many studies as effective screening tools to detect advanced fibrosis.[31-34] Despite the relatively good diagnostic performance of existing noninvasive serologic panels, they still have several limitations. APRI has the disadvantage of decreased accuracy in detecting early stages of fibrosis and has a high proportion of patients with undetermined results (approximately 30%).[33,35] FIB-4 and NFS are the most extensively validated tests for NAFLD, but they can produce scores in the "indeterminate" range for at least 30% of cases.[31,36] According to one study, FIB-4 and NFS show reduced specificity in patients aged ≥65 years, leading to the proposal of new cutoff values for this age group.[37] In patients aged <35 years, these tests have shown decreased accuracy, prompting the use of alternative assessments.[37] The utility of NFS may be limited in some cases because of the large variation in score depending on the presence or absence of IGT and DM diagnoses.[38]

Modified M2BP proteins, which undergo specific glycosylation and sugar chain structural changes, are closely associated with the activation of hepatic stellate cells and the progression of liver fibrosis.[14,39] This is because M2BPGi levels act as juxtacrine-acting messengers between hepatic stellate cells and Kupffer cells.[39] The degree of fibrosis progression is significantly correlated with the levels of these modified M2BP proteins. In the present study, the diagnostic performance of serum M2BPGi in detecting advanced fibrosis in NAFLD (AUROC=0.823) was similar to that reported in previous studies (AUROC=0.876, 0.842, and 0.740).[13,40,41] Additionally, other studies have shown that serum M2BPGi performs similarly to FIB-4 and NFS in the diagnosis of liver fibrosis grade.[13,40,41] Therefore, serum M2BPGi can be used as an additional option for the examination of degree of liver fibrosis due to its convenience and efficiency compared to other serological panels that require various factors. Moreover, the subgroup analysis in our study revealed that for 64 individuals aged <35 years, the diagnostic performance of serum M2BPGi (AUROC=0.735) in detecting significant fibrosis (≥F2) was superior to that of age-incorporated FIB-4 (AUROC=0.686) and NFS (AUROC=0.558) (Supplementary Table 1). Therefore, as the prevalence of NAFLD is increasing among younger age groups, serum M2BPGi holds promise as a useful diagnostic tool, and further research is warranted.[42]

Recently, clinical studies have recommended the development of two-step algorithms to compensate for the limitations of a single noninvasive method for diagnosing advanced fibrosis in patients with NAFLD.[21-23,30] The primary aim of such algorithms is to exclude patients with advanced fibrosis and prevent unnecessary testing and monitoring. The recently proposed two-step algorithms all start with FIB-4 as the first step, followed by a recommendation for VCTE or ELF in the next step, thereby reducing the classification of patients as intermediate stage and improving the diagnostic analysis. A previous analysis using histological samples from a subgroup of 3,202 patients in the STELLAR trials demonstrated that using VCTE or ELF in the indeterminate group after classifying patients with FIB-4 or NFS could reduce the indeterminate group by 20%.[43] In a prospective longitudinal cohort study of 3,012 individuals using an algorithm comprising the FIB-4 followed by ELF, unnecessary referrals were reduced by 80%, and the diagnosis of advanced fibrosis and cirrhosis were improved 5-fold and 3-fold compared to that before the algorithm was used, respectively.[24] However, the use

of VCTE or ELF in the second step of these two-step algorithms have limitations in their ability to distinguish advanced fibrosis at the primary medical center. VCTE may not be feasible in 5% to 13% of cases due to obesity and additional equipment is required to perform the test, and ELF is relatively expensive compared to other methods.[33,44] All of these limitations make it difficult to conduct tests to exclude advanced fibrosis in primary medical centers.

The results of our study suggest that sequential application of M2BPGi with FIB-4 has a comparable accuracy of 0.814 to the subgroup analysis of 90 patients who underwent VCTE in our study (0.811). This accuracy is similar to the diagnostic accuracy of FIB-4 followed by VCTE in the STELLAR trial (sensitivity, specificity, and accuracy: 0.77, 0.89, and 0.83, respectively).[43] Although the optimal cutoff for advanced fibrosis as a single method was suggested to be 0.71 in our study, a cutoff of 0.6 for serum M2BPGi was recommended in the second step of our algorithm to improve sensitivity while maintaining a high accuracy and reducing false negative rates. Serum M2BPGi, which is relatively simple, inexpensive, and does not require additional equipment, may be useful as an algorithm for differentiating liver fibrosis in primary medical centers.

This study has several limitations. First, this was a retrospective study that analyzed the data from two institutions, resulting in a relatively long study duration. Second, it is important to consider that all participants in this study belonged to the Korean ethnic group, which may have limited the diversity of the data. However, this reduced the potential for racial bias in the study, which may be advantageous in certain cases. Third, there may be concerns regarding the quality of the histological results due to the lack of pathological agreement and standardization between the two institutions. However, both institutions had experienced liver pathologists who conducted at least two tissue reviews for each sample, ensuring high quality of the results. Lastly, we did not perform validation of our proposed diagnostic algorithm for advanced fibrosis. We will make efforts to conduct a prospective validation study in the future.

In conclusion, serum M2BPGi is a simple test with sufficient diagnostic ability for advanced fibrosis that can be easily performed in primary medical centers for patients with NAFLD. Furthermore, the two-step algorithm that sequentially applies FIB-4 and serum M2BPGi provides improved performance and similar efficacy to the previously proposed algorithm and improved performance, while being simpler and relatively inexpensive, making it more suitable for primary medical centers.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This research was supported by Dong-A University Research Fund.

AUTHOR CONTRIBUTIONS

Study concept and design: S.Y.M., Y.H.B., S.Y.J. Data acquisition: S.Y.M., Y.H.B., S.Y.J., D.W.J., K.T.Y., Y.Y.C., H.G.J. Data analysis and interpretation: S.Y.M., Y.H.B., S.Y.J., D.W.J. Drafting of the manuscript; critical revision of the manuscript for important intellectual content: S.Y.M., Y.H.B. Statistical analysis: S.Y.M., Y.H.B., A.J.J. Administrative, technical, or material support; study supervision: Y.H.B. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl230128.

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