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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Impact of cardiometabolic criteria on the pathogenesis of MASLD and diagnostic performance of non-invasive tests

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Abstract

Background The impact of each factor and the number of positive criteria on pathogenesis and the diagnostic performance of non-invasive tests (NITs) in patients with metabolic dysfunction-associated steatotic liver disease (MASLD) remains unclear. This study aimed to investigate the association between the cardiometabolic criteria and the clinical features.

Methods This retrospective study investigated clinicopathological characteristics and the diagnostic performance of the Enhanced Liver Fibrosis (ELF) test, fibrosis-4 (FIB-4) index, and nonalcoholic fatty liver disease fibrosis score (NFS) according to the factors and the number of positive cardiometabolic criteria in 1,038 patients with biopsy-proven MASLD.

Results Hypertension is a significant risk factor for progression to advanced fibrosis and at-risk metabolic dysfunction-associated steatohepatitis (MASH) in male patients. In contrast, no significant risk factor was identified in female patients. The incidence of advanced fibrosis increased significantly in patients with three or more positive criteria. Similarly, the incidence of at-risk MASH increased significantly in a stepwise manner with the number of positive cardiometabolic criteria. The diagnostic performance of the ELF test for advanced fibrosis exceeded that of the FIB-4

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index and NFS for all factors except hypertension. Regardless of the number of positive cardiometabolic criteria, the ELF test demonstrated a superior diagnostic performance for advanced fibrosis.

Conclusions MASLD with hypertension in male patients or a higher number of positive cardiometabolic criteria constitutes a significant risk for advanced fibrosis or at-risk MASH. The ELF test is a valuable tool for diagnosing advanced fibrosis progression regardless of individual factors or the number of positive cardiometabolic criteria.

Keywords MASLD · Cardiometabolic criteria · NITs · ELF test

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUROC	Area under receiver-operating characteristic
BMI	Body mass index
CI	Confidence intervals
ELF	Enhanced liver fibrosis
FIB-4	Fibrosis-4
γGT	γ-Glutamyl transferase
HbA1c	Hemoglobin A1c
HCC	Hepatocellular carcinoma
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
MASLD	Metabolic dysfunction-associated steatotic liver disease
MASH	Metabolic dysfunction-associated steatohepatitis
NAS	Nonalcoholic fatty liver disease activity score
NITs	Non-invasive tests
NFS	Nonalcoholic fatty liver disease fibrosis score
OR	Odds ratio
ROC	Receiver-operating characteristic
SLD	Steatotic liver disease
T2DM	Type 2 diabetes mellitus

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is defined as steatotic liver disease (SLD) without moderate or high alcohol consumption, along with one or more positive cardiometabolic criteria [1, 2]. The progression of hepatic fibrosis is linked to liver-related events, such as liver failure and hepatocellular carcinoma (HCC), and has a significant impact on the prognosis of patients with MASLD [3–7]. Multiple studies have demonstrated the association of metabolic dysfunction—including obesity [8–10], type 2 diabetes mellitus (T2DM) [11–16], hypertension [17–19], and dyslipidemia [19]—with the progression of hepatic fibrosis and development of HCC. In addition, a stepwise increase in metabolic dysfunction accelerates the progression of liver-related diseases [19]. However, the specific impact of each cardiometabolic factor used as a diagnostic criterion for MASLD on clinical characteristics at the time of diagnosis has not yet been thoroughly assessed. Although the assessment of hepatic fibrosis has gained importance in clinical practice in recent years, we have reported the usefulness of various non-invasive tests (NITs)—including the Agile score [20], the Fibrosis-3 (FIB-3) index [21], and the Enhanced Liver Fibrosis (ELF) test [22, 23]—as alternatives to liver biopsy for predicting advanced fibrosis in patients with MASLD. T2DM affects the accuracy of NITs [24–27] in patients with MASLD. We demonstrated that the ELF test has high diagnostic performance for predicting advanced hepatic fibrosis in patients with MASLD, even in those with T2DM [23]. However, the impact of factors other than T2DM, as well as the number of positive cardiometabolic criteria, on the prediction of hepatic fibrosis progression using NITs in patients with MASLD remains unknown. Therefore, in this study, we aimed to evaluate the clinicopathological characteristics and diagnostic potential of NITs in patients with biopsy-proven MASLD according to each factor and the number of positive cardiometabolic criteria.

Materials and methods

Patient population

A total of 1,361 patients with steatotic liver disease, diagnosed by liver biopsy and without moderate or high alcohol consumption, were retrospectively enrolled from 14 hospitals, which are university hospitals or general hospitals of equivalent scale (Hamamatsu University Hospital,

University Hospital, Kyoto Prefectural University of Medicine, Saiseikai Suita Hospital, Gifu Municipal Hospital, Fukui-ken Saiseikai Hospital, Nippon Medical School Hospital, Ogaki Municipal Hospital, Osaka Metropolitan University Hospital, National Defense Medical College Hospital, Tokyo Medical University Ibaraki Medical Center, Kawasaki Medical School General Medical Center, Kagawa University Hospital, Yokohama City University Hospital, and Saga University Hospital). Among the 1,361 enrolled patients, 259 were excluded for the following reasons: 1) age < 20 years ($n = 15$); 2) missing data on body mass index (BMI) ($n = 13$), high-density lipoprotein (HDL) cholesterol ($n = 172$), or triglycerides ($n = 7$); and 3) unknown status of prediabetes/T2DM ($n = 7$). Therefore, 1,090 patients were diagnosed with MASLD, and 52 were excluded because of missing data on the ELF test, fibrosis-4 (FIB-4) index, or nonalcoholic fatty liver disease fibrosis score (NFS). Finally, the records of the remaining 1,038 patients were evaluated (Fig. 1).

