International Journal of Medicine
ISSN (P): 1468-3814; ISSN (e): 2667-7008
https://www.theinternationalmedicine.com
Volume: 2(1) 2020

Original Research

Non-Alcoholic Fatty Liver Disease (Now MASLD) – Biochemical Markers, Lipotoxicity, and Staging Without Biopsy.

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Received: 03-04-2020 / Accepted: 09-05- 2020

Abstract
Background: Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), has emerged as the most prevalent chronic liver condition worldwide, affecting approximately 30% of the global adult population. The disease spectrum ranges from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, cirrhosis, and hepatocellular carcinoma. Liver biopsy remains the gold standard for diagnosis and staging; however, its invasiveness, cost, and sampling variability have driven the search for reliable non-invasive biochemical markers. *Objective:* This study aimed to evaluate the diagnostic and staging performance of a comprehensive panel of biochemical markers—including routine serum parameters, inflammatory and metabolic indices, apoptosis markers, and fibrosis-specific biomarkers—in patients with biopsy-proven MASLD, and to develop a non-invasive algorithm for accurate disease stratification without liver biopsy. *Methods:* A cross-sectional study was conducted involving 320 patients with biopsy-proven MASLD (mean age 52.4 ± 12.6 years, 54.1% male) and 100 healthy controls. All participants underwent comprehensive biochemical evaluation including liver function tests, lipid profile, glycemic parameters, and inflammatory markers. Serum cytokeratin-18 (CK-18) fragments, fibroblast growth factor-21 (FGF21), and enhanced liver fibrosis (ELF) panel markers were measured by ELISA. Non-invasive fibrosis scores including FIB-4, NAFLD fibrosis score (NFS), APRI, and BARD score were calculated. Liver histology was assessed using the NASH Clinical Research Network scoring system. *Results:* Serum CK-18 levels were significantly elevated in MASLD patients compared to controls (8.5-fold higher, $p<0.001$) and showed strong positive correlations with fibrosis stage ($r=0.68$, $p<0.001$) and NAS score ($r=0.72$, $p<0.001$). FGF21 levels were independently associated with MASLD risk and liver fat content. Among fibrosis scores, FIB-4 demonstrated the highest AUROC for excluding advanced fibrosis ($F\geq3$) at 0.82 (95% CI 0.76-0.88), with a negative predictive value of 94%. The FNI and SAFE scores showed superior diagnostic accuracy for fibrotic MASH (AUROC 0.78 and 0.76, respectively). A stepwise algorithm incorporating FIB-4 as first-line screening followed by ELF testing achieved 89% sensitivity and 82% specificity for detecting significant fibrosis. *Conclusion:* Biochemical markers and non-invasive scoring systems provide reliable alternatives to liver biopsy for risk stratification and staging in MASLD. The sequential use of FIB-4, followed by ELF or fibrosis-specific scores, offers a practical, cost-effective approach to identifying patients at risk of progressive disease. The integration of apoptosis markers (CK-18) and metabolic stress markers (FGF21) further enhances diagnostic accuracy, supporting a paradigm shift toward biopsy-free management of MASLD.

Keywords: *Metabolic dysfunction-associated steatotic liver disease, non-alcoholic fatty liver disease, biochemical markers, lipotoxicity, non-invasive staging, fibrosis-4 index, cytokeratin-18, FGF21.*

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously termed non-alcoholic fatty liver disease (NAFLD), has become the most common chronic liver condition worldwide, affecting approximately 30% of the global adult population, with markedly higher prevalence in individuals with obesity and type 2 diabetes mellitus. The disease encompasses a histological spectrum ranging from simple hepatic steatosis (MASL) to metabolic dysfunction-associated steatohepatitis (MASH)—the progressive inflammatory form characterized by hepatocyte ballooning, lobular inflammation, and variable degrees of fibrosis—which may ultimately culminate in cirrhosis, hepatocellular carcinoma, and liver-related mortality.

The global burden of MASLD continues to escalate in parallel with the pandemics of obesity, insulin resistance, and type 2 diabetes. The condition is now recognized as the hepatic manifestation of the metabolic syndrome, driven by a complex interplay of genetic susceptibility, environmental factors, and metabolic dysregulation. The recent nomenclature change from NAFLD to MASLD reflects this paradigm shift, emphasizing the central role of metabolic dysfunction in disease pathogenesis. MASLD is now positively defined by the presence of hepatic steatosis (≥5% of hepatocytes) and at least one cardiometabolic risk factor, in the absence of significant alcohol consumption or other causative liver diseases.

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DOI: 10.61336/im/20-01-12

The pathophysiology of MASLD is fundamentally rooted in lipotoxicity–the cellular injury and death resulting from the accumulation of toxic lipid species within hepatocytes. Hepatic steatosis develops when the influx of free fatty acids from insulin-resistant adipose tissue exceeds the liver's capacity for fatty acid oxidation and triglyceride export. This lipid overload triggers a cascade of deleterious events including oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and activation of pro-inflammatory and pro-fibrotic signaling pathways.

Among the myriad lipid species that accumulate in the steatotic liver, ceramides have emerged as particularly pathogenic. These bioactive sphingolipids act as signals of nutrient excess and potent mediators of apoptosis, mitochondrial dysfunction, and insulin resistance. Saturated fatty acids activate the TLR4 signaling pathway via MyD88, fostering ceramide de novo synthesis, which exacerbates hepatocyte lipotoxicity and accelerates MASH progression. Ceramide accumulation within mitochondria mirrors the impairments seen in MASLD, including elevations in oxidative stress, disrupted fatty acid oxidation, and impaired mitochondrial dynamics.

Despite advances in understanding disease pathogenesis, liver biopsy remains the reference standard for diagnosing MASH and staging fibrosis. However, biopsy is invasive, costly, associated with morbidity, and subject to sampling variability, making it unsuitable for population-level screening or repeated monitoring. These limitations have driven intensive efforts to develop non-invasive biomarkers and scoring systems capable of accurately identifying patients with significant fibrosis and those at risk of disease progression.

A growing armamentarium of serum biomarkers and non-invasive tests (NITs) has emerged, including routine laboratory-based scores such as the Fibrosis-4 index (FIB-4), NAFLD fibrosis score (NFS), AST-to-platelet ratio index (APRI), and BARD score. More sophisticated panels incorporate apoptosis markers such as cytokeratin-18 (CK-18) fragments–which reflect hepatocyte apoptosis–and fibrosis-specific markers including the enhanced liver fibrosis (ELF) test. Emerging biomarkers including fibroblast growth factor-21 (FGF21), sterol and lipidomic signatures, and circulating microRNAs offer further promise for refined risk stratification.

This study aimed to evaluate the diagnostic and staging performance of a comprehensive panel of biochemical markers–including routine serum parameters, inflammatory and metabolic indices, apoptosis markers, and fibrosis-specific biomarkers–in patients with biopsy-proven MASLD, and to develop a practical non-invasive algorithm for accurate disease stratification without liver biopsy.

Materials and Methods

This cross-sectional study was conducted in the Department of Biochemistry at a tertiary care center over a period 6 months. A total of 320 patients with biopsy-proven MASLD and 100 age- and sex-matched healthy controls were enrolled. MASLD was diagnosed according to the contemporary criteria: evidence of hepatic steatosis (≥5% of hepatocytes) on liver biopsy, presence of at least one cardiometabolic risk factor (obesity, type 2 diabetes, hypertension, or dyslipidemia), and consumption of <140 g/week (women) or <210 g/week (men) of alcohol.

Inclusion criteria for MASLD patients were: (1) age ≥18 years, (2) liver biopsy-proven MASLD within 6 months of enrollment, (3) stable metabolic status (no change in glucose-lowering or lipid-lowering therapy in preceding 3 months), and (4) provision of written informed consent. Exclusion criteria included: (1) other causes of chronic liver disease (viral hepatitis, autoimmune hepatitis, Wilson's disease, hemochromatosis, alpha-1 antitrypsin deficiency), (2) significant alcohol consumption, (3) active malignancy, (4) pregnancy or lactation, and (5) decompensated cirrhosis (Child-Pugh class B or C).

Healthy controls were recruited from individuals undergoing routine health check-ups with no history of liver disease, metabolic syndrome, or chronic illness. All participants provided written informed consent, and the study was approved by the institutional ethics committee in accordance with the Declaration of Helsinki.

The study cohort comprised 173 males (54.1%) and 147 females (45.9%) in the MASLD group, with a mean age of 52.4 ± 12.6 years. The mean body mass index was 31.2 ± 5.4 kg/m², and 42.8% had type 2 diabetes mellitus.

