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Validation of the diagnostic accuracy of the acFibroMASH index for at-risk MASH in patients with metabolic dysfunction-associated steatotic liver disease

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Abstract

Objective The objective of this study was to validate the diagnostic accuracy of the acFibroMASH index in a population of metabolic dysfunction-associated steatotic liver disease (MASLD) patients with at-risk metabolic dysfunction-associated steatohepatitis (MASH) and to compare it with other scoring systems.

Methods 394 patients with biopsy-proven MASLD were retrospectively enrolled. The patients were divided into the at-risk MASH (NAFLD activity score ≥ 4 and significant fibrosis) group ($n = 103$) and the non-at-risk MASH group ($n = 291$). The diagnostic performance of the acFibroMASH index was compared to that of fibroScan-aspartate aminotransferase (FAST) and other noninvasive fibrosis scores by plotting the receiver operating characteristic curve (ROC), including the area under the curve (AUC), sensitivity, and specificity. Cut-offs of the acFibroMASH index for sensitivity (≥ 0.90) and specificity (≥ 0.90) were obtained in our cohort.

Results The AUC of the acFibroMASH index in assessing at-risk MASH was 0.780, while the AUC of FAST was 0.770. The comparison of acFibroMASH with FAST showed no significant difference ($P = 0.542$). When the cut-off value for acFibroMASH was < 0.15, 95.5% of at-risk MASH patients could be excluded in 89 patients correctly. Conversely, when the cut-off value was set at > 0.39, 49.3% of at-risk MASH patients could be diagnosed in 140 patients correctly. When the NPV was set at 0.900, the critical value for exclusion was determined to be 0.23, with a sensitivity of 0.835 and a specificity of 0.526.

Conclusion This study validated the efficacy of the acFibroMASH index in predicting at-risk MASH in a population of MASLD patients, demonstrating comparable performance to that of the FAST. The acFibroMASH index may provide a valuable clinical basis for screening and identifying at-risk MASH in primary care settings.

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Keywords Metabolic dysfunction-associated steatotic liver disease, Liver biopsy, Significant liver fibrosis, At-risk MASH, AcFibroMASH index

Introduction

Non-alcoholic fatty liver disease (NAFLD) affects over 30% of the global adult population, making it the most prevalent liver disease worldwide [1]. In China, its prevalence is as high as 29.2% [2]. Recently, three major pan-national liver associations, namely the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver, and the Latin American Association for the Study of Liver Diseases, have proposed a new nomenclature for steatosis liver disease (SLD). The presence of at least one related cardiac metabolic risk factor (overweight or obesity, type 2 diabetes, hypertension, dyslipidemia) is defined as metabolic dysfunction-associated steatotic liver disease (MASLD) [3]. Approximately 20% of patients with MASLD progress to MASH, a process that is strongly associated with metabolic risk factors such as obesity, insulin resistance, hypertriglyceridemia, and hypertension. These factors contribute to disease progression through the following mechanisms: obesity and insulin resistance lead to elevated levels of free fatty acids and triglycerides, which increase hepatic fat synthesis and inhibit its breakdown, resulting in hepatocellular fat accumulation. Fat accumulation and the ensuing insulin resistance trigger a cascade of events that leads to oxidative stress, the generation of reactive oxygen species, and subsequent damage to hepatocytes. The activation of Kupffer cells and hepatic stellate cells, in turn, releases inflammatory factors, thereby triggering a chronic inflammatory response that promotes hepatic fibrosis. This process, if left unaddressed, can lead to cirrhosis or even hepatocellular carcinoma. Acknowledging the pivotal role of metabolic risk factors in the pathogenesis of MASLD is imperative. These factors not only contribute to the development of MASH but also accelerate its progression, ultimately resulting in severe liver disease [4, 5]. Patients with both MASH and significant fibrosis (NAFLD activity score ≥ 4 and fibrosis stage ≥ 2), also known as 'at-risk MASH', are at the highest risk of disease progression and have a significantly increased risk of liver-related death [6, 7]. Therefore, they are considered a key target for drug development. Timely identification of at-risk patients is crucial for risk stratification and management [8, 9]. Early screening and treatment are expected to reduce the disease burden and improve patients' quality of life.

Liver biopsy histology is the gold standard for differentiating simple steatosis from MASH and assessing the extent of fibrosis. However, its invasiveness, potential complications, and difficulty performing the procedure have limited its widespread clinical use [10]. Furthermore, it is essential to note that biopsy samples only represent a small fraction of the liver volume, approximately 1/5000, and are therefore subject to sampling error. Currently, diagnostic models for fibrotic MASH are primarily based on MASLD fibrosis, and there is a lack of a single accurate indicator to distinguish at-risk MASH in clinical practice. Researchers have recently explored non-invasive indicators, such as serology, imaging, genetics, and proteomics, to assess the severity of MASH and liver fibrosis. Several non-invasive models for identifying at-risk MASH (e.g., fibroScan-aspartate aminotransferase (FAST), MRI-aspartate aminotransferase (MAST), metabolomics-advanced steatohepatitis fibrosis score (MASEF), MACK3, NIS4, etc.) have been developed and validated [9, 11–14]. However, many of these models contain indicators that are not easily accessible in clinical practice. Thus, identifying at-risk NASH through non-invasive models based on readily available indicators remains a current research topic.

The acFibroMASH index comprises the acMASH index and the liver stiffness measurement (LSM), with the acMASH index itself being based on serum creatinine(SCr) and aspartate aminotransferase (AST) concentrations. LSM serves as a reliable, non-invasive metric for evaluating the extent of liver fibrosis and is a core indicator for diagnosing at-risk NASH. AST reflects the importance of liver cell damage and is a key indicator for evaluating NASH activity. SCr is included in the acFibroMASH index because it is closely related to metabolic syndrome and insulin resistance. It reflects renal function and correlates with systemic metabolic dysfunction. Studies have demonstrated a correlation between SCr and hepatic fibrosis and insulin resistance, which may indicate the impact of metabolic dysfunction on the liver [15]. The index integrates liver stiffness, serological indicators, and metabolic factors to comprehensively reflect the pathologic features of MASH, thereby improving diagnostic accuracy and reducing the risk of misdiagnosis. Through the optimization of component weights and combinations, the acFibroMASH index demonstrated commendable diagnostic efficacy in both the derivation and validation cohorts. In the study by Feng G et al., the acFibroMASH index exhibited superior diagnostic efficacy compared to the FAST score in identifying at-risk MASH, with an area under the receiver operating characteristic curve (AUC) of 0.808 in the derivation cohort and 0.800 in the validation cohort. The study demonstrated that the acFibroMASH index exhibited superior diagnostic accuracy for at-risk MASH when compared to existing models. The study further

noted that the acFibroMASH index's simplicity, derived from routine laboratory indicators, renders it a promising candidate for broad clinical application. The model exhibited notable efficacy in identifying patients with at-risk MASH. In light of the encouraging preliminary outcomes, there is a pressing need to further substantiate the findings through external validation and evaluate the index compared with other non-invasive diagnostic scoring systems. Accordingly, this study aimed to validate the accuracy of the acFibroMASH index in diagnosing at-risk MASH. To this end, a systematic comparison was conducted between the index and the non-invasive diagnostic scores that have been widely used, namely FAST, fibrosis-4 index (FIB-4), the NAFLD fibrosis score (NFS), and AST to platelet ratio index (APRI). Furthermore, this study investigated whether demographic characteristics and metabolic factors significantly impact the efficiency of the acFibroMASH index through subgroup analyses to provide additional evidence to support the clinical application of this model.

Materials and methods

Study population

Retrospective inclusion of patients who were diagnosed with hepatic steatosis underwent liver biopsy in Changzhou Third People's Hospital from Feb 2017 to Oct 2023. The diagnostic criteria of MASLD are based on evidence of biopsy-proven hepatic steatosis, combined with evidence of metabolic dysfunction, at least 1 out of 5: [1] body mass index (BMI) ≥ 23 kg/m² [Asia] or waist circumference (WC) > 94 cm (male) 80 cm (female) [2] fasting plasma glucose (FPG) ≥ 5.6 mmol/L or 2-hour post-load glucose levels ≥ 7.8 mmol/L or hemoglobin A1c (HbA1c) ≥ 5.7% or type 2 diabetes mellitus (T2DM) or treatment for T2DM [3] blood pressure ≥ 130/85 mmHg or specific antihypertensive drug treatment [4] plasma triglycerides (TG) ≥ 1.70 mmol/L or lipid-lowering treatment [5] plasma high-density lipoprotein-cholesterol (HDL-c) ≤ 1.0 mmol/L (male) and ≤ 1.3 mmol/L (female) or lipid-lowering treatment [3]. Exclusion criteria were: [1] age under 18 [2], weekly alcohol consumption equivalent to more than 140 g of ethanol for males or 70 g for females [3], history of liver disease including co-infection with hepatitis virus, autoimmune hepatitis, or drug-induced hepatitis [4], chronic kidney disease, cardiovascular disease, or tumors, and [5] missing data.

The study followed the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Changzhou Third People's Hospital. All subjects were informed of the study before participation and provided written consent.

Liver puncture biopsy and pathology scoring

Liver puncture biopsy, 10% formaldehyde fixation, dehydration, paraffin embedding, sectioning, and hematoxylin-eosin staining. For liver puncture biopsies, case sections were analyzed independently by two pathologists concurrently. During the analysis, the two pathologists were unaware of each other's diagnostic results and were blinded to the patient's clinical information and measurement data. In the event of a discrepancy, the sections were re-examined collectively, and a consensus was reached through discussion. The NAS scoring system was employed to determine NASH, with the scoring criteria encompassing steatosis, intralobular inflammation, and hepatocellular ballooning. Hepatocellular steatosis: Scores were based on the percentage of hepatocellular steatosis, with scores of 0 (< 5%), 1 (5-33%), 2 (34-66%), and 3 (> 66%). Intralobular inflammation: Scores were based on the number of foci of inflammatory necrosis at 20x, with scores of 0 (none), 1 (< 2), 2 [2–4], and 3 (> 4). Hepatocellular ballooning was evaluated based on the severity of ballooning, scored as 0 (none), 1 (rare), or 2 (frequent). The sum of these three scores yielded the total NAS score, ranging from 0 to 8. The assessment of fibrosis followed the NASH Clinical Research Network (NASH CRN) scoring system, which categorizes fibrosis into five stages: F0: no fibrosis; F1: perisinusoidal or periportal/periportal fibrosis; F2: perisinusoidal and periportal/periportal fibrosis; F3: bridging fibrosis; and F4: cirrhosis. The term "high-risk NASH" was defined as steatohepatitis (NAS ≥ 4, at least 1 point for each component) and significant fibrosis (F ≥ 2) [16]. In the present study, the NAS score itself was a continuous variable covering a composite score of three pathologic features: hepatocellular ballooning, intralobular inflammation, and hepatocellular steatosis. However, to differentiate the degree of risk for MASH patients, we analyzed the NAS score as ≥ 4 as a classification criterion and combined it with a fibrosis stage of ≥ 2 to define it as "at-risk MASH" for further analysis [7]. This classification method aims to more clearly define the group of MASH patients with a significant risk of fibrosis, thus providing a more transparent basis for subsequent studies and clinical applications.

Non-invasive assessment of liver stiffness

Trained nurses measured LSM and controlled attenuation parameter (CAP) using the FibroScan-502 M probe (Echosens, France). Patients were instructed to fast for at least 8 h before the examination. The patient is supine with their right arm in maximum abduction, and the probe is placed vertically. The right liver lobe is scanned through the intercostal space for measurement. To ensure adequate detection of LSM and CAP, the success rate of the procedure must be set at ≥ 60%, and the

interquartile range (IQR)/median (IQR/M) must be ≤ 0.3. The final value will be the median of ten successful detections. The measurements for LSM and CAP should be completed within one week before the liver biopsy.

Body measurements and laboratory tests

Patient height and weight were routinely measured, and their BMI was calculated using the formula BMI = weight/height^2 (kg/m^2). Patients fasted for 8–10 h before having early morning fasting venous blood drawn. Liver function markers, including alanine aminotransferase (ALT), AST, gamma-glutamyl transferase (GGT), as well as total bilirubin (TBIL), total cholesterol(TC), TG, HDL-c, and low-density cholesterol (LDL-c), FPG, Creatinine, estimated glomerular filtration rate (eGFR), uric acid (UA) and C-reactive protein (CRP) were detected by the International Federation of Clinical Chemistry and Laboratory Medicine method using Photopure Pharmaceutical Co., LTD reagent. Meikang Biological Technology Co., Ltd. reagents were used to measure FPG using the dry chemical method. These biochemical markers are analyzed in serum. The biochemical indicators were detected using Hitachi LABOSPECT008AS. Platelet count (PLT) was determined using the SYSMEX xn3000 complete blood cell analyzer. HbA1c was measured through high-performance liquid chromatography.

Non-invasive diagnostic biomarkers

acMASH was calculated using the formula: acMASH = AST/ Creatinine×10 [17].

acFibroMASH index was calculated using the formula: acFibroMASH = e^(-3.956 + 0.305 × LSM + 0.065 × acMASH) / [1 + e^(-3.956 + 0.305 × LSM + 0.065 × acMASH)] [18].

FAST score was calculated using [e^(-1.65 + 1.07 × In(LSM) + 2.66 × 10^{-8} × CAP^3 − 63.3 × AST^{-1})]/[1 + e^(-1.65 + 1.07 × In(LSM) + 2.66 × 10^{-8} × CAP^3 − 63.3 × AST^{-1})] [12].

FIB-4 was calculated using the formula: FIB-4 = (age × AST) / (PLT × $ALT^{1/2}$) [19].

NFS was calculated using the formula: NFS = -1.675 + 0.037 × age + 0.094 × BMI + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST /ALT − 0.013 × PLT − 0.66 × albumin [20].

APRI was calculated using the formula: APRI = (AST / upper limit of normal AST range) / PLT × 100 [21].

Statistical analysis

The Shapiro-Wilk test was employed to ascertain whether continuous variables exhibited characteristics consistent with a normal distribution. In particular, variables that showed a normal distribution were expressed as mean ± standard deviation. Conversely, these variables were described using the median and the 25th and 75th percentiles. Differences between the groups were compared using the Mann–Whitney U test for continuous data and the chi-square test for categorical data. The odds ratio (OR) and 95% confidence interval (CI) were estimated using logistic regression models. Moreover, receiver operating characteristic (ROC) curve analysis was employed to evaluate the predictive values of FAST, FIB-4, NFS, APRI, and acFibroMASH index for at-risk MASH. The diagnostic accuracy was assessed by calculating the AUC, as well as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The calibration of the acFibroMASH index was graphically assessed using a bootstrap resampling approach with relocations. A decision curve analysis curve was also employed to determine the net clinical benefits of acFibroMASH. A comparison of AUROCs was carried out using paired methods using the DeLong test. By the established cut-off values from prior studies, we calculated the sensitivity, specificity, positive predictive value, and negative predictive value, respectively. Cut-offs of the acFibroMASH index for sensitivity (≥ 0.90) and specificity (≥ 0.90) were obtained in this cohort. Subgroup analyses were conducted to examine further the models' performance in different demographic and clinical subgroups. The assessment efficacy was expressed as the AUC with 95% CI. In two-sided tests, a p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using R 4.4.0 (http://www.R-project.org, The R Foundation) and SPSS version 23.0 (SPSS, IBM, Corp., Armonk, NY, USA) software. We plot the figures using GraphPad Prism version 9.0 (GraphPad Software, USA).

Results

Study design and patient population

Using retrospective analysis, this study included 472 patients diagnosed with MASLD by hepatic puncture biopsy. After applying strict exclusion criteria, such as excessive alcohol consumption, viral hepatitis, autoimmune diseases, active malignant tumors, and incomplete data, 394 eligible participants were finally screened. The study included liver puncture samples with at least ten lobules available for evaluation. A total of 263 patients were diagnosed with MASH. The distribution of fibrosis stage ranged from F0 to F4, with F0 (n = 80), F1 (n = 199), F2 (n = 57), F3 (n = 26), and F4 (n = 32) cases. The number of patients with F ≥ 2 was 115. The subjects were further categorized into at-risk MASH and non-at-risk MASH groups based on the definition of at-risk MASH (Fig. 1).

Comparative analysis of the clinical characteristics of the subjects

The mean age of the subjects was 49.00 (38.00-56.75) years, and 61.42% were female. The prevalence of

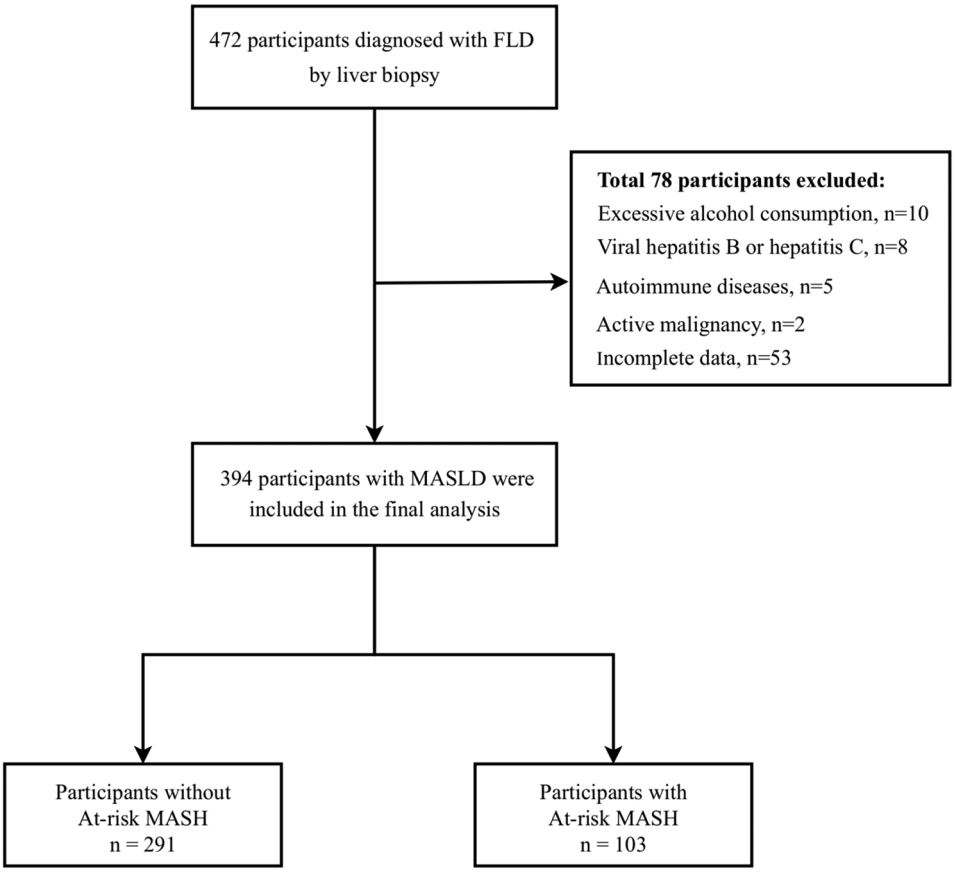

Fig. 1 Study flowchart. Abbreviations: FLD, Fatty liver disease; MASLD, Metabolic dysfunction-associated steatotic liver disease; MASH, metabolic-associated steatohepatitis

overweight/obesity, T2DM, hypertension, and dyslipidemia in this group was 303 (76.90%), 59 (14.97%), 127 (32.23%), and 254 (64.47%), respectively. Notably, the at-risk MASH group had a mean age of 51.00 (39.00–60.00) years, and 66.99% were female. The at-risk MASH group had significantly higher values for several indices, including BMI, ALT, AST, FPG, CRP, LSM, CAP, FAST, FIB-4, NFS, and APRI, compared to the non-at-risk MASH group (all $p < 0.05$). Conversely, the at-risk MASH group had significantly lower values for PLT and HDL-c (all $p < 0.05$). Additionally, the prevalence of T2DM was significantly higher in the at-risk MASH group ($p < 0.01$) (Table 1).

Performance evaluation of acfibromash index in predicting high-risk MASH

By plotting the ROC curves, it was determined that the novel prediction model had a good predictive ability for at-risk MASH, with an AUC (95% CI) of 0.780 [0.729,0.831] ($p < 0.001$) (Table 2; Fig. 2A). The diagnostic performance of acFibroMASH index in terms of sensitivity and specificity by threshold is shown in Fig. 2B. The study utilized a calibration curve to compare the predicted probability of at-risk MASH (horizontal axis) with the observed probability of at-risk MASH after bias correction (vertical axis), which was obtained from 1000 bootstrap resamplings. The calibration curves of the acFibroMASH index demonstrated good agreement between the training and validation sets (Fig. 2C). The clinical decision curve analysis indicated a better net clinical benefit of the acFibroMASH index (Fig. 2D).

Comparison of performance with existing forecasting models

The AUC of acFibroMASH (95% CI) for predicting at-risk MASH in patients with MASLD was 0.780 [0.729,0.831], with a corresponding optimal cut-off value of 0.35. FAST, FIB-4, NFS, and APRI also showed diagnostic potential for predicting at-risk MASH (all $P < 0.01$). The AUC (95% CI) of these models were 0.770 [0.717,0.823], 0.670 [0.605,0.735], 0.607 [0.542,0.672], and 0.703 [0.646,0.760], respectively. The optimal FAST, FIB-4, NFS, and APRI cut-off values were 0.61, 1.29, -1.99 and 0.79, respectively (Table 2). There was no difference in AUC between acFibroMASH and FAST ($P = 0.542$). The AUC of acFibroMASH was higher than the above non-invasive indices,

Table 1 Demographic, clinical and laboratory data from the study cohort

Variable	Total ($n=394$)	Participants without At-risk MASH ($n=291$)	Participants with At-risk MASH ($n=103$)	*P*
GENDER (Male), n (%)	152 (38.58%)	118 (40.55%)	34 (33.01%)	0.177
AGE (years)	49.00 (38.00, 56.75)	49.00 (38.00, 55.00)	51.00 (39.00, 60.00)	0.087
BMI (kg/m^2)	24.98 (23.20, 27.22)	24.73 (23.21, 26.77)	25.64 (23.37, 28.65)	0.012
WC (cm)	90.10 (84.38, 97.35)	89.50 (83.87, 96.75)	91.80 (86.90, 102.74)	0.011
PLT (10^9/L)	204.00 (171.00, 252.75)	211.00 (178.50, 258.50)	183.00 (158.50, 222.50)	< 0.001
ALT (U/L)	86.85 (50.80, 139.90)	81.00 (48.80, 136.15)	101.90 (62.35, 143.60)	0.030
AST (U/L)	50.00 (35.00, 77.00)	46.00 (32.50, 70.50)	70.35 (44.00, 97.00)	< 0.001
GGT (U/L)	92.55 (52.35, 152.98)	92.70 (49.65, 157.90)	92.00 (57.85, 136.03)	0.739
TBIL (μmol/L)	13.10 (10.20, 16.65)	12.70 (10.25, 16.20)	13.90 (10.10, 18.30)	0.075
TC (mmol/L)	4.88 (4.30, 5.34)	4.90 (4.31, 5.35)	4.72 (4.29, 5.32)	0.521
TG (mmol/L)	1.87 (1.28, 2.54)	1.88 (1.25, 2.54)	1.83 (1.42, 2.51)	0.605
HDL-c (mmol/L)	1.18 (1.03, 1.27)	1.18 (1.05, 1.29)	1.13 (0.98, 1.20)	0.027
LDL-c (mmol/L)	3.17 (2.76, 3.37)	3.17 (2.83, 3.42)	3.05 (2.59, 3.34)	0.130
FPG (mmol/L)	5.20 (4.80, 5.80)	5.12 (4.80, 5.70)	5.30 (4.81, 6.05)	0.020
HbA1c	6.20 (5.90, 6.40)	6.20 (5.90, 6.40)	6.22 (5.92, 6.52)	0.299
Creatinine (μmol/L)	61.00 (50.90, 71.27)	61.00 (51.15, 72.75)	60.60 (48.50, 69.00)	0.158
eGFR (ml/min/1.73m^2)	107.47 (96.89, 118.63)	107.78 (97.75, 118.15)	106.21 (94.98, 119.22)	0.525
UA (μmol/L)	359.95 (293.83, 422.17)	361.66 (293.90, 423.10)	342.80 (291.30, 418.00)	0.368
CRP (mg/L)	1.81 (0.97, 3.40)	1.59 (0.81, 3.26)	2.25 (1.57, 3.60)	< 0.001
LSM (kpa)	7.60 (5.60, 10.38)	6.70 (5.30, 8.80)	11.30 (7.90, 15.60)	< 0.001
CAP (dB/m)	305.00 (277.25, 340.00)	302.00 (271.50, 333.50)	315.38 (285.50, 344.50)	0.008
FAST	0.52 (0.32, 0.69)	0.46 (0.27, 0.62)	0.71 (0.53, 0.80)	< 0.001
FIB4	1.25 (0.84, 1.93)	1.13 (0.79, 1.67)	1.75 (1.11, 2.86)	< 0.001
NFS	-2.60 (-4.28, -1.29)	-2.75 (-4.37, -1.45)	-1.79 (-3.75, -0.56)	0.001
APRI	0.52 (0.33, 0.81)	0.47 (0.30, 0.70)	0.74 (0.49, 1.19)	< 0.001
acFibroMASH	0.28 (0.16, 0.55)	0.22 (0.14, 0.37)	0.56 (0.32, 0.88)	< 0.001
Overweight/Obesity, n (%)	303 (76.9%)	225 (77.32%)	78 (75.73%)	0.742
Type 2 diabetes, n (%)	59 (14.97%)	35 (12.03%)	24 (23.30%)	0.006
Hypertension, n (%)	127 (32.23%)	87 (29.90%)	40 (38.83%)	0.095
Dyslipidemia, n (%)	254 (64.47%)	186 (63.92%)	68 (66.02%)	0.702

Data are shown as median (25th, 75th percentiles) or percentages, $p<0.05$ considered statistically significant

Abbreviations: MASH, metabolic-associated steatohepatitis; BMI, Body mass index; WC, waist circumference; PLT, Platelet count; ALT, Alanine transaminase; AST, Aspartate aminotransferase; GGT, Glutamyl transferase; TBIL, Total bilirubin; TC, Total cholesterol; TG, Triglyceride; HDL-c, High-density lipoprotein cholesterol; LDL-c, Low-density cholesterol; FPG, Fasting plasma-glucose; HbA1c, Hemoglobin A1c; eGFR, Estimated glomerular filtration rate; UA, Uric acid; CRP, C-reactive protein; LSM, Liver stiffness measurement; CAP, Controlled attenuation parameter; FAST, FibroScan-AST; FIB-4, Fibrosis-4 index; NFS, Nonalcoholic fatty liver disease fibrosis score; APRI, AST to platelet ratio index

Table 2 Performance assessment of FAST, FIB-4, NFS, APRI, and acfibromash for predicting At-risk MASH

Variables	AUC [95%CI]	*p*	Cutoff value	Sensitivity	Specificity	PPV	NPV	Accuracy
FAST	0.770 [0.717,0.823]	**< 0.001**	0.61	0.680	0.739	0.479	0.867	0.723
FIB-4	0.670 [0.605,0.735]	**< 0.001**	1.29	0.699	0.588	0.374	0.843	0.617
NFS	0.607 [0.542,0.672]	**0.001**	-1.99	0.631	0.557	0.335	0.810	0.576
APRI	0.703 [0.646,0.760]	**< 0.001**	0.79	0.495	0.811	0.481	0.819	0.728
acFibroMASH	0.780 [0.729,0.831]	**< 0.001**	0.35	0.735	0.718	0.490	0.881	0.731

The bold values indicated statistically significant

Abbreviations: FAST, FibroScan-AST; FIB-4, Fibrosis-4 index; NFS, Nonalcoholic fatty liver disease fibrosis score; APRI, AST to platelet ratio index; MASH, metabolic-associated steatohepatitis; AUC, Area under receiver operating characteristics curve; CI, Confidence interval; PPV, Positive predictive value; NPV, Negative predictive value

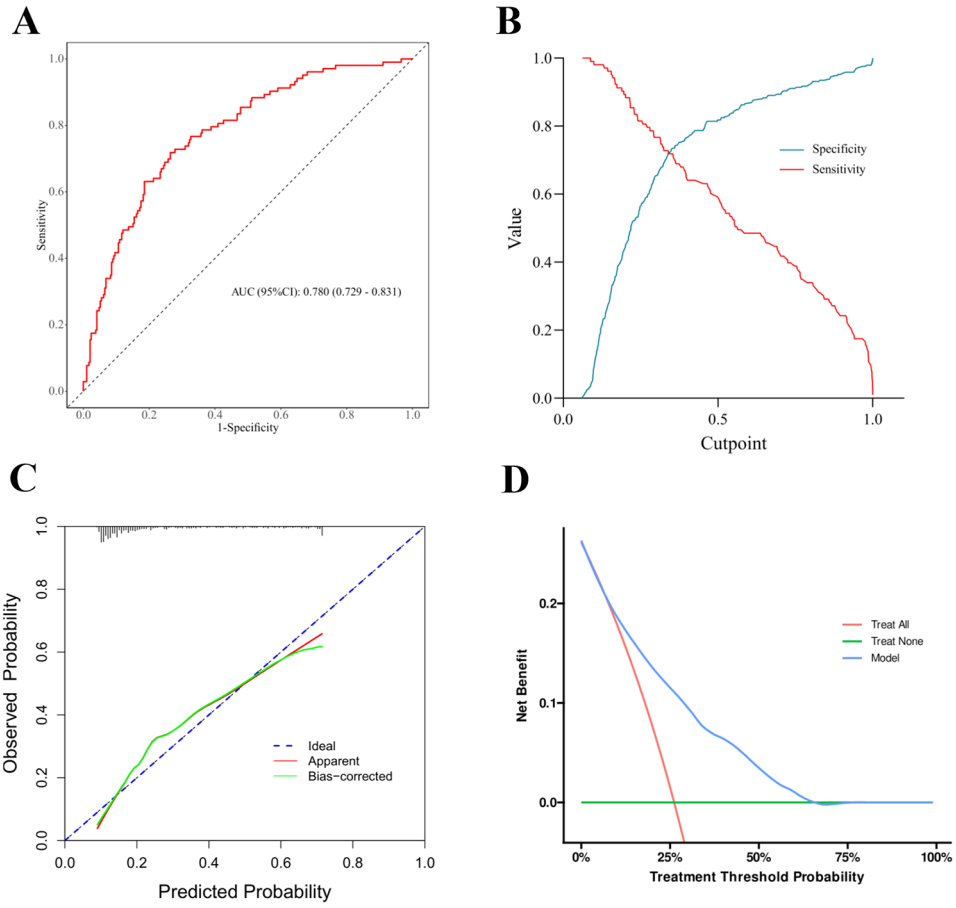

Fig. 2 Diagnostic performance of the acFibroMASH for the diagnostic of At-risk MASH. (**A**) Receiver operating characteristic curve. (**B**) Sensitivity and specificity of the acFibroMASH for the diagnostic of At-risk MASH. Individual plots were derived from all individual score cutoffs covering the range where sensitivity was 100% to where specificity was 100%, followed by smoothening of the graph to cover the dynamic range of scores for their intended uses. (**C**) The calibration curves for acFibroMASH (1000 bootstrap resamples) for predicting At-risk MASH in MASLD. AcFibroMASH-predicted probability of significant fibrosis is plotted on the x-axis; actual probability is plotted on the y-axis. (**D**) Decision curve analysis curve of the acFibroMASH for the diagnostic of At-risk MASH. Abbreviations: MASH, metabolic-associated steatohepatitis; AUC, Area under receiver operating characteristics curve

Table 3 The comparation of AUCs of FAST, FIB-4, NFS, APRI, and acfibromash for predicting At-risk MASH

Variables	AUC1 [95%CI]	AUC2 [95%CI]	*p*
acFibroMASH VS FAST	0.780 [0.729,0.831]	0.770 [0.717,0.823]	0.542
acFibroMASH VS FIB4	0.780 [0.729,0.831]	0.670 [0.605,0.735]	**< 0.001**
acFibroMASH VS NFS	0.780 [0.729,0.831]	0.607 [0.542,0.672]	**< 0.001**
acFibroMASH VS APRI	0.780 [0.729,0.831]	0.703 [0.646,0.760]	**0.005**
FAST VS FIB4	0.770 [0.717,0.823]	0.670 [0.605,0.735]	**0.003**
FAST VS NFS	0.770 [0.717,0.823]	0.607 [0.542,0.672]	**< 0.001**
FAST VS APRI	0.770 [0.717,0.823]	0.703 [0.646,0.760]	**0.005**
FIB4 VS NFS	0.670 [0.605,0.735]	0.607 [0.542,0.672]	0.071
FIB4 VS APRI	0.670 [0.605,0.735]	0.703 [0.646,0.760]	0.197
NFS VS APRI	0.607 [0.542,0.672]	0.703 [0.646,0.760]	**0.014**

The bold values indicated statistically significant

Abbreviations: FAST, FibroScan-AST; FIB-4, Fibrosis-4 index; NFS, Nonalcoholic fatty liver disease fibrosis score; APRI, AST to platelet ratio index; MASH, metabolic-associated steatohepatitis; AUC, Area under receiver operating characteristics curve; CI, Confidence interval

and the differences were statistically significant when compared to FIB-4, NFS, and APRI. (Table 3; Fig. 3).

The double cut-off of acfibromash index to predict at-risk MASH

The AUC of the acFibroMASH index in distinguishing at-risk MASH was 0.780. When the rule-out cutoff (acFibroMASH < 0.15) was used, the NPV reached 0.955. The sensitivity was 0.961, while the specificity was 0.292. The PPV was 0.493 (sensitivity: 0.670, specificity: 0.756) when the rule-in cutoff (acFibroMASH > 0.39) was employed. In the study cohort, 154 patients (39.1%) were correctly classified according to histologic criteria, while 165 patients (41.9%) were indeterminate, lacking a precise classification. Upon recalculation of the optimal cutoff for the study cohort, the NPV using the rule-out cutoff (acFibroMASH < 0.20) was 0.926 (sensitivity: 0.900, specificity: 0.432), while the PPV using the rule-in cutoff (acFibroMASH > 0.70) was 0.597 (sensitivity:

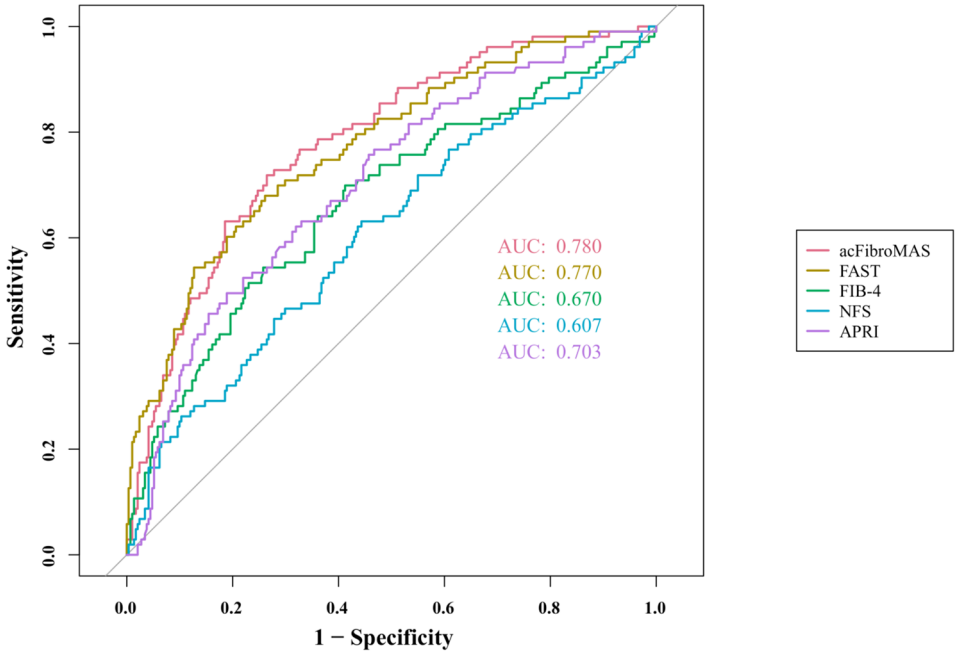

Fig. 3 Receiver operating characteristics (ROC) curves for predicting At-risk MASH. Abbreviations: AUC, Area under receiver operating characteristics curve; FAST, FibroScan-AST; FIB-4, Fibrosis-4 index; NFS, Nonalcoholic fatty liver disease fibrosis score; APRI, AST to platelet ratio index

0.417, specificity: 0.900). The application of these revised cutoff values resulted in the correct classification of 174 patients (44.2%) according to histologic criteria, while 182 patients (46.2%) remained in the indeterminate category. The NPV and PPV values were set at 0.900, and the critical values for the acFibroMASH index were calculated using these values. The derived critical value for exclusion was 0.23, with a sensitivity of 0.835 and a specificity of 0.526. The derived inclusion threshold was determined to be 0.9998, with a PPV of 0.750, a sensitivity of 0.029, and a specificity of 0.997 (Table 4).

Subgroup analysis of acfibromash index and other models for diagnosis of at-risk MASH

The analysis of the ROC curves revealed that the acFibroMASH index exhibited satisfactory predictive efficacy for at-risk MASH in all subgroups. The predictive efficacy was not significantly affected by patient gender, age, BMI, T2DM, hypertension, or dyslipidemia (Table 5).

Discussion

The study's main findings indicate that liver function indices, metabolic abnormalities, and hepatic fibrosis were higher in the at-risk MASH group compared to the non-at-risk MASH group. The diagnostic model acFibroMASH index constructed from AST, Creatinine, and LSM had an AUC (95% CI) of 0.780 [0.729,0.831]. The diagnostic accuracy of acFibroMASH and FAST was superior to that of FIB-4, NFS, and APRI. The non-invasive model acFibroMASH index, designed to assess at-risk MASH, demonstrates satisfactory performance, and its effectiveness remains unaffected by factors such as patient gender, age, BMI, and metabolism.

The main harm of MASLD lies in the progression of necrosis, inflammation, and fibrosis. MASH patients' fibrosis progresses faster due to necrotizing inflammation. On average, MASLD patients deteriorate to the next stage of fibrosis in 14.3 years, while MASH patients progress in 7.1 years [22]. It is important to note that approximately 20% of MASH patients are categorized as 'rapid progressors' whose fibrosis stage evolves in less than seven years. To define at-risk MASH, it is critical to combine the characteristics of MASH and fibrosis since predictors of rapid fibrosis progression have not been fully quantified [23]. In this study cohort, at-risk MASH patients represented only 26.14% (103/394) but were significant during MASLD. The U.S. Food and Drug Administration and the European Medicines Agency prefer MASH status without worsening fibrosis or improved fibrosis without worsening of MASH as a key histologic endpoint for conditional approval of new drugs [24]. Early and accurate identification of at-risk MASH has far-reaching implications for improving treatment outcomes and prognosis. Non-invasive diagnostic scoring systems for at-risk MASH are limited in current medical practice. Scoring tools such as NIS4, MACK-3, MAST, and MASEF play an essential role in assessing an individual's risk, but their application is subject to limitations. The NIS4 algorithm includes four biomarkers: miR-34a-5p, alpha-2 macroglobulin, CHI3L1, and glycosylated

Table 4 Performance assessment of FAST, FIB-4, NFS, APRI, and acfibromash for predicting At-risk MASH using dual cut-offs

Variables	AUC [95%CI]	Diagnostic performance using dual cut-offs		
		"rule-out" zone	"grey" zone	"rule-in" zone
FAST	0.770 [0.717,0.823]	**< 0.35**	**0.35–0.67**	**> 0.67**
		N = 114 (28.9%)	*N* = 165 (41.9%)	*N* = 115 (29.2%)
		Sensitivity = 0.903		Sensitivity = 0.583
		Specificity = 0.357		Specificity = 0.811
		NPV = 0.912		PPV = 0.522
FIB-4	0.670 [0.605,0.735]	**< 1.30**	**1.30–2.67**	**> 2.67**
		N = 209 (53.0%)	*N* = 133 (33.8%)	*N* = 52 (13.2%)
		Sensitivity = 0.660		Sensitivity = 0.272
		Specificity = 0.598		Specificity = 0.918
		NPV = 0.833		PPV = 0.538
NFS	0.607 [0.542,0.672]	**<-1.455**	**-1.455-0.676**	**> 0.676**
		N = 285 (72.3%)	*N* = 53 (13.5%)	*N* = 56 (14.2%)
		Sensitivity = 0.350		Sensitivity = 0.204
		Specificity = 0.749		Specificity = 0.880
		NPV = 0.765		PPV = 0.375
APRI	0.703 [0.646,0.760]	**< 0.57**	**0.57–0.84**	**> 0.84**
		N = 218 (55.3%)	*N* = 81 (20.6%)	*N* = 95 (24.1%)
		Sensitivity = 0.641		Sensitivity = 0.456
		Specificity = 0.622		Specificity = 0.835
		NPV = 0.830		PPV = 0.495
acFibroMASH	0.780 [0.729,0.831]	**< 0.15**	**0.15–0.39**	**> 0.39**
		N = 89 (22.6%)	*N* = 165 (41.9%)	*N* = 140 (35.5%)
		Sensitivity = 0.961		Sensitivity = 0.670
		Specificity = 0.292		Specificity = 0.756
		NPV = 0.955		PPV = 0.493
		< 0.20	**0.20–0.70**	**> 0.70**
		N = 141 (35.8%)	*N* = 182 (46.2%)	*N* = 71 (18.0%)
		Sensitivity = 0.900		Sensitivity = 0.417
		Specificity = 0.432		Specificity = 0.900
		NPV = 0.926		PPV = 0.597
		< 0.23	**0.23-0.9998**	**> 0.9998**
		N = 170 (43.1%)	*N* = 182 (55.8%)	*N* = 4 (1.1%)
		Sensitivity = 0.835		Sensitivity = 0.029
		Specificity = 0.526		Specificity = 0.997
		NPV = 0.900		PPV = 0.750

Abbreviations: FAST, FibroScan-AST; FIB-4, Fibrosis-4 index; NFS, Nonalcoholic fatty liver disease fibrosis score; APRI, AST to platelet ratio index; MASH, metabolic-associated steatohepatitis; AUC, Area under receiver operating characteristics curve; CI, Confidence interval; NPV, Negative predictive value; PPV, Positive predictive value

hemoglobin [13]. Meanwhile, MACK-3 is based on HOMA, AST, and CK18 markers [14]. The MAST scoring methodology combines magnetic resonance imaging techniques with serologic markers. The MASEF is a highly specific metabolomics-driven score based on 12 lipids, body mass index, aspartate aminotransferase, and alanine aminotransferase [11]. However, the complexity of these scoring systems and the unconventional nature of specific biomarkers have limited their widespread use in clinical practice. The FAST score has recently emerged as a new tool for non-invasive diagnosis of at-risk MASH [12].

In this study, we validated the performance of the noninvasive diagnostic model acFibroMASH index in predicting at-risk MASH based on pathological diagnosis of liver tissue biopsies, with an AUC of 0.780. This value suggests that the model has excellent diagnostic ability to differentiate at-risk MASH accurately. Additionally, the diagnostic accuracy of other noninvasive fibrosis indicators was evaluated. Among the evaluated models, the AUC of FAST was 0.770 (95% CI: 0.717, 0.823), comparable to the diagnostic performance of acFibroMASH. FAST, a validated model designed explicitly for identifying at-risk MASH, was established by British academics by combining LSM and CAP, measured by FibroScan and AST [12]. It has been subjected to several external validations [25–27]. Notably, the diagnostic efficacy of acFibroMASH and FAST was superior to that of FIB-4, NFS, and

Table 5 The AUCs of FAST, FIB-4, NFS, APRI, and acfibromash for predicting At-risk MASH in different subgroups

Subgroups	AUC [95%CI]				
	FAST	FIB-4	NFS	APRI	acFibroMASH
Gender					
Male	0.753 (0.660,0.846)	0.597 (0.492,0.702)	0.540 (0.424,0.655)	0.653 (0.550,0.757)	0.765 (0.673,0.857)
Female	0.777 (0.710,0.844)	0.721 (0.646,0.796)	0.620 (0.539,0.702)	0.728 (0.658,0.798)	0.784 (0.722,0.846)
Age (years)					
< 65	0.769 (0.711,0.827)	0.658 (0.590,0.727)	0.587 (0.516,0.659)	0.692 (0.630,0.754)	0.780 (0.726,0.835)
≥ 65	0.758 (0.586,0.930)	0.727 (0.539,0.915)	0.546 (0.336,0.756)	0.773 (0.602,0.944)	0.708 (0.527,0.889)
BMI (kg/m^2)					
< 23	0.773 (0.663,0.883)	0.773 (0.671,0.875)	0.719 (0.600,0.839)	0.700 (0.580,0.820)	0.790 (0.690,0.890)
≥ 23	0.772 (0.710,0.834)	0.635 (0.559,0.711)	0.557 (0.478,0.635)	0.703 (0.636,0.771)	0.779 (0.720,0.838)
Type 2 diabetes					
No	0.767 (0.706,0.829)	0.637 (0.563,0.711)	0.599 (0.525,0.674)	0.681 (0.613,0.749)	0.775 (0.718,0.833)
Yes	0.750 (0.621,0.879)	0.807 (0.689,0.926)	0.650 (0.490,0.810)	0.761 (0.638,0.883)	0.785 (0.662,0.907)
Hypertension					
No	0.757 (0.685,0.828)	0.657 (0.577,0.736)	0.618 (0.535,0.670)	0.703 (0.630,0.776)	0.776 (0.711,0.842)
Yes	0.789 (0.705,0.873)	0.688 (0.584,0.793)	0.597 (0.484,0.710)	0.700 (0.601,0.798)	0.778 (0.693,0.863)
Dyslipidemia					
No	0.709 (0.604,0.815)	0.596 (0.484,0.707)	0.601 (0.483,0.718)	0.602 (0.496,0.707)	0.725 (0.627,0.824)
Yes	0.801 (0.742,0.861)	0.706 (0.629,0.783)	0.614 (0.532,0.675)	0.754 (0.687,0.822)	0.810 (0.753,0.868)

Abbreviations: AUC, Area under receiver operating characteristics curve; FAST, FibroScan-AST; FIB-4, Fibrosis-4 index; NFS, Nonalcoholic fatty liver disease fibrosis score; APRI, AST to platelet ratio index; MASH, metabolic-associated steatohepatitis; CI, Confidence interval; BMI, Body mass index

APRI [28]. According to the AACE guidelines, the FIB-4 score is recommended to assess the risk of liver fibrosis in patients with MASLD. The specific recommendations are: FIB-4 < 1.3: low risk, FIB-4 > 2.67: high risk, FIB-4 1.3–2.67: uncertain risk. We, therefore, analyzed at-risk MASH by borrowing the two cut-off values for FIB-4 [29]. FIB-4, NFS and APRI are noninvasive diagnostic models based on routine laboratory parameters that are widely used in clinical settings, especially in primary care and resource-limited settings, because of their low cost and ease of use. However, these models were initially designed for advanced liver fibrosis, are not sensitive enough for early liver fibrosis, are prone to underdiagnose patients with mild fibrosis, and do not reflect the heterogeneity and dynamic changes of MASH. In contrast, the acFibroMASH and FAST models, which integrate LSM data, have higher diagnostic accuracy and are notably superior to FIB-4, NFS, and APRI in identifying at-risk MASH. However, their reliance on the FibroScan device may increase the cost of the assay, limiting its application in resource-limited settings. However, with the increasing adoption of liver elastometry, there is a growing expectation that the applications of acFibroMASH and FAST will expand, leading to an enhancement in the diagnostic process and an improvement in the early diagnosis rate and management efficiency of patients with at-risk MASH.

As Feng G. et al. recommended, we employed a double cutoff value strategy. When the rule-out cutoff (acFibroMASH: <0.15) was utilized, the model demonstrated a sensitivity of 0.961, indicating its capacity to correctly exclude 96.1% of the actual non-at-risk MASH patients. Concurrently, the specificity at this threshold was 0.292. As a crucial metric for evaluating the model's capacity to identify the disease accurately, high sensitivity indicates a reduced likelihood of false-negative outcomes. When the rule-in cutoff (acFibroMASH:>0.39) is employed, the model exhibits a specificity of 0.756, signifying its capacity to accurately identify 75.6% of the actual at-risk MASH patients. Concurrently, the sensitivity is 0.670. As a crucial metric for evaluating the model's capacity to differentiate between healthy populations, a high specificity indicates reduced false-positive outcomes. Based on the NPV and PPV calculations, 85 subjects could be excluded, and the diagnosis was confirmed for 69 subjects. Thus, 154 subjects (39.1%) were correctly classified based on the histology, while 165 subjects (41.9%) fell into the gray area.

The new predictive model must undergo comprehensive validation in a diverse study population to guarantee its generalizability. Given the relatively low PPV of the patients in our study cohort based on the acFibroMASH inclusion cutoff proposed by Feng G et al., we recalculated the rule-out and rule-in cutoffs of the acFibroMASH index applicable to our patient cohort. Upon analysis, we determined that an optimal exclusion threshold of 0.20 would be most appropriate. The NPV reached 0.926 at this threshold, and the model sensitivity was 0.900. This implies that the model could accurately exclude 90.0% of the study population with actual non-at-risk MASH, while the specificity was 0.432. Conversely, when the rule-in cutoff was employed (acFibroMASH > 0.70), the

PPV was 0.597, and the model specificity was enhanced to 0.900. This indicates that the model could confirm the diagnosis of 90.0% of study subjects who were, in fact, at-risk MASH. Based on the NPV and PPV calculation results, 131 subjects could be excluded, and 43 diagnoses could be confirmed. This allowed for the correct classification of 174 subjects (44.2%) according to histologic criteria. However, 182 subjects (46.2%) remained in the gray area, i.e., they could not be definitively classified. Despite the limitations of its sensitivity, the high NPV of the acFibroMASH index model suggests that the model performs well in ruling out at-risk MASH, similar to the cut-off value proposed by Feng G et al.

In this study, our primary focus was on the sensitivity and specificity of the acFibroMASH index. However, we also comprehensively evaluated its negative predictive value (NPV) and positive predictive value (PPV) to ensure its suitability for clinical applications. The low threshold value of the acFibroMASH index was determined to be 0.23, at which point the NPV reached 0.900. This threshold, while maintaining a high NPV, is suitable for the initial screening phase, as it can efficiently exclude low-risk patients and reduce unnecessary tests and interventions, thus avoiding the waste of medical resources. However, we encountered some challenges when attempting to determine a high critical value of the acFibroMASH index by high PPV to confirm the diagnosis of at-risk MASH. When a critical value close to 1 was incorporated, the PPV was only 0.750, and only three patients were diagnosed with at-risk MASH. The analysis revealed that the acFibroMASH index exhibited different diagnostic efficacies at varying critical value settings. While it is challenging for a single threshold to satisfy both high NPV and high PPV, optimizing the threshold setting can better balance the sensitivity and specificity of diagnosis in clinical practice.

This property makes the model a useful noninvasive diagnostic tool in clinical practice, which can safely eliminate the need for liver puncture in some patients, thus assisting clinical decision-making and reducing disease burden. However, in clinical practice, it is not possible for diagnostic tools to completely avoid false-positive and false-negative results. False-negative patients may be misclassified as healthy, leading to missed diagnoses, delayed referrals to specialists, missed optimal interventions, and accelerated disease progression to advanced fibrosis or cirrhosis, affecting prognosis and increasing healthcare costs. Conversely, false-positive patients may be misclassified as at-risk MASH patients, leading to unnecessary further investigations (e.g., liver biopsy, imaging, etc.), resulting in healthcare resource wastage and financial burden. The acFibroMASH Index maximizes diagnostic efficacy and reduces misdiagnosis by optimizing threshold values to balance sensitivity and specificity. Lower thresholds enhance sensitivity but concomitantly elevate the probability of false- positive outcomes. Conversely, higher thresholds improve specificity but concomitantly increase the likelihood of false- negative results. Future studies must further refine the critical value setting of the acFibroMASH index to address the diverse clinical scenarios that arise in clinical practice.

Accordingly, in practical application, it should be combined with other clinical information and diagnostic tools to facilitate more accurate diagnostic decisions. Furthermore, the acFibroMASH index exhibited robust predictive efficacy in all subgroup analyses, demonstrating no correlation with patients' gender, age, or metabolic factors (e.g., BMI, DM, hypertension, and dyslipidemia). This finding further confirms the broad applicability of this index in patients with MASLD.

Regarding diagnosis, the acFibroMASH index is based on routine laboratory indicators and non-invasive tests, which are simple, easy to use, and suitable for broad application in primary care and liver disease specialty outpatient clinics. It can accurately risk-stratify MASH patients, help identify high-risk patients, and optimize resource allocation while reflecting dynamic changes in liver lesions for long-term follow-up and treatment effect assessment. Regarding treatment decision-making, the model avoids over-treatment by accurately identifying low-risk patients while focusing resources on high-risk patients. It also assists in the early identification of high-risk patients, initiating timely interventions to slow disease progression. For example, early identification of fibrosis risk and intervention can significantly improve prognosis. Subsequent studies will validate the acFibroMASH Index's applicability in a broader range of clinical settings and explore its potential for application in different treatment phases. These efforts aim to optimize the management of MASH patients and enhance the precision and efficiency of clinical decision-making.

Nevertheless, it should be noted that this study is not without limitations. Firstly, the prevalence of at-risk MASH in this study (26.1%) reflects the characteristics of a tertiary care center cohort and may be influenced by referral bias. This limitation restricts the generalizability of the findings to community or primary care settings, where the prevalence of at-risk MASH may be lower. Future studies should include additional patient populations in community or primary care settings to assess the broader applicability of the findings. Secondly, while the validity of the acFibroMASH index has been validated to a certain extent in this study, future studies must include more comorbidities (e.g., type 2 diabetes mellitus, cardiovascular disease, etc.), more regions, and stratify the different stages of fibrosis (e.g., F2-F3 and F4). The generalizability and predictive accuracy of the acFibroMASH index can be further enhanced by conducting larger

and more diverse cohort studies from multiple perspectives to improve the robustness of the findings. Thirdly, this study was a cross-sectional study constrained by the practicalities of hepatic puncture biopsy. Consequently, a cohort study could not be conducted to verify the diagnostic validity of the model dynamically. Accordingly, a prospective cohort design may be deemed appropriate for future studies to evaluate the long-term diagnostic performance of the model comprehensively.

Conclusions

In conclusion, this study validated the good diagnostic efficacy of the acFibroMASH index in detecting at-risk MASH in the MASLD population using liver puncture histology as the gold standard. Furthermore, the performance of the acFibroMASH index was comparable to that of FAST. This non-invasive model is readily accessible in clinical practice and can effectively identify at-risk MASH patients without liver biopsy, thus enhancing cost-effectiveness. The acFibroMASH index, when used in conjunction with FAST, can provide a robust clinical foundation for screening and identifying at-risk MASH patients in primary care settings, which can optimize the treatment process and enhance the quality of patient management.

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Author contributions
Yunfei Wu reviewed and searched the literature, made pathological diagnoses, discussed the findings, and drafted the manuscript. Yan Han collected cases, discussed the findings, and drafted the manuscript. Liming Zheng collated the data. Longgen Liu collected cases. Wenjian Li collated the data, made statistical analysis, and revised the manuscript. Fan Zhang provided ideas, experimental guidance and revised the manuscript. All authors have made an intellectual contribution to the manuscript and approved the submission.

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Data availability
The data used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Changzhou Third People's Hospital (No. 2020002) and was performed by the 2013 Declaration of Helsinki. All subjects were informed of the study before participation and provided written consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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