Laboratory tests

Information on physical findings, medical history, lifestyle, and laboratory data was collected retrospectively from medical records. Hematological and biochemical parameters were measured using standard laboratory methods at each hospital. The ELF test, FIB-4 index, and NFS were calculated as follows: 1) ELF test = 2.278 + 0.851 ln ($C_{\text{hyaluronic acid}}$) + 0.751 ln ($C_{\text{N-terminal peptide of procollagen III}}$) + 0.394 ln ($C_{\text{tissue inhibitor of metalloproteinase-1}}$) (Siemens Health Care Diagnostics Inc., Tokyo, Japan); 2) FIB-4 index = age (years) × [aspartate aminotransferase (AST) (U/L)/platelet count ($\times 10^9$/L)] × [alanine aminotransferase (ALT) (U/L)]$^{1/2}$; and 3) NFS = −1.675 + 0.037—age + 0.094—BMI + 1.13 × impaired fasting glucose/diabetes + 0.99 × AST/ALT ratio—0.013 × platelet count—0.66 × serum albumin.

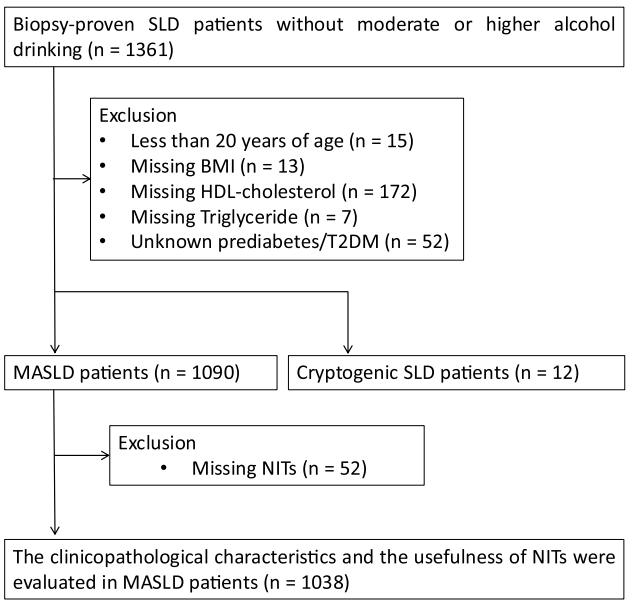

Fig. 1 Flowchart of the patient selection process. *BMI* body mass index, *HDL* high-density lipoprotein, *MASLD* metabolic dysfunction-associated steatotic liver disease, *NITs* non-invasive tests, *SLD* steatotic liver disease, *T2DM* type 2 diabetes mellitus

Liver histology

Percutaneous liver biopsies under ultrasonic guidance were performed as part of routine practice under the supervision of hepatologists at each hospital. All liver samples were evaluated by an experienced pathologist (S.A.) who was blinded to the clinical laboratory data and served as the central pathology reader. Histological evaluation was performed using the nonalcoholic fatty liver disease activity score (NAS), including steatosis, lobular inflammation, hepatocellular ballooning [28], and hepatic fibrosis staging based on the Brunt criteria [29]. Fibrosis progression beyond stage 3 was defined as advanced fibrosis. Some patients scored 0 for steatosis but were considered either improved with treatment or formally diagnosed as burned-out. Metabolic dysfunction-associated steatohepatitis (MASH) was defined according to the fatty liver inhibition of progression algorithms, based on steatosis, activity, and fibrosis scores [30]. At-risk MASH was defined as an NAS score ≥ 4 with at least one point for steatosis, lobular inflammation, ballooning, and fibrosis stage 2 or higher [31].

Definition of the cardiometabolic criteria

According to the MASLD diagnostic criteria proposed by a multisociety Delphi consensus statement [1, 2], positive cardiometabolic criteria were defined when the following conditions were met. BMI criterion: BMI ≥ 23 kg/m^2. Prediabetes/T2DM criterion: fasting plasma glucose ≥ 100 mg/dL, HbA1c ≥ 5.7%, a previous diagnosis of T2DM, or ongoing treatment for T2DM. Hypertension criterion: blood pressure ≥ 130/85 mmHg or ongoing antihypertensive treatment. Hypertriglyceridemia criterion: fasting triglyceride ≥ 150 mg/dL or ongoing lipid-lowering treatment. Hypo-HDL cholesterolemia criteria: HDL cholesterol < 40 mg/dL in men or < 50 mg/dL in women, or ongoing treatment for hypo-HDL cholesterolemia.

Ethics statement

This study was approved by the Ethics Committee of each participating institution.

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. Informed consent was obtained

from enrolled patients through the opt-out method on the website of each participating institution.

Statistical analyses

Statistical analyses were performed using GraphPad Prism version 10.5.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics version 29.0.2.0 for Macintosh (IBM Corp., Armonk, NY, USA). Data on patient characteristics are presented as numbers for categorical variables and medians with interquartile ranges for continuous variables. Categorical data were compared between groups using the chi-squared test. Continuous variables were evaluated using the Mann–Whitney U test. Correlations between the number of positive cardiometabolic criteria and the prevalence of steatosis, lobular inflammation, ballooning, advanced fibrosis, and at-risk MASH were analyzed using the Cochran–Armitage test for trend. The diagnostic performance of the FIB-4 index, ELF test, and NFS for advanced fibrosis was assessed by the area under the receiver-operating characteristic (AUROC) curves with 95% confidence intervals (CIs). The DeLong test with Bonferroni correction was used to compare the AUROCs. Univariate and multivariate logistic regression analyses were performed after propensity score matching (PSM), using standardized mean differences in age < 0.2 to assess independent risk factors for cardiometabolic criteria associated with progression to advanced fibrosis and at-risk MASH by gender. A p value < 0.05 was considered statistically significant.

Results

Clinical and pathological characteristics of all patients with MASLD

Among the 1,038 patients, 461 were male and 577 were female. Their median age and BMI were 60 years and 27.6 kg/m^2. The median values of the ELF test, FIB-4 index, and NFS were 9.9, 1.84, and −0.654, respectively. On pathological examination, 180 patients (17%) had stage 0 fibrosis; 350 (34%), stage 1; 276 (27%), stage 2; 196 (19%), stage 3; and 36 (4%), stage 4. In addition, 351 patients (34%) met the diagnostic criteria for at-risk MASH (Table 1).

Clinical and pathological characteristics of patients with MASLD by the factor of cardiometabolic criteria

BMI criterion

The prevalence of BMI ≥ 23 kg/m^2 was 89.9% ($n = 933$). The proportion of males, body weight, BMI, hypertension rate, and serum low-density lipoprotein (LDL) cholesterol level were significantly higher in patients with BMI ≥ 23 kg/m^2. Patients with BMI ≥ 23 kg/m^2 were significantly younger than those with BMI < 23 kg/m^2. The FIB-4 index levels were significantly lower, while the NFS levels were significantly higher in patients with BMI ≥ 23 kg/m^2; however, there were no differences in the progression of liver fibrosis between the two groups on pathological evaluation. Meanwhile, steatosis was significantly more advanced in patients with MASLD with BMI ≥ 23 kg/m^2 than in those with BMI < 23 kg/m^2. However, there were no significant differences in lobular inflammation scores, ballooning scores, or the rate of at-risk MASH between the two groups (Fig. 2 and Supplemental Table 1).

Prediabetes/T2DM criterion

The prevalence of prediabetes/T2DM was 85.5% ($n = 887$). Age, BMI, hypertension rate, and γ-glutamyl transferase (γGT) levels were significantly higher in patients with prediabetes/T2DM than in those without prediabetes/T2DM. The results of the ELF test, FIB-4 index, and NFS were all higher, and liver fibrosis was more advanced on pathological evaluation in patients with prediabetes/T2DM. Ballooning was significantly more advanced, and the rate of at-risk MASH was significantly higher in patients with prediabetes/T2DM. However, there were no significant differences in steatosis or lobular inflammation scores between the two groups (Fig. 2 and Supplemental Table 2).

Hypertension criterion

The prevalence of hypertension was 54.6% ($n = 567$). Age, female sex, BMI, total protein, fasting glucose, and hemoglobin A1c (HbA1c) levels were significantly higher in patients with hypertension. In contrast, the levels of ALT, γGT, total cholesterol, and LDL cholesterol were significantly lower. The ELF test, FIB-4 index, and NFS were all higher, and liver fibrosis was more advanced on pathological evaluation in patients with hypertension. Lobular inflammation and ballooning were significantly more advanced in patients with hypertension than in those without hypertension. Additionally, the rate of at-risk MASH was significantly higher in patients with hypertension. In contrast, steatosis was significantly more advanced in patients without hypertension than in those with hypertension (Fig. 2 and Supplemental Table 3).

Triglyceride criterion

The prevalence of hypertriglyceridemia was 43.1% ($n = 447$). The male ratio, weight, platelet count, and the levels of ALT, γGT, total protein, albumin, total cholesterol, LDL cholesterol, and fasting glucose were significantly higher in patients with hypertriglyceridemia than in those with

Table 1 Clinical and pathological characteristics of all patients with MASLD

Parameters	
Gender, male/female (*n*)	461/577
Age (years)	60 (48–67)
Weight (kg)	70.6 (61.6–81.2)
BMI (kg/m^2)	27.6 (25.2–30.8)
Hypertension, no/yes (*n*)	471/567
Platelets (10^4/μL)	20.1 (16.3–24.6)
AST (U/L)	47 (34–69)
ALT (U/L)	61 (38–94)
γGT (U/L)	60 (38–97)
Total protein (g/dL)	7.2 (6.9–7.6)
Albumin (g/dL)	4.3 (4.0–4.5)
Total cholesterol (mg/dL)	191 (169–217)
Triglyceride (mg/dL)	138 (99–187)
HDL-cholesterol (mg/dL)	46 (40–55)
LDL-cholesterol (mg/dL)	120 (99–142)
Fasting glucose (mg/dL)	107 (97–127)
HbA1c (%)	6.1 (5.6–6.9)
ELF test	9.9 (9.2–10.8)
FIB-4 index	1.84 (1.10–2.87)
NFS	−0.654 (−1.688–−0.452)
Pathological evaluations	
Steatosis score 0/1/2/3, *n* (%)	42/740/178/78 (4/71/17/8)
Lobular inflammation score 0/1/2/3, *n* (%)	39/649/281/69 (4/63/27/7)
Ballooning score 0/1/2, *n* (%)	337/473/228 (32/46/22)
Fibrosis stage 0/1/2/3/4, *n* (%)	180/350/276/196/36 (17/34/27/19/4)
At-risk MASH, *n* (%)	351 (34)

Data are presented as numbers or medians (interquartile ranges)

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BMI* body mass index, *ELF* enhanced liver fibrosis, *FIB-4* fibrosis-4 index, *γGT* γ-glutamyl transferase, *HbA1c* hemoglobin A1c, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *MASLD* metabolic dysfunction-associated steatotic liver disease, *MASH* metabolic dysfunction-associated steatohepatitis, *NFS* nonalcoholic fatty liver disease fibrosis score

normal triglyceridemia. In contrast, age and high-density lipoprotein-cholesterol levels were significantly lower in patients with hypertriglyceridemia than in those with normal triglyceridemia. The ELF test, FIB-4 index, and NFS were all higher in patients with hypertriglyceridemia than in those without hypertriglyceridemia; however, liver fibrosis progression did not differ between the two groups on pathological evaluation. In addition, there were no significant differences in steatosis, lobular inflammation, or ballooning scores between the two groups (Fig. 2 and Supplemental Table 4).

High-density lipoprotein-cholesterol criterion

The prevalence of hypo-HDL cholesterolemia was 45.6% ($n=473$). The female ratio and the levels of total protein, triglycerides, and HbA1c were significantly higher in patients with MASLD with hypo-HDL-cholesterolemia than in those with normal-HDL cholesterolemia. Total cholesterol levels were significantly lower in patients with hypo-HDL cholesterolemia than in those with normal-HDL cholesterolemia. The ELF test, FIB-4 index, NFS, and liver fibrosis progression did not differ between the two groups. Steatosis and lobular inflammation were significantly more advanced in patients with hypo-HDL cholesterolemia than in those with normal-HDL cholesterolemia. However, there were no significant differences in ballooning scores or the rate of at-risk MASH between the two groups (Fig. 2 and Supplemental Table 5).

Risk factors of cardiometabolic criteria for the progression of hepatic fibrosis and at-risk MASH

The characteristics of patients, categorized by gender, with or without advanced fibrosis, as well as those with or without at-risk MASH of PSM-unmatched and PSM-matched data, are shown in Supplemental Table 6. PSM analysis identified 188 male and 272 female patients with and

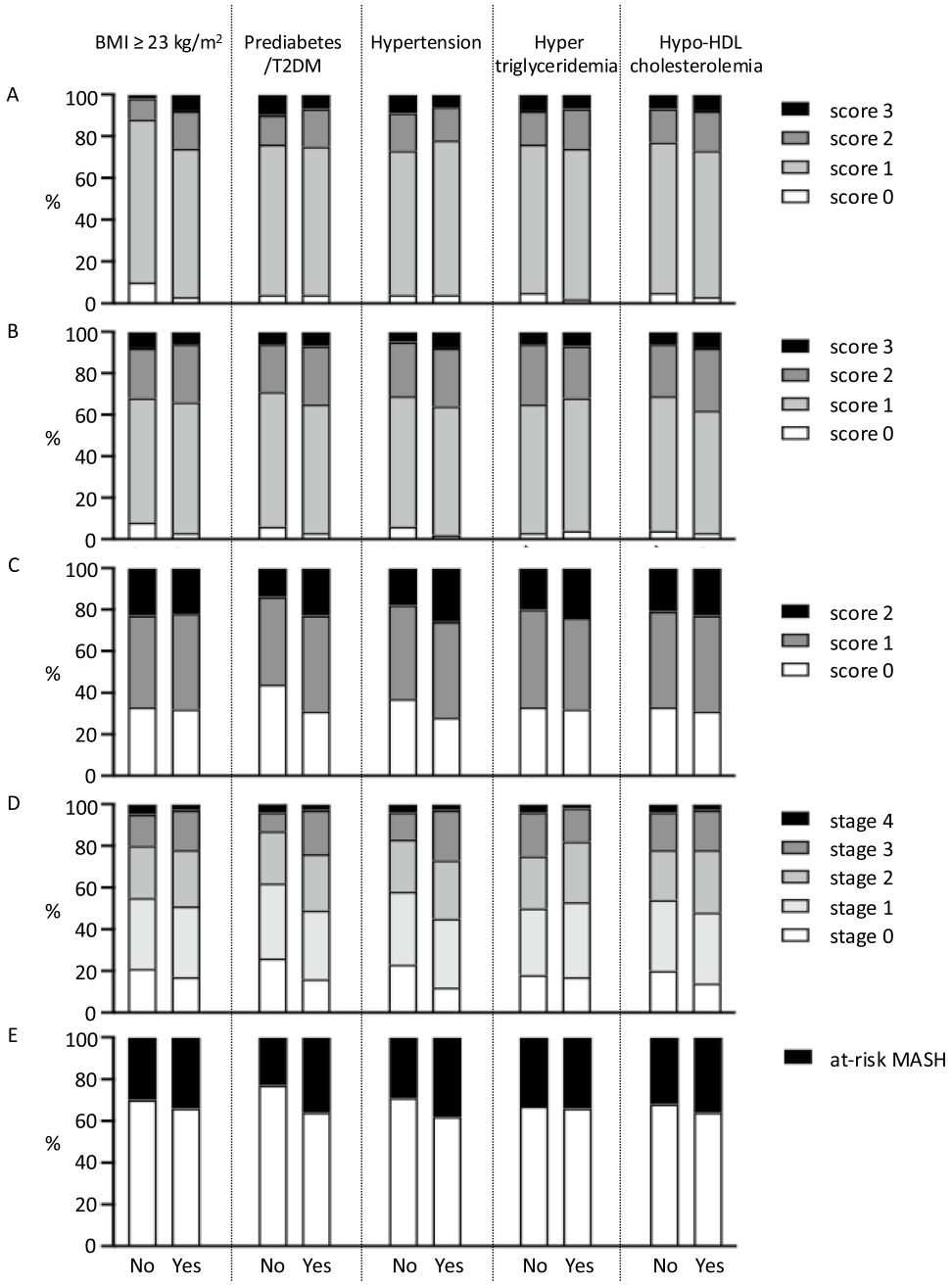

Fig. 2 Pathological evaluations of liver pathology in patients with MASLD according to each factor of the cardiometabolic criteria, Hepatic steatosis (**A**), lobular inflammation (**B**), ballooning (**C**), stage of hepatic fibrosis (**D**), and rate of at-risk MASH (**E**) in patients with MASLD. *BMI* body mass index, *HDL* high-density lipoprotein, *MASLD* metabolic dysfunction-associated steatotic liver disease, *MASH* metabolic dysfunction-associated steatohepatitis, *T2DM* type 2 diabetes mellitus

without advanced fibrosis and 268 male and 432 female patients with and without at-risk MASH. Univariate and multivariate analyses were performed using PSM-matched data to examine the risk of developing individual cardiometabolic criteria for advanced fibrosis and at-risk MASH. Among the cardiometabolic criteria, hypertension was identified as a significant risk factor for advanced fibrosis (OR 2.233, 95% CI, 1.217–4.094; $p < 0.01$) and at-risk MASH (OR 1.684, 95% CI, 1.010–2.808; $p < 0.05$) in male patients. Meanwhile, there was no significant risk factor in female patients (Table 2).

Clinical and pathological characteristics of patients with MASLD by the number of positive cardiometabolic criteria

Weight, BMI, and the levels of triglycerides, fasting glucose, and HbA1c increased, whereas HDL-cholesterol levels decreased, with a stepwise increase in the number of positive cardiometabolic criteria. However, age and other laboratory data, including the ELF test, FIB-4 index, and NFS, did not differ with the stepwise increase in the number of positive cardiometabolic criteria. The rate of advanced fibrosis

Table 2 Univariate and multivariate analyses to identify risk factors for the progression of advanced fibrosis and at-risk MASH using data matched for age with PSM

	Advanced fibrosis						At-risk MASH					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	*p value*	OR	95% CI	*p value*	OR	95% CI	*p value*	OR	95% CI	*p value*
Male												
BMI ≥ 23 kg/m^2	2.441	0.612–9.741	0.206	2.176	0.509–9.315	0.294	1.463	0.540–3.966	0.454	1.229	0.443–3.410	0.692
Prediabetes or T2DM	2.605	0.880–7.713	0.084	2.602	0.838–8.082	0.098	2.317	1.080–4.969	< 0.05	1.875	0.848–4.150	0.121
Hypertension	2.323	1.284–4.204	< 0.01	2.233	1.217–4.094	< 0.01	1.841	1.130–3.000	< 0.05	1.684	1.010–2.808	< 0.05
Hypertriglyceridemia	0.843	0.475–1.495	0.559	0.814	0.433–1.530	0.524	1.270	0.786–2.053	0.328	1.168	0.700–1.955	0.552
Hypo-HDL-cholesterolemia	0.758	0.417–1.377	0.363	0.783	0.405–1.513	0.467	1.289	0.786–2.113	0.315	1.300	0.765–2.211	0.333
Female												
BMI ≥ 23 kg/m^2	1.278	0.642–2.546	0.485	1.216	0.601–2.459	0.587	1.443	0.821–2.534	0.202	1.441	0.817–2.540	0.207
Prediabetes or T2DM	1.076	0.509–2.273	0.849	1.046	0.492–2.222	0.907	1.207	0.661–2.203	0.541	1.199	0.653–2.203	0.558
Hypertension	1.211	0.738–1.988	0.449	1.185	0.714–1.966	0.511	1.019	0.694–1.498	0.922	0.980	0.663–1.449	0.921
Hypertriglyceridemia	0.900	0.535–1.514	0.691	0.896	0.528–1.519	0.683	1.334	0.896–1.988	0.156	1.323	0.880–1.990	0.179
Hypo-HDL-cholesterolemia	0.971	0.603–1.563	0.903	1.003	0.617–1.629	0.991	1.097	0.752–1.601	0.630	1.036	0.703–1.526	0.859

BMI body mass index, *CI* confidence interval, *HDL* high-density lipoprotein, *MASH* metabolic dysfunction-associated steatohepatitis, *OR* odds ratio, *PSM* propensity score matching, *T2DM* type 2 diabetes mellitus

increased significantly in patients with three or more positive factors. There were no significant differences in steatosis and lobular inflammation scores. Interestingly, the rates of ballooning and at-risk MASH significantly increased with a stepwise increase in the number of positive cardiometabolic criteria (Fig. 3 and Supplemental Table 7).

Diagnostic performance of NITs in patients with MASLD by the factor and the number of positive cardiometabolic criteria

The AUROC values for the ELF test, FIB-4 index, and NFS were 0.833, 0.753, and 0.757 for advanced fibrosis, respectively. The diagnostic performance of the ELF test was significantly higher than that of the FIB-4 index and NFS in patients with advanced fibrosis (Supplemental Fig. 1).

The median levels of the ELF test, FIB-4 index, and NFS in patients with MASLD according to the cardiometabolic criteria are shown in Supplemental Tables 1–5. The diagnostic performance of the ELF test for predicting advanced fibrosis was significantly higher than that of the FIB-4 index and NFS in patients with MASLD, regardless of the positive factor of cardiometabolic criteria, except for hypertension (Fig. 4 and Supplemental Fig. 2).

The median values of the ELF test, FIB-4 index, and NFS in patients with MASLD according to the number of positive cardiometabolic criteria are shown in Supplemental Table 7. The diagnostic performance of the ELF test for advanced fibrosis tended to be greater than that of the FIB-4 index and NFS in patients with MASLD who had more than two positive cardiometabolic factors (Fig. 5 and Supplemental Fig. 3). Thus, the ELF test demonstrated superior diagnostic performance for advanced fibrosis compared with the FIB-4

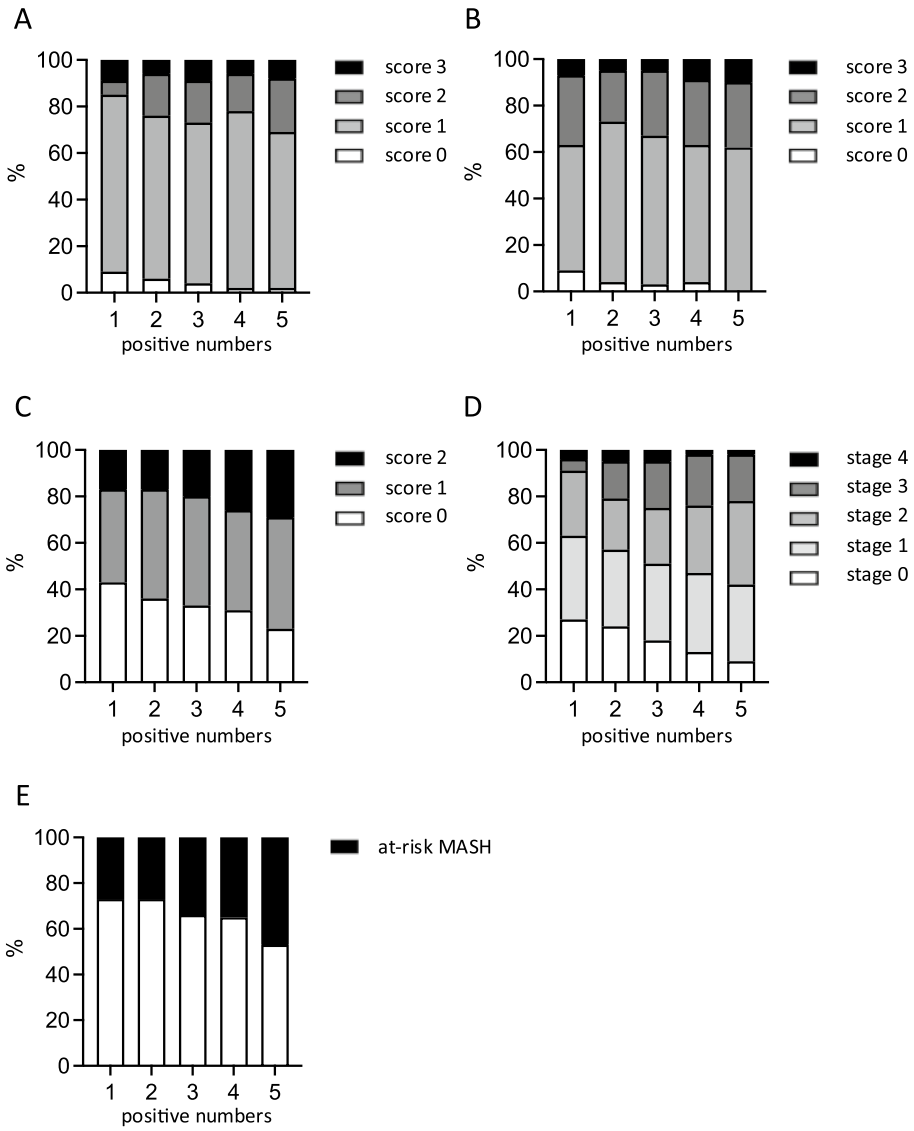

Fig. 3 Pathological characteristics in patients with MASLD according to the number of positive cardiometabolic criteria, Hepatic steatosis (**A**), lobular inflammation (**B**), ballooning (**C**), stage of hepatic fibrosis (**D**), and the rate of at-risk MASH (**E**) in patients with MASLD according to the number of positive cardiometabolic criteria. *MASLD* metabolic dysfunction-associated steatotic liver disease, *MASH* metabolic dysfunction-associated steatohepatitis

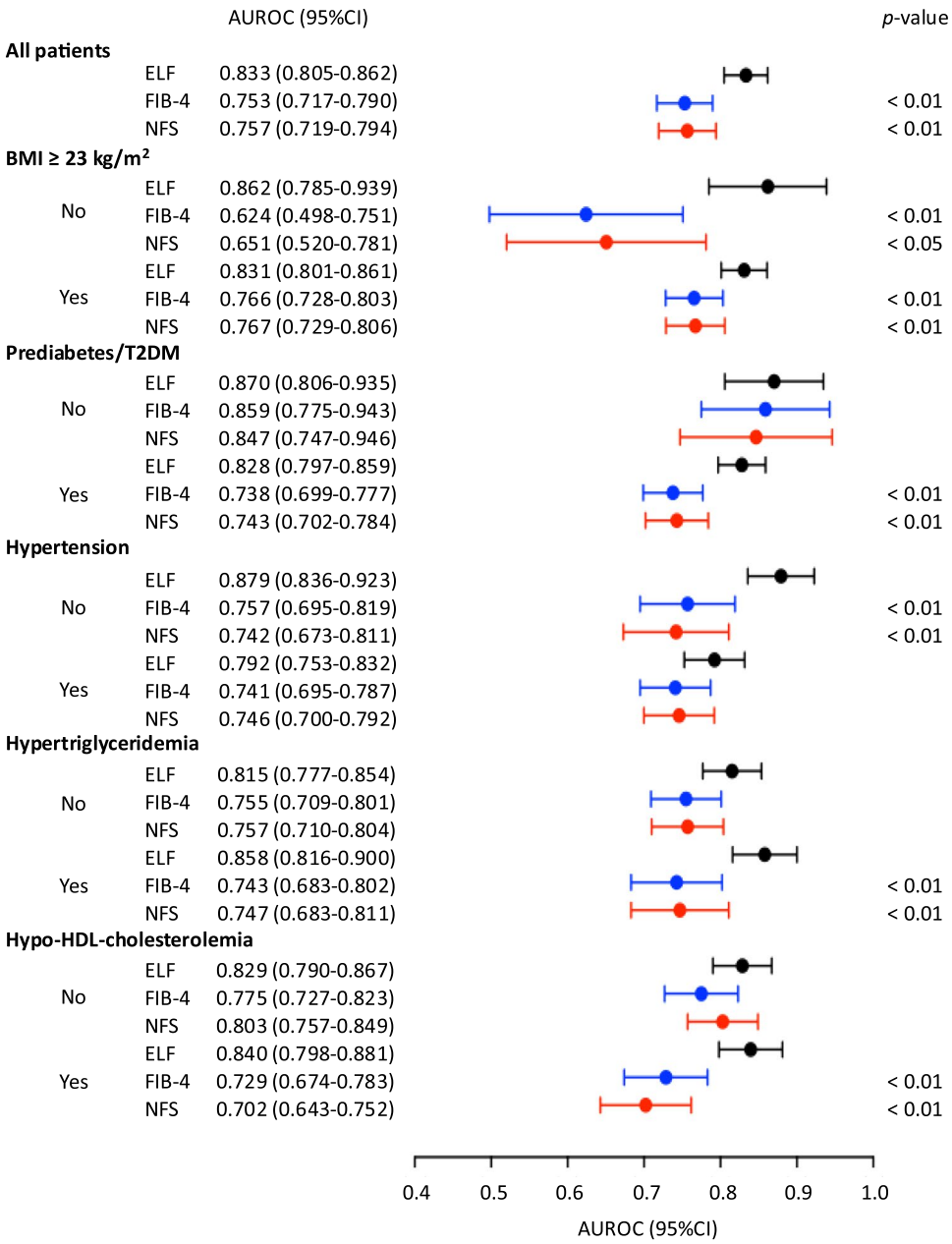

Fig. 4 AUROCs of NITs for predicting advanced fibrosis in patients with MASLD according to each factor of the cardiometabolic criteria. Forest plots showing AUROCs with 95% CI for predicting more than stage 3 fibrosis in patients with MASLD with or without positive cardiometabolic criteria. $p < 0.05$ and $p < 0.01$ were determined using the Delong test with Bonferroni correction to compare the AUROCs to the ELF test. *AUROC* area under the receiver-operating characteristic, *BMI* body mass index, *CI* confidence interval, *ELF* enhanced liver fibrosis, *FIB-4* fibrosis-4, *HDL* high-density lipoprotein, *MASLD* metabolic dysfunction-associated steatotic liver disease, *NITs* non-invasive tests, *NFS* nonalcoholic fatty liver disease fibrosis score, *T2DM* type 2 diabetes mellitus

index and NFS, regardless of the specific factors or the total number of positive cardiometabolic criteria.

Discussion

In this multicenter retrospective observational study, we examined the clinicopathological characteristics and diagnostic performance of NITs in patients with MASLD according to each factor and the number of positive cardiometabolic criteria. Pathologic evaluation revealed significant progression of hepatic steatosis in patients with BMI ≥ 23 kg/m^2 or hypo-HDL-cholesterolemia; lobular inflammation in patients with hypertension or hypo-HDL-cholesterolemia; ballooning in patients with prediabetes/T2DM or hypertension; and hepatic fibrosis in patients with prediabetes/T2DM or hypertension, compared with patients without each factor. Among the five cardiometabolic criteria, hypertension was a significant risk factor for advanced fibrosis and at-risk MASH in male patients, while no significant risk factor was identified in female patients. Similar to the stepwise increase in the number of positive cardiometabolic criteria, there were gradual changes in associated factors, such as body weight, BMI, triglyceride levels, and high-density lipoprotein-cholesterol levels; however, no significant alterations were observed in other laboratory findings.

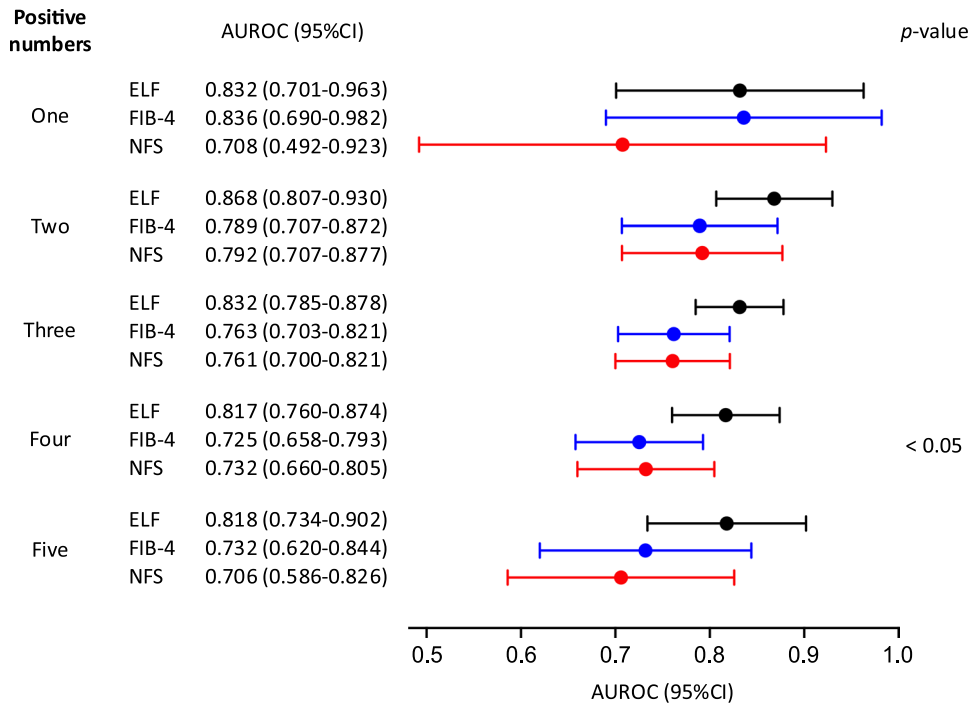

Fig. 5 AUROCs of NITs for predicting advanced fibrosis in patients with MASLD according to the number of positive cardiometabolic criteria. Forest plots showing AUROCs with 95% CI for predicting more than stage 3 fibrosis in patients with MASLD with each number of positive cardiometabolic criteria. $p < 0.05$ was determined using the DeLong test with Bonferroni correction to compare the AUROCs with the ELF test. *AUROC* area under receiver-operating characteristic, *CI* confidence interval, *ELF* Enhanced liver fibrosis, *FIB-4* fibrosis-4, *MASLD* metabolic dysfunction-associated steatotic liver disease, *NFS* nonalcoholic fatty liver disease fibrosis score, *NITs* non-invasive tests

Hepatic ballooning and fibrosis progressed, with a stepwise increase in the number of cardiometabolic criteria. Notably, compared with patients with one or two cardiometabolic criteria, the prevalence of advanced fibrosis increased significantly in those with three or more. Additionally, a stepwise increase in the number of positive cardiometabolic criteria was associated with an increased risk of at-risk MASH. Regarding the diagnostic performance of NITs, the ELF test showed better performance for advanced hepatic fibrosis than the FIB-4 index and NFS for most factors of the five cardiometabolic criteria. Moreover, its diagnostic performance remained unaffected by an increase in the number of positive cardiometabolic criteria.

From the vast amount of health checkup data in Japan, two or more positive factors of the cardiometabolic criteria, and hypertriglyceridemia in males or hypo-HDL-cholesterolemia in females, were significant risk factors for developing MASLD in individuals without steatosis [32]. Based on the previous report and our study, an increase in the number of positive cardiometabolic criteria strongly suggests involvement not only in disease onset but also in hepatic fibrosis progression in MASLD. However, individual factors influencing the onset and progression of liver fibrosis differ between males and females. The prevalence and severity of MASLD vary across genders and age groups, primarily due to differences in metabolism and hormone levels [33]. These findings indicate that gender-specific factors contribute to the onset and progression of MASLD.

According to reports from Western countries, the progression of hepatic fibrosis is strongly associated with liver-related events, as well as all-cause and liver-related mortality in patients with MASLD [3–7]. Additionally, mortality risk increases exponentially with advancing stages of hepatic fibrosis [7]. In an Asian cohort, we reported that hepatic fibrosis progression was associated with liver-related events but not with overall mortality, based on a multicenter retrospective study conducted by the Japan Study Group for NAFLD [34]. These findings underscore the importance of identifying risk factors involved in the progression of liver fibrosis in MASLD. While ALT levels and age have been suggested to correlate with hepatic fibrosis progression in patients with MASLD [35], the present study focused on identifying factors related to liver fibrosis progression using the cardiometabolic criteria alone. The results indicate that male patients with MASLD who have hypertension, as well as all patients with an increased number of positive cardiometabolic criteria, are at elevated risk for advanced fibrosis or at-risk MASH. Recent studies have reported that hypertension is a risk factor for the progression of liver fibrosis in MASLD [17, 18]. Hypertension contributes to liver fibrosis progression through various mechanisms, such as increased production of inflammatory cytokines and oxidative stress, endothelial dysfunction, systemic vascular remodeling, reduced hepatic blood flow, worsening insulin resistance, and dyslipidemia [18]. Additionally, hypertension increases the risk of adverse clinical outcomes, including all-cause mortality, cardiovascular events, and progression of liver fibrosis in longitudinal observational studies [18]. Furthermore, an association has been reported between the number of positive cardiometabolic criteria and the cumulative

incidence of major adverse cardiovascular events, but not liver-related events [36]. Although further investigation is required to clarify these relationships, patients with MASLD and hypertension or three or more positive factors should be monitored closely in clinical practice.

Therefore, regular monitoring of liver fibrosis progression using NITs is crucial. This study demonstrated that the ELF test provides higher diagnostic performance than the FIB-4 index and NFS. Regardless of the factor or number of positive cardiometabolic criteria, the AUROCs of the ELF test were > 0.80 in all cases, except 0.79 in patients with hypertension. In contrast, the AUROCs of the FIB-4 index and NFS were < 0.8 in most cases and decreased progressively with an increasing number of positive cardiometabolic criteria. Both the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL) guidelines have recommended the FIB-4 index as the initial step in the diagnostic algorithm for assessing hepatic fibrosis progression. As a second step, the ELF tests or imaging techniques, such as transient elastography, are recommended for evaluating hepatic fibrosis [31, 37]. As approximately 75% of patients with MASLD in this study met three or more positive cardiometabolic criteria, a multistep diagnostic approach using the FIB-4 index, a simple test, followed by the ELF test—which remains unaffected by cardiometabolic criteria—is effective for assessing hepatic fibrosis stages.

As limitations, liver biopsies were conducted under the supervision of hepatologists at general hospitals of similar size in a multicenter clinical setting, and all specimens were subsequently reevaluated by an experienced central pathologist. Nevertheless, the possibility of center-specific biases between facilities cannot be entirely excluded. This retrospective observational study analyzed data exclusively from Japanese patients; therefore, BMI criteria were based on Asian standards. Additionally, the focus was exclusively on the factors and number of positive cardiometabolic criteria and not on severity, medications, or treatment outcomes. Therefore, these excluded variables may affect liver inflammation and fibrosis, potentially leading to an underestimation of the association between cardiometabolic criteria and MASLD pathophysiology. Furthermore, in accordance with the cardiometabolic criteria, the state of prediabetes prior to the onset of T2DM was also included among the positive factors. Therefore, while recent studies have indicated a strong association between T2DM and liver fibrosis [11–16], prediabetes/T2DM may not have emerged as a risk factor in this cohort. Additionally, the potential effects of other variables, such as ALT levels and age, were not assessed. In clinical practice, it is important to consider not only the positive cardiometabolic criteria but also other relevant factors, including ALT levels and age. Meanwhile, all patients in this study underwent liver biopsy, and this cohort may have a relatively high proportion of advanced fibrosis and at-risk MASH compared to outpatients with MASLD who did not undergo biopsy. Therefore, the current results may not be directly generalizable to typical outpatients with MASLD or to primary-care screening settings. Furthermore, the long-term prognosis has not yet been studied fully. These aspects should be investigated on a larger scale with an unselected population and through prospective studies as the validation cohort.

Conclusions

Hypertension in male patients and an increase in the number of positive cardiometabolic criteria affect the progression of hepatic fibrosis in patients with MASLD. The diagnostic performance of the ELF test for advanced hepatic fibrosis was maintained regardless of the factor or the number of positive cardiometabolic criteria. Individual factors and the number of positive cardiometabolic criteria should be considered in MASLD practice.

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Declarations

Conflict of interest Kazuhito Kawata received honoraria from AbbVie GK. Hirokazu Takahashi received research grants from Takeda Pharmaceutical Company Limited, Sysmex Corporation, Kracie, Ltd., and MiZ Company Limited and received honoraria from Novo Nordisk Pharma Ltd., AbbVie GK, and Eli Lilly Japan K.K. Hidenori Toyoda received lecture fees from AbbVie GK, Gilead Sciences Inc., Kowa Co. Ltd., Terumo Corporation, FUJIFILM Wako Pure Chemical Corporation, and AstraZeneca K.K. Masato Yoneda received a research grant from Gilead Sciences Inc. and received honoraria from Kowa Co. Ltd. Miwa Kawanaka received honoraria from AbbVie GK and a research grant from Fujirebio Inc. Yoshihiro Kamada received honoraria from Kowa Co. Ltd. and Sysmex Corporation and received contract research funding from Nippon Boehringer Ingelheim Co., Ltd. Yoshio Sumida received honoraria from Taisho Pharmaceutical Co., Ltd, Kowa Co. Ltd., and Novo Nordisk Pharma Ltd. Hideaki Fukushima is an employee of Siemens Healthcare Diagnostics K.K. The other authors declare no conflicts of interest.

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