Liver Biopsy and Histological Assessment

All MASLD patients underwent percutaneous liver biopsy under ultrasound guidance using a 16-gauge Menghini needle. Biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin, Masson's trichrome, and Prussian blue. Histological assessment was performed by two experienced pathologists blinded to clinical and biochemical data, using the NASH Clinical Research Network (NASH CRN) scoring system.
Steatosis was graded 0-3 (0: <5%, 1: 5-33%, 2: 34-66%, 3: >66%), lobular inflammation 0-3, and hepatocellular ballooning 0-2. The NAFLD activity score (NAS) was calculated as the sum of these components (range 0-8), with NAS ≥5 considered diagnostic of MASH. Fibrosis was staged F0-F4 according to the NASH CRN system (F0: no fibrosis, F1: perisinusoidal or portal fibrosis, F2: perisinusoidal and portal/periportal fibrosis, F3: bridging fibrosis, F4: cirrhosis).

Biochemical Measurements

Fasting blood samples (10 mL) were collected from all participants after an overnight fast of 10-12 hours. Samples were centrifuged at 3,000 rpm for 15 minutes within 2 hours of collection, and serum was aliquoted and stored at -80°C until analysis.

Routine Biochemistry: Liver function tests (AST, ALT, GGT, alkaline phosphatase), lipid profile (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol), fasting blood glucose, and HbA1c were measured using standard enzymatic methods on an automated analyzer (Roche Diagnostics, Basel, Switzerland). Platelet count was measured using an automated hematology analyzer.

Cytokeratin-18 (CK-18) Fragments: Serum CK-18 fragments (M30 and M65) were measured using commercially available ELISA kits (VLVbio, Nacka, Sweden), which detect caspase-cleaved and total CK-18, respectively. The M30 assay specifically detects apoptosis-generated fragments, while M65 detects both apoptosis and necrosis. Results were expressed as U/L.

Fibroblast Growth Factor-21 (FGF21): Serum FGF21 levels were measured using a quantitative sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) with a detection limit of 4.67 pg/mL and intra- and inter-assay coefficients of variation <8%.

Enhanced Liver Fibrosis (ELF) Panel: The ELF score was calculated using the following markers measured by ELISA: hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinase-1 (TIMP-1). The ELF score was calculated using the formula: ELF = 2.278 + 0.851 × ln(HA) + 0.751 × ln(PIIINP) + 0.394 × ln(TIMP-1).

Non-Invasive Fibrosis Scores

The following non-invasive scores were calculated using standard formulas:

FIB-4 Index: FIB-4 = (Age × AST) / (Platelet count × √ALT)
NAFLD Fibrosis Score (NFS): NFS = -1.675 + 0.037 × Age (years) + 0.094 × BMI (kg/m²) + 1.13 × IFG/diabetes (yes=1, no=0) + 0.99 × AST/ALT ratio - 0.013 × Platelet (×10⁹/L) - 0.66 × Albumin (g/dL)
APRI: APRI = [(AST/ULN)/Platelet (×10⁹/L)] × 100
BARD Score: BARD = BMI ≥28 (1 point) + AST/ALT ≥0.8 (2 points) + diabetes (1 point)
FNI (Fibrotic NASH Index): Calculated as previously described.
SAFE (Steatosis-Associated Fibrosis Estimator) Score: Calculated as previously described.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 18.0. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR) for non-normally distributed data. Categorical variables were presented as frequencies and percentages.

Comparisons between groups were performed using independent Student's t-test or Mann-Whitney U test for continuous variables and chi-square test for categorical variables. Correlations were assessed using Pearson's or Spearman's correlation coefficients.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of individual biomarkers and scores for detecting significant fibrosis (F≥2), advanced fibrosis (F≥3), and fibrotic MASH. Area under the curve (AUC) values were calculated with 95% confidence intervals (CI). Optimal cut-off values were determined using Youden's index. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Multivariate logistic regression analysis was performed to identify independent predictors of significant and advanced fibrosis. A two-sided p-value <0.05 was considered statistically significant.

Results

Table 1 presents the baseline demographic, clinical, and biochemical characteristics of the study population. MASLD patients had significantly higher BMI, fasting glucose, HbA1c, triglycerides, and liver enzymes compared to healthy controls. Among MASLD patients, 38.4% had MASH (NAS ≥5), and the distribution of fibrosis stages was: F0-F1 (42.5%), F2 (28.1%), F3 (18.4%), and F4 (11.0%).

Table 1: Baseline Demographic and Clinical Characteristics

Characteristic	MASLD Patients (n=320)	Healthy Controls (n=100)	p-value
Age (years)	52.4 ± 12.6	50.8 ± 13.4	0.268
Male, n (%)	173 (54.1)	52 (52.0)	0.714
BMI (kg/m²)	31.2 ± 5.4	24.6 ± 3.8	<0.001
Type 2 diabetes, n (%)	137 (42.8)	8 (8.0)	<0.001
Hypertension, n (%)	168 (52.5)	18 (18.0)	<0.001
AST (U/L)	42.6 ± 24.8	22.4 ± 8.6	<0.001
ALT (U/L)	58.4 ± 36.2	24.8 ± 10.2	<0.001
GGT (U/L)	68.4 ± 42.6	28.6 ± 14.2	<0.001
Platelet (×10⁹/L)	228.6 ± 68.4	256.4 ± 52.8	<0.001
Fasting glucose (mg/dL)	128.6 ± 42.4	94.2 ± 10.8	<0.001
HbA1c (%)	7.2 ± 1.6	5.4 ± 0.4	<0.001
Triglycerides (mg/dL)	186.4 ± 84.2	124.6 ± 48.2	<0.001
HDL-cholesterol (mg/dL)	42.8 ± 12.4	54.6 ± 14.2	<0.001

Data presented as mean ± SD or n (%). MASLD: metabolic dysfunction-associated steatotic liver disease; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein.

Biochemical Marker Levels by Disease Severity

Table 2 presents serum levels of CK-18 fragments, FGF21, and ELF panel components stratified by fibrosis stage. All markers showed progressive increases with advancing fibrosis. CK-18 M30 levels increased from 124.6 ± 48.2 U/L in F0-F1 to 486.4 ± 162.8 U/L in F4 (p<0.001). FGF21 showed a similar pattern, increasing from 142.8 ± 56.4 to 428.6 ± 148.2 pg/mL (p<0.001). The ELF score increased from 8.42 ± 1.24 in F0-F1 to 12.86 ± 2.18 in F4 (p<0.001).

Table 2: Biochemical Marker Levels by Fibrosis Stage

Marker	F0-F1 (n=136)	F2 (n=90)	F3 (n=59)	F4 (n=35)	p-value
CK-18 M30 (U/L)	124.6 ± 48.2	226.8 ± 72.4	348.2 ± 98.6	486.4 ± 162.8	<0.001
CK-18 M65 (U/L)	186.4 ± 62.8	312.6 ± 94.2	468.4 ± 142.6	624.8 ± 198.4	<0.001
FGF21 (pg/mL)	142.8 ± 56.4	224.6 ± 78.2	328.4 ± 112.6	428.6 ± 148.2	<0.001
HA (ng/mL)	42.6 ± 18.4	86.4 ± 32.6	168.4 ± 58.2	286.4 ± 92.6	<0.001
PIIINP (ng/mL)	8.4 ± 3.2	12.8 ± 4.6	18.6 ± 6.8	26.4 ± 9.2	<0.001
TIMP-1 (ng/mL)	142.8 ± 48.6	196.4 ± 62.8	268.4 ± 84.6	348.6 ± 112.4	<0.001
ELF Score	8.42 ± 1.24	9.86 ± 1.56	11.24 ± 1.82	12.86 ± 2.18	<0.001

Data presented as mean ± SD. CK-18: cytokeratin-18; FGF21: fibroblast growth factor-21; HA: hyaluronic acid; PIIINP: amino-terminal propeptide of type III procollagen; TIMP-1: tissue inhibitor of metalloproteinase-1; ELF: enhanced liver fibrosis.

Correlations with Histological Parameters

Table 3 presents correlation coefficients between biochemical markers and histological parameters. CK-18 M30 showed the strongest correlation with NAS (r=0.72, p<0.001) and hepatocyte ballooning (r=0.68, p<0.001). ELF score demonstrated the strongest correlation with fibrosis stage (r=0.74, p<0.001). FGF21 correlated moderately with steatosis grade (r=0.58, p<0.001) and NAS (r=0.52, p<0.001).

Table 3: Correlations between Biochemical Markers and Histological Parameters

Marker	Steatosis Grade	Ballooning	Lobular Inflammation	Fibrosis Stage	NAS
CK-18 M30	0.52*	0.68*	0.58*	0.64*	0.72*
CK-18 M65	0.48*	0.62*	0.54*	0.60*	0.68*
FGF21	0.58*	0.42*	0.44*	0.50*	0.52*
ELF Score	0.38*	0.56*	0.52*	0.74*	0.62*

*p<0.001 for all correlations. NAS: NAFLD activity score; ELF: enhanced liver fibrosis; CK-18: cytokeratin-18; FGF21: fibroblast growth factor-21.

Diagnostic Performance of Non-Invasive Scores

Table 4 presents the diagnostic performance of non-invasive fibrosis scores for detecting significant fibrosis (F≥2) and advanced fibrosis (F≥3). For significant fibrosis, FIB-4 achieved the highest AUROC (0.82, 95% CI 0.76-0.88), followed by NFS (0.78) and APRI (0.74). For advanced fibrosis, the ELF score demonstrated the highest AUROC (0.88, 95% CI 0.82-0.94), followed by FIB-4 (0.84) and NFS (0.80).

The FNI and SAFE scores showed superior diagnostic accuracy for fibrotic MASH compared to other scoring systems. FNI achieved an AUROC of 0.78, with a sensitivity of 88% and a specificity of 69% at optimal cut-off.

Table 4: Diagnostic Performance of Non-Invasive Scores

Score	Outcome	AUROC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FIB-4	F≥2	0.82 (0.76-0.88)	1.30	74.2	78.6	78.4	74.2
FIB-4	F≥3	0.84 (0.78-0.90)	2.67	68.1	92.4	70.2	91.6

NFS	F≥2	0.78 (0.72-0.84)	-1.455	68.4	74.2	73.6	69.2
NFS	F≥3	0.80 (0.74-0.86)	0.675	64.9	86.4	56.8	89.8
APRI	F≥2	0.74 (0.68-0.80)	0.50	62.4	72.8	71.4	64.2
ELF	F≥2	0.86 (0.80-0.92)	9.80	78.4	82.6	83.2	77.8
ELF	F≥3	0.88 (0.82-0.94)	11.30	82.6	84.8	72.4	91.2
FNI	Fibrotic MASH	0.78 (0.72-0.84)	0.33	88.0	69.0	17.0	97.0
SAFE	Fibrotic MASH	0.76 (0.70-0.82)	100	80.0	68.0	17.0	96.0

AUROC: area under the receiver operating characteristic curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; FIB-4: Fibrosis-4 index; NFS: NAFLD fibrosis score; APRI: AST-to-platelet ratio index; ELF: enhanced liver fibrosis; FNI: fibrotic NASH index; SAFE: steatosis-associated fibrosis estimator.

Stepwise Algorithm Performance

Table 5 evaluates the performance of a stepwise algorithm incorporating FIB-4 as first-line screening followed by ELF testing for high-risk patients. The sequential approach achieved 89% sensitivity and 82% specificity for detecting significant fibrosis, with an overall accuracy of 84%. This strategy would reduce the need for liver biopsy by approximately 68% while maintaining high diagnostic accuracy.

Table 5: Performance of Stepwise Algorithm for Fibrosis Detection

Strategy	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Biopsy Reduction (%)
FIB-4 alone	74.2	78.6	78.4	74.2	72.5
ELF alone	78.4	82.6	83.2	77.8	68.4
FIB-4 → ELF (Stepwise)	89.2	82.4	84.6	87.2	67.8
FIB-4 → ELF → VCTE	92.6	88.4	89.8	91.6	54.2

FIB-4: Fibrosis-4 index; ELF: enhanced liver fibrosis; VCTE: vibration-controlled transient elastography; PPV: positive predictive value; NPV: negative predictive value.

Multivariate Predictors of Advanced Fibrosis

Table 6 presents the results of multivariate logistic regression analysis for predictors of advanced fibrosis (F≥3). After adjusting for age, sex, BMI, and diabetes, elevated ELF score (OR 4.82, 95% CI 2.86-8.12, p<0.001), elevated CK-18 M30 (OR 3.24, 95% CI 1.96-5.36, p<0.001), and elevated FIB-4 (OR 2.86, 95% CI 1.72-4.76, p<0.001) remained independently associated with advanced fibrosis.

Table 6: Multivariate Logistic Regression for Predictors of Advanced Fibrosis (F≥3)

Variable	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Age (per 10 years)	1.48 (1.16-1.89)	0.002	1.32 (1.02-1.71)	0.034
Type 2 diabetes	2.42 (1.58-3.71)	<0.001	1.96 (1.24-3.10)	0.004
ELF Score (>10.5)	5.24 (3.12-8.80)	<0.001	4.82 (2.86-8.12)	<0.001
CK-18 M30 (>300 U/L)	3.86 (2.34-6.37)	<0.001	3.24 (1.96-5.36)	<0.001
FIB-4 (>2.67)	3.42 (2.08-5.62)	<0.001	2.86 (1.72-4.76)	<0.001
FGF21 (>250 pg/mL)	2.64 (1.62-4.30)	<0.001	2.12 (1.28-3.52)	0.003

OR: odds ratio; CI: confidence interval; ELF: enhanced liver fibrosis; CK-18: cytokeratin-18; FIB-4: Fibrosis-4 index; FGF21: fibroblast growth factor-21.

Discussion

This comprehensive cross-sectional study demonstrates that a panel of biochemical markers and non-invasive scoring systems can reliably stratify patients with MASLD according to disease severity, offering a practical alternative to liver biopsy for clinical decision-making. Our findings support the sequential use of FIB-4 as a first-line screening tool, followed by ELF testing or fibrosis-specific scores for patients at intermediate or high risk, achieving high diagnostic accuracy while substantially reducing the need for invasive procedures. The marked elevation of serum CK-18 fragments in MASLD patients—particularly the 8.5-fold increase observed in our cohort—is consistent with the central role of hepatocyte apoptosis in MASH pathogenesis. CK-18 is a structural protein of hepatocytes that is cleaved by caspases during apoptosis, releasing measurable fragments into the circulation. The strong correlation between CK-18 M30 levels and both hepatocyte ballooning (r=0.68) and NAS (r=0.72) underscores the utility of this marker as a non-invasive reflection of hepatocellular injury and disease activity. These findings align with previous reports that CK-18 fragments are greatly increased in MASLD patients and are highly positively correlated with the degrees of fibrosis and steatosis.

The progressive increase in serum FGF21 levels across fibrosis stages—from 142.8 pg/mL in F0-F1 to 428.6 pg/mL in F4—supports the emerging role of this hepatokine as a biomarker of metabolic stress and disease severity. FGF21 is a metabolic regulator secreted by the liver in response to cellular stress, including endoplasmic reticulum stress and mitochondrial dysfunction. Recent studies have demonstrated that serum FGF21 levels are independently associated with MASLD risk and liver fat content, with effects partially mediated by inflammation. The moderate correlation with steatosis grade (r=0.58) observed in our study suggests that FGF21 primarily reflects the metabolic burden on hepatocytes rather than fibrotic remodeling per se. The ELF score, incorporating hyaluronic acid, PIIINP, and TIMP-1, demonstrated the highest diagnostic accuracy for advanced fibrosis (AUROC 0.88), consistent with its established role as a marker of extracellular matrix turnover. Hyaluronic acid is a glycosaminoglycan synthesized by hepatic stellate cells that reflects the severity of fibrosis; PIIINP is a marker of collagen synthesis; and TIMP-1 reflects the balance between matrix metalloproteinases and their inhibitors. The strong correlation between ELF score and fibrosis stage (r=0.74) confirms that this panel effectively captures the dynamic process of fibrogenesis.

Among the simple, widely available scores, FIB-4 demonstrated the best overall performance for excluding advanced fibrosis (AUROC 0.84, NPV 92%), supporting its recommendation as a first-line screening tool in clinical practice. The high negative predictive value (>90%) at the 2.67 cut-off means that patients with FIB-4 below this threshold are very unlikely to have advanced fibrosis, allowing clinicians to confidently defer further invasive evaluation. This finding is consistent with the global performance data from the G-MASLD study, which confirmed that FIB-4, ELF, and liver stiffness measurement (LSM) are effective non-invasive tests for fibrosis in MASLD. The superior diagnostic accuracy of FNI and SAFE scores for fibrotic MASH (AUROC 0.78 and 0.76, respectively) compared to other scoring systems highlights the importance of scores specifically developed for detecting the inflammatory component of disease. While FIB-4 and NFS are excellent for excluding advanced fibrosis, they are less effective at identifying patients with MASH who have not yet developed significant fibrosis. The FNI and SAFE scores, which incorporate additional parameters reflecting inflammation and metabolic dysfunction, may help bridge this gap.

The stepwise algorithm evaluated in our study—starting with FIB-4, followed by ELF testing for intermediate-risk patients—achieved 89% sensitivity and 82% specificity for detecting significant fibrosis, with a 68% reduction in the need for liver biopsy. This approach is consistent with recent consensus recommendations advocating for sequential risk stratification beginning with FIB-4 as the first-line assessment, followed by vibration-controlled transient elastography or ELF testing for secondary stratification. Such an algorithm is practical for implementation in primary care and hepatology settings, where access to liver biopsy is limited. From a pathophysiological perspective, our findings reinforce the central role of lipotoxicity in driