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Diagnostic accuracy of enhanced liver fibrosis test for nonalcoholic steatohepatitis-related fibrosis: Multicenter study

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver operator characteristic curve; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; ELF, enhanced liver fibrosis; FIB-4, Fibrosis-4; HCC, hepatocellular carcinoma; LC, liver cirrhosis; M2BPGi, Mac-2-binding protein glycosylation isomer; MRE, magnetic resonance elastography; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operator characteristic.

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Abstract

Aim: The enhanced liver fibrosis (ELF) test is a noninvasive method for diagnosing hepatic fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). This multicenter cohort study aimed to evaluate the accuracy of the ELF test and compare it with other noninvasive tests in Japan.

Methods: We analyzed 371 Japanese patients with biopsy-proven NAFLD. We constructed area under the receiver operator characteristic curves (AUROC) to determine the diagnostic accuracies of the ELF test, the Mac-2-binding protein glycosylation isomer (M2BPGi), the Fibrosis-4 (FIB-4) index, and combinations of these indices.

Results: In patients with F0/F1/F2/F3/F4 fibrosis, the median values of the ELF test were 8.98/9.56/10.39/10.92/11.41, respectively. The AUROCs of the ELF test for patients with F0 versus F1–4, F0–1 versus F2–4, F0–2 versus F3–4, and F0–3 versus F4 fibrosis were 0.825/0.817/0.802/0.812, respectively. The AUROCs of the ELF test were greater than those of the FIB-4 index and M2BPGi at each fibrosis stage. Respective low and high cut-off values yielded sensitivities and specificities for predicting advanced fibrosis (≥F3) of 91.1% and 50.8%, and 38.5% and 92.8%, respectively. For F3 or F4 fibrosis, the combined values from the ELF test and FIB-4 index showed a sensitivity of 98.5%, and the combined values from the ELF test and M2BPGi assay showed a specificity of 97.5%.

Conclusions: In Japan, the ELF test predicts NAFLD-related fibrosis from its early stages. The diagnostic ability of the ELF test was not inferior to that of other indices, and the combined values of ELF plus other indices were more accurate.

KEYWORDS
advanced fibrosis, AUROC, ELF test, FIB-4, liver fibrosis, M2BPGi, nonalcoholic fatty liver disease

INTRODUCTION

Nonalcoholic fatty liver disease is the most common cause of chronic liver disease, and leads to serious public health problems in many countries worldwide.[1] Nonalcoholic fatty liver disease is recognized as the hepatic manifestation of metabolic syndrome. It is commonly complicated by obesity, dyslipidemia, hypertension, and DM.[2–4] Nonalcoholic fatty liver disease encompasses a wide spectrum of liver pathology, ranging from NAFL to NASH, which is characterized by steatosis, lobular inflammation, and hepatocellular injury, and may progress to LC, hepatic failure, and HCC. Although the progression of fibrosis is slow in NAFLD, each fibrosis stage in NASH progresses over a 7-year period.[5] Because hepatic fibrosis is the strongest predictor of liver-related events and mortality,[6,7] preventing the progression of fibrosis is an important issue. The predictors of progression are not clear, but elevated liver enzyme levels, the presence of DM, and a family history of LC are associated.[8,9] Accurately estimating the degree of fibrosis is important for preventing the severe complications of NAFLD.

Liver biopsy remains the gold standard for the diagnosis of NASH. However, liver biopsy is invasive and has several problems, including its invasiveness, cost, sampling errors, and inter- and intraobserver differences.[10] Noninvasive approaches for the evaluation of the stages of hepatic fibrosis have used combinations of clinical parameters with imaging. The ELF test is a predictive formula that is calculated from three serum markers: hyaluronic acid, tissue inhibitor of metalloproteinase-1, and N-terminal peptide of procollagen III.[11] The ELF test is used to evaluate hepatic fibrosis not only for chronic liver disease associated with NAFLD, but also other etiologies.

Sharma et al. found that the ELF test showed a good diagnostic performance for advanced fibrosis and cirrhosis in several patient cohorts of chronic liver disease. The AUROCs were >0.80.[12] The ELF test has been recommended as a noninvasive test by the guidelines of the European Association for the Study of the Liver[13] and the Practice Guidance for NAFLD published by the American Association for the Study of Liver Diseases.[14] However, because of the lack of sufficient evidence, the ELF test has not been recommended as a noninvasive test by the Japanese guidelines.[15] In contrast, the FIB-4

index and the M2BPGi biomarker are recommended by the Japanese guidelines and are widely used. Therefore, comparing the ELF test with these indices in Japanese patients with NAFLD is reasonable.

This study aimed to study Japanese patients with NAFLD to estimate the diagnostic performance of the ELF test and compare it with other noninvasive tests. We also evaluated whether the established cut-off values are appropriate for the Japanese population.

MATERIALS AND METHODS

Patients

A total of 371 consecutive patients with well characterized, liver biopsy-confirmed NAFLD were enrolled in this multicenter study. They were enrolled from January 1990 to February 2020 at six centers in Japan: Saga University, Kyoto Prefectural University of Medicine, Yokohama City University, Saiseikai Suita Hospital, Ogaki Municipal Hospital, and Gifu Municipal Hospital. The exclusion criteria were as follows: (1) daily alcohol consumption >20 g for female patients and >30 g for male patients; (2) other liver diseases, including viral hepatitis, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hemochromatosis, α-1-antitrypsin deficiency-associated liver disease, Wilson disease, and drug-induced liver disease; and (3) evidence of HCC, biliary tract cancer, or pancreatic cancer. Patients whom researchers judged inappropriate for this study were also excluded. The study was carried out in accordance with the Declaration of Helsinki and approved by the institutional review boards at all participating institutions.

Laboratory and clinical parameters

Laboratory assays included blood cell counts and measurements of serum concentrations of total protein, albumin, AST, ALT, bilirubin, alkaline phosphatase, γ-glutamyltranspeptidase, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, hemoglobin A1c, and M2BPGi. These parameters were measured with standard clinical chemistry laboratory techniques using samples obtained at liver biopsy. For all patients, ELF and M2BPGi were measured at the same time. The ELF test and M2BPGi measurements were undertaken using serum that was stored with consent when the liver biopsy was carried out. The ELF score was calculated as follows: 2.278 + 0.851 $\log_{10}$ (hyaluronic acid) + 0.751 $\log_{10}$ (N-terminal peptide of procollagen III) + 0.394 $\log_{10}$ (tissue inhibitor of metalloproteinase-1) after the analysis of serum samples (Siemens Health Care Diagnostics Inc.). The FIB-4 index was calculated as follows: age (years) × AST (IU/L)/(platelet count [10^9/L] × ALT [IU/L]$^{1/2}$), and low (1.3) and high (2.67) cut-off values were used to analyze the generated data.[16] Clinical parameters, such as body measurements (height, weight) were also collected. The BMI was calculated as weight in kilograms/(height in meters)^2, and obesity was defined as a BMI >25 kg/m^2.

Liver histology

All enrolled patients underwent a percutaneous liver biopsy under ultrasonic guidance with a 16G needle. The liver specimens were embedded in paraffin and stained with hematoxylin and eosin and Masson-trichrome. Digital images of biopsy samples were obtained using a batch slide scanner (NanoZoomer 3.2.15; Hamamatsu Photonics) and then transported for central reading and scoring by an experienced pathologist (S.A.) at Saga University, who was blinded to the patients' clinical and laboratory data and the clinical findings. Hepatic steatosis, lobular inflammation, and hepatocyte ballooning were evaluated to yield a NAFLD activity score.[17] The stage of liver fibrosis was classified according to the methods of Brunt et al.[18] Nonalcoholic steatohepatitis was diagnosed according to the fatty liver inhibition of progression algorithm.[19]

Statistical analysis

Results are presented as numbers of patients for qualitative data or as medians for quantitative data. Statistical differences were determined by the Wilcoxon signed-rank test for quantitative data and Fisher's exact probability test or the χ^2-test for categorical data.

To assess the accuracy of the ELF test and other noninvasive tests, we calculated the sensitivity and specificity of each value of each test, and then constructed ROC curves. The diagnostic performance of the scoring systems was assessed by the AUROC, with values close to 1.0 indicating high diagnostic accuracy. The optimal cut-off values were chosen to maximize the sum of the sensitivity and specificity in the Youden index.[20] To analyze whether the same cut-off values can be applied in Japan as in foreign countries, we used the established cut-off values of ELF test and FIB-4 index. The cut-off value of M2BPGi was calculated as that which satisfied a sensitivity or specificity of 90% for identifying each fibrosis stage. The DeLong test was used to compare the AUROCs for ELF and M2BPGi measurements and the FIB-4 index.[21] SPSS version 25 (SPSS Inc.) was used to undertake statistical analyses.

RESULTS

Patient characteristics

Table 1 summarizes the demographic profiles and laboratory and histologic data of the study patients. Of the 371 patients, 211 (56.9%) were female. The median age of the patients was 61 years. With regard to fibrosis stage, 32 patients (8.6%) had stage 0, 98 patients (26.4%) had stage 1, 106 patients (28.6%) had stage 2, 105 patients (28.3%) had stage 3, and 30 (8.1%) had cirrhosis (stage 4). The median ELF score, FIB-4 index, and M2BPGi level were 10.34, 2.16, and 0.93, respectively. Figure 1 shows the level of indices at each stage. The ELF scores of stages 0/1/2/3/4 were 8.98/9.56/10.39/10.92/11.41, respectively (Figure 1a). The FIB-4 indices of stages 0/1/2/3/4 were

1.00/1.40/2.11/2.80/3.61, respectively (Figure 1b). The M2BPGi levels of stages 0/1/2/3/4 were 0.68/0.73/0.90/1.40/2.45, respectively (Figure 1c).

Diagnostic accuracy of parameters for differentiating NASH-related fibrosis from NAFLD without fibrosis

We calculated the sensitivity and specificity of the scoring systems for differentiating NASH-related fibrosis from NAFLD without fibrosis. Figure 2 shows the ROC curves of the ELF test for differentiating fibrosis stages 1–4 from stage 0, stages 2–4 from stages 0–1, stages 3–4 from stages 0–2, and stage 4 from stages 0–3. The AUROCs of each stage were 0.825 (95% CI, 0.747–0.903)/0.817 (95% CI, 0.771–0.863)/0.802 (95% CI, 0.757–0.847)/0.812 (95% CI, 0.739–0.885), respectively. Figure S1 shows the AUROCs for each components of ELF test including hyaluronic acid (Figure S1a), N-terminal peptide of procollagen III (Figure S1b), and tissue inhibitor of metalloproteinase-1 (Figure S1c) for the diagnosis of fibrosis stage ≥3. Figures S2 and S3 show the diagnostic accuracies of the FIB-4 index and M2BPGi. At all fibrosis stages, the ELF test showed superior diagnostic ability over the other indices. The AUROC (0.802 [95% CI, 0.757–0.847]) of the ELF test for diagnosing advanced fibrosis was higher than that of the FIB-4 index (0.775 [95% CI, 0.727–0.823]) and M2BPGi (0.765 [95% CI, 0.717–0.814]) (Figure 3). There were no statistically significant differences by the Delong test ($p = 0.288$, $p = 0.128$). The cut-off value that satisfied a sensitivity of 90% for identifying stage ≥3 fibrosis was 9.81, and the cut-off value that satisfied a 90% specificity was 11.22. Because this cut-off value is almost the same as the established one, we used the established cut-off value to analyze whether the same cut-off values can be applied to Japanese as to foreign countries. At a low cut-off value of 9.80, the sensitivities of the ELF test for ≥stage 3 and ≥stage 4 fibrosis were 91.1% and 96.7%, respectively. At a high cut-off value of 11.30, the specificities of the ELF test for ≥stage 3 and ≥stage 4 fibrosis were 92.8% and 84.8%, respectively (Table 2). We also evaluated the diagnostic accuracies of the FIB-4 index and M2BPGi. Established cut-off values were used for the FIB-4 index, and cut-off values with at least 90% sensitivity and specificity were chosen for M2BPGi. The FIB-4 index showed the highest sensitivity for ≥stage 3 (93.3%) fibrosis, and both the FIB-4 index and ELF test showed the highest sensitivity for ≥stage 4 fibrosis (96.7%). The high cut-off value of M2BPGi showed the highest specificity for ≥stage 4 (90.3%) fibrosis.

TABLE 1 Characteristics of patients with nonalcoholic fatty liver disease in this study

Parameters	*N* = 371
Female, *n* (%)	211 (56.9)
Diabetes mellitus, *n* (%)	203 (54.7)
Age (years)	61 (17–85)
BMI (kg/m^2)	28.1 (16.8–62.8)
AST (U/L)	49 (13–608)
ALT (U/L)	58 (10–324)
ALP (U/L)	85.4 (17.5–283.6)
GGT (U/L)	63 (9–568)
TP (g/dl)	7.2 (4.4–8.6)
Albumin (g/dl)	4.3 (1.7–6.0)
TC (mg/dl)	187 (93–347)
LDL-C (mg/dl)	116 (17–259)
HDL-C (mg/dl)	48 (16–115)
TG (mg/dl)	136 (39–751)
Platelet count ($\times 10^4$/μl)	19.5 (5.8–63.7)
FPG (mg/dl)	108 (72–374)
HbA1c (%)	6.2 (4.1–11.0)
ELF score	10.34 (7.90–14.55)
FIB-4 index	2.16 (0.23–12.56)
M2BPGi, C.O.I.	0.93 (0.21–6.65)
Steatosis score, 0/1/2/3 (*n*)	5/268/68/30
Inflammation score, 0/1/2/3 (*n*)	3/220/122/26
Ballooning score, 0/1/2 (*n*)	87/176/108
NAS total, 0–2/3–4/5–7 (*n*)	56/197/118
Fibrosis stage, 0/1/2/3/4	32/98/106/105/30

Note: Data are presented as median (range) unless otherwise indicated.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; C.O.I., cut off index; ELF, enhanced liver fibrosis; FIB-4, Fibrosis-4; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; M2BPGi, Mac-2-binding protein glycosylation isomer; NAS, nonalcoholic fatty liver disease activity score; TC, total cholesterol; TG, triglyceride; TP, total protein.

Performance of combined and sequential procedures for diagnosing advanced fibrosis

To establish a more accurate diagnostic method, we combined two indices in order of high sensitivity/specificity. The FIB-4 index was combined with M2BPGi or the ELF test (Table 3). The ELF test (cut-off 9.8) combined with the FIB-4 index (cut-off 1.30) yielded a sensitivity of 98.5% (133/135 patients) for ≥stage 3 and 100% (30/30 patients) for ≥stage 4 fibrosis. These sensitivities were greater than those for the ELF test or FIB-4 index alone and other combinations.

The ELF test (cut-off 11.3) combined with M2BPGi (cut-off 1.85) yielded a specificity of 97.5% (230/236 patients) for ≥stage 3 fibrosis. The ELF test (cut-off 11.3) combined with M2BPGi (cut-off 2.20) yielded a specificity of 94.7% (323/341 patients) for ≥stage 4 fibrosis. These specificities were also greater than those for the ELF test or M2BPGi alone. Next, we examined the sequential procedure, in which the patients were first selected by FIB-4 index and then further

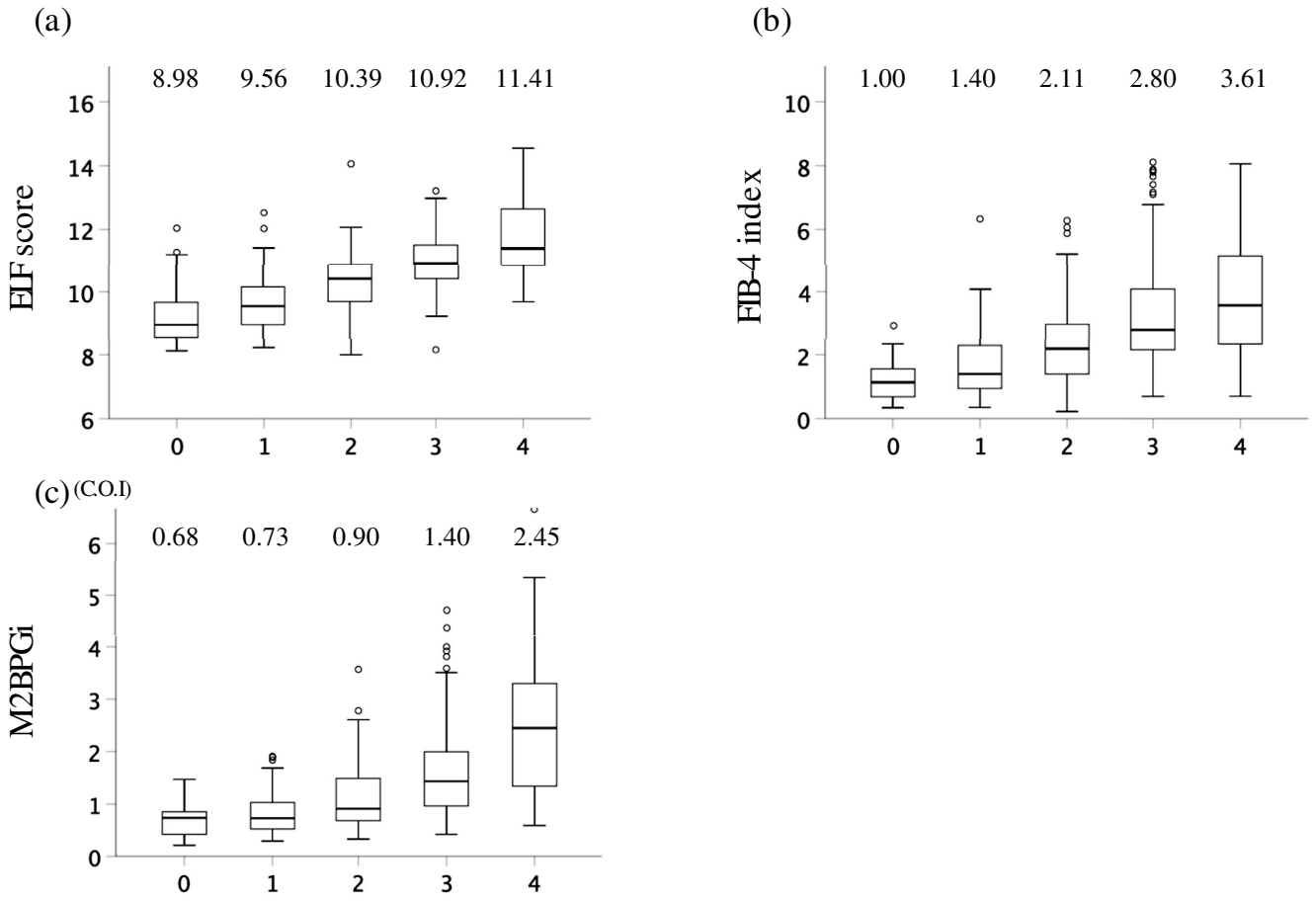

FIGURE 1 Correlations between fibrosis stage (horizontal axis) and the median levels of (a) enhanced liver fibrosis (ELF) test, (b) Fibrosis-4 (FIB-4) index, and (c) Mac-2-binding protein glycosylation isomer (M2BPGi). Horizontal lines indicate medians; boxes, interquartile ranges; whiskers, 95% confidence intervals; points, outliers from 95% confidence intervals. C.O.I., cut off index.

narrowed down by ELF test. Of the 270 patients with FIB-4 index >1.30, 126 had ≥stage 3, 209 had ELF test >9.8, and 116 had ELF >9.8 and ≥stage 3. The sensitivity/specificity/PPV/NPV for predicting ≥stage 3 were 92.1% (116/126 patients)/35.4% (51/144 patients)/55.5% (116/209 patients)/83.6% (51/61 patients). Of the 130 patients with FIB-4 index >2.67, 79 had ≥stage 3, 49 had ELF test >11.3, and 39 had ELF >11.3 and ≥stage 3. The sensitivity/specificity/PPV/NPV for predicting ≥stage 3 were 49.4% (39/49 patients)/80.4% (41/51 patients)/79.6% (39/49 patients)/50.6% (41/81 patients).

Only six among 236 patients without advanced fibrosis showed both elevated values for the ELF test (>11.3) and M2BPGi level (>1.85). Table 4 shows the characteristics of these patients. Three patients had elevated measurements of liver stiffness (>11.8 kPa) as determined by elastography, and two patients had low platelet counts ($<10 \times 10^4/\mu l$) (Table 4).

DISCUSSION

To explore appropriate noninvasive markers that could distinguish NASH-related fibrosis from nonfibrotic NAFLD, we validated the accuracy of the ELF test and chose the FIB-4 index and the biomarker M2BPGi as benchmark indices. In this large, well-characterized cohort of Japanese patients with biopsy-proven NAFLD from multiple centers, we found that the ELF test provided sufficient accuracy for predicting NASH-related fibrosis compared to other established scoring systems.

As shown in Figure 1, the ELF scores increased with increases in the stages of fibrosis, with less overlapping of scores at different stages. There was less overlap in the ELF scores between stage 0 and stage 1 fibrosis compared to the values for the FIB-4 index and M2BPGi. In clinical settings, these three noninvasive tests are used to identify patients with advanced fibrosis; however, the ELF test with a cut-off value <9.8 has been thought to have high diagnostic ability for distinguishing NAFL from NASH. The ELF test showed AUROC values >0.8 for the diagnosis of fibrosis stages ≥1, 2, 3, and 4. The AUROC values of the ELF test for these fibrosis stages were higher than those of the values for the FIB-4 index and M2BPGi. At the cut-off value of <9.8, the sensitivity and NPV of the ELF test for the diagnosis of stage ≥3 fibrosis were 91.1% and 90.9%, respectively. The specificity and PPV of the ELF test for the diagnosis of stage ≥3 fibrosis were 92.8% and 75.4%, respectively.

Guha et al. previously reported that the ELF test produced an AUROC of 0.82 for the diagnosis of stage ≥3 fibrosis.[22] Anstee et al.

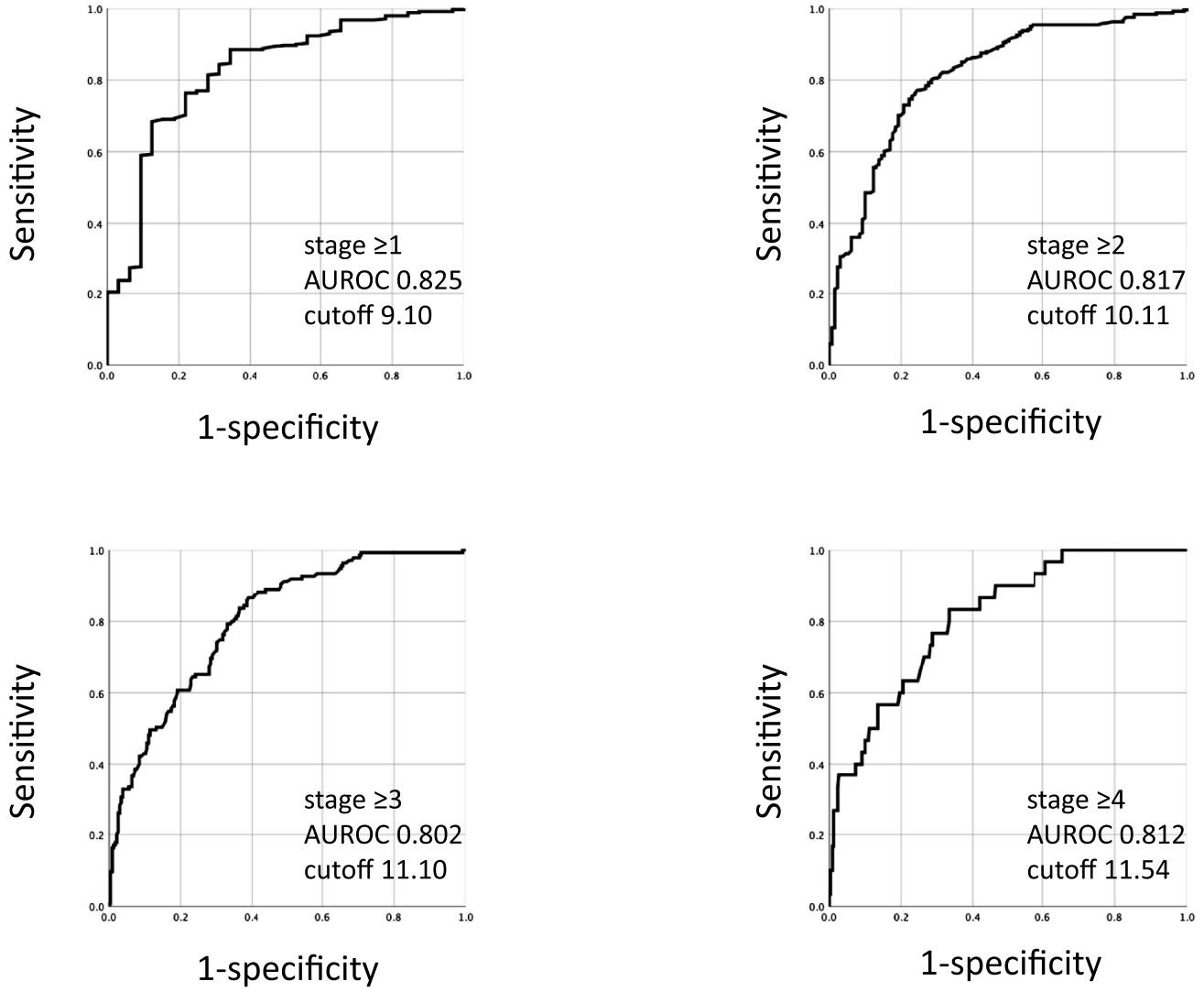

FIGURE 2 Area under the receiver operating characteristic curve (AUROC) for the enhanced liver fibrosis test for the diagnosis of fibrosis stage.

reported that ELF test produced an AUROC of 0.80 for the diagnosis of stage ≥3 fibrosis.[23] In their study, an ELF test with a cut-off value <9.8 yielded a sensitivity, specificity, PPV, and NPV of 74%, 73%, 87%, and 53%, respectively; and an ELF test with a cut-off value of 11.3 yielded a sensitivity, specificity, PPV, and NPV of 20%, 98%, 95%, and 33% respectively. A meta-analysis from a review by Sharma et al. showed AUROCs of the ELF test for detecting advanced fibrosis in NAFLD patients ranging from 0.78 to 0.97.[12] The diagnostic ability of the ELF test in our study is consistent with those of these previous studies.

We used the established cut-off values for ELF of 9.8 for the low cut-off and 11.3 for the high cut-off in our study. The cut-off value determined by this study that satisfied a sensitivity of 90% for identifying stage ≥3 fibrosis was 9.81, and the cut-off value determined by this study that satisfied a 90% specificity was 11.22. This cut-off value is almost the same as the established one and it suggests that the same cut-off values can be applied to Japan as to foreign countries. A high FIB-4 index cut-off value of 2.67 showed a specificity of 78.4% for the diagnosis of stage ≥3 fibrosis. A FIB-4 index cut-off value of 3.49 was needed to obtain at least 90% specificity in this study, and that cut-off value (3.49) provided a sensitivity of 38.5%. This result suggests that at least at high cut-off values, a unique Japanese cut-off value for the FIB-4 index could be needed. A previous Japanese validation study of the FIB-4 index showed an NPV of 98% for advanced fibrosis, but the FIB-4 index cut-off value used in that study was 1.45.[24]

Together, the findings on cut-off values for ELF, M2BPGi, and the FIB-4 index suggest that the same ELF cut-off values can be used for Japanese patients. Additionally, the ELF test was found to be useful to "rule out" NAFLD patients with advanced fibrosis. Although the FIB-4 index and M2BPGi are inexpensive and easy to use, the ELF test showed a diagnostic performance on the more accurate as other indices in this study.

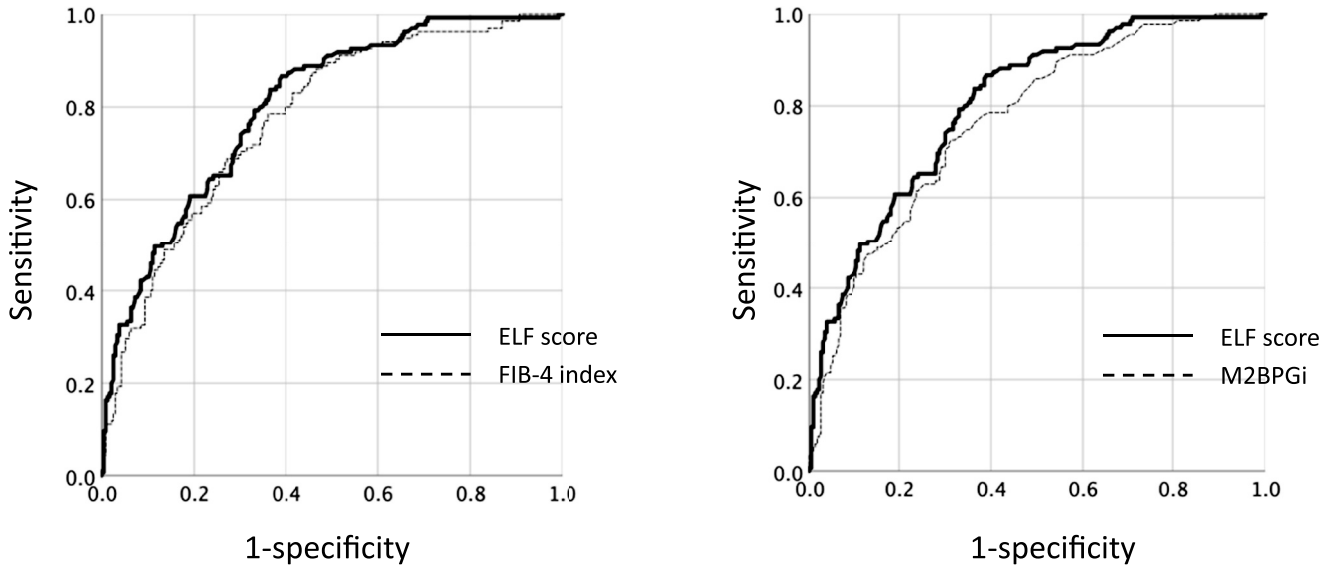

FIGURE 3 Area under the receiver operating characteristic curve (AUROC) for the enhanced liver fibrosis (ELF) test, Fibrosis-4 (FIB-4) index, and Mac-2-binding protein glycosylation isomer (M2BPGi) for diagnosing fibrosis stage ≥3.

TABLE 2 Diagnostic accuracies of the enhanced liver fibrosis (ELF) test and other assessments according to different cut-off values

Fibrosis stage	Cut-off value	Se (%)	Sp (%)	PPV (%)	NPV (%)
ELF test					
≥3	9.80	91.1	50.8	51.5	90.9
	11.30	38.5	92.8	75.4	72.5
≥4	9.80	96.7	38.4	12.1	99.2
	11.30	56.7	84.8	24.6	95.7
FIB-4 index					
≥3	1.30	93.3	39.0	46.7	91.1
	2.67	58.5	78.4	60.8	76.8
≥4	1.30	96.7	29.3	10.7	99.0
	2.67	70.0	68.0	16.2	96.3
M2BPGi					
≥3	0.74	90.4	44.1	48.0	88.9
	1.85	40.0	90.3	73.1	69.4
≥4	0.74	90.0	33.4	10.6	97.4
	2.20	56.7	90.3	34.0	95.9

Abbreviations: FIB-4, fibrosis-4; M2BPGi, Mac-2-binding protein glycosylation isomer; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

In Europe, the FIB-4 index is primarily used as a triaging tool, and ELF test has become established as a secondary triaging tool.[25] The FIB-4-ELF sequential algorithm has been shown to reduce the number of unnecessary referrals by 80%.[26] In our study, we found that ELF test values >9.8 combined with FIB-4 index values >1.30 were more sensitive than that of the FIB-4 index alone (98.5% vs. 92.8%, respectively) for detecting advanced fibrosis. The ELF test values >11.3 combined with M2BPGi values >1.85 showed higher specificity for ≥stage 3 fibrosis than that of M2BPGi alone (97.5% vs. 90.3%, respectively). These results suggest that the ELF test combined with other noninvasive tests is suitable to use for both ruling in and ruling out advanced fibrosis in NAFLD patients. In this study, FIB-4-ELF sequential analysis with high cut-off values showed higher predictive ability than the FIB-4 index alone. This suggests that a sequential method with high cut-off values could be useful in making the decision not to perform unnecessary liver biopsies.

FibroScan elastography and MRE have also been recognized as important noninvasive methods for diagnosing liver fibrosis. The common challenges of FibroScan and MRE are limited access and high cost. Although they certainly have high diagnostic performance for liver fibrosis, they cannot be used for assessing the very large numbers of patients with NAFLD in primary care. Because of that, combinations of or sequential diagnostic algorithms have been explored for the detection of liver fibrosis.[13,27] Jung et al. have shown that in cohorts in the United States and Japan, the combination of MRE and the FIB-4 index (MEFIB) showed PPVs of 97.1% and 91.0%, respectively, for detecting fibrosis stages ≥2.[28] Other combination scoring systems have also been proposed, including the FAST score (FibroScan and AST),[29] and the MAST score (MRE and AST).[30] Further study is warranted to estimate the predictive performance of combining the ELF test with elastography.

This study had several limitations. It was a retrospective, hospital-based cohort study, which involved selection bias. The majority of real-world patients with NAFLD who have NAFL show a lower prevalence of advanced fibrosis than the prevalence of advanced fibrosis seen in our study patients. Especially, the numbers

TABLE 3 Diagnostic performance of combinations of assessments used for the diagnosis of advanced liver fibrosis

Assessment	Stage	Se (%)	Sp (%)	PPV (%)	NPV (%)
Combination					
ELF test >9.8 or FIB-4 index >1.30	≥3	98.5	29.2	44.3	97.2
ELF test >9.8 or FIB-4 index >1.30	≥4	100	20.8	10.0	100
ELF test >11.3 and M2BPGi >1.85	≥3	25.9	97.5	85.4	76.7
ELF test >11.3 and M2BPGi >2.20	≥4	46.7	94.7	43.8	95.3
Sequential					
FIB-4 index >1.30 first, ELF test >9.8	≥3	92.1	35.4	55.5	83.6
FIB-4 index >2.67 first, ELF test >11.3	≥3	49.4	80.4	79.6	50.6

Note: Data are percentages.

Abbreviations: ELF, enhanced liver fibrosis; FIB-4, Fibrosis-4; M2BPGi, Mac-2-binding protein glycosylation isomer; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

TABLE 4 Characteristics of patients without advanced fibrosis who showed both elevated enhanced liver fibrosis (ELF) test values and Mac-2-binding protein glycosylation isomer (M2BPGi) scores

Age (years)	Sex	BMI	Fibrosis stage	ELF test	AST (U/L)	ALT (U/L)	Platelet count (10^4/hl)	M2BPGi (C.O.I)	FIB-4 index	LSM (kPa)	CAP (dB/m)
76	F	20.7	1	12.03	74	68	63.7	1.91	1.07	6.80	299
68	F	24.2	2	11.41	138	112	24.0	2.17	3.69	17.80	215
81	M	25.5	2	11.99	59	46	9.0	2.27	7.83	6.40	272
72	F	25.0	2	12.07	68	62	9.9	4.14	6.28	9.70	242
63	F	28.6	2	11.39	50	46	22.8	2.78	2.04	15.10	278
52	F	31.4	2	14.06	608	276	31.4	4.81	6.06	31.20	320

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; C.O.I., cut off index; F, female; FIB-4, Fibrosis-4; LSM, liver stiffness measurement; M, male.

of patients with fibrosis stages 0 and 4 are too small to confirm conclusions. We must validate the performance of the ELF test and compare it to the performance of other indices. Furthermore, it is debatable whether a more accurate diagnosis can be made by a single pathologist or by consensus or average of multiple pathologists in consultation. However, the strengths of this study are that it included a large number of patients from multiple centers in Japan.

In conclusion, the ELF test can be used to detect liver fibrosis, especially advanced fibrosis, in Japanese patients with NAFLD. The most important role of noninvasive tests in the practice of NAFLD is whether they can predict prognosis. In a large Japanese study, liver fibrosis was associated with liver-related events but was not a prognostic factor.[31] Future long-term studies are needed to determine whether the ELF test is useful for estimating prognosis as well as the risk for liver-related adverse events in patients with NAFLD.

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CONFLICT OF INTEREST

Hideaki Fukushima is an employee of Siemens Healthcare Diagnostics K.K. The other authors declare no conflict of interests for this article.

ETHICS STATEMENTS

Approval of the research protocol: This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review boards at all participating institutions.

Informed consent: This multicenter registry-based historical cohort study was approved by the institutional review board of Saga University Hospital, Saga, Japan (approval no. 2020-04-R-02; June 30, 2020), which waived the requirement for informed consent due to the use of pre-existing data. All patients provided written informed consent at the time of liver biopsy.

Registry and registration no. of the study/trial: N/A.

Animal studies: N/A.

Research involving recombinant DNA: N/A.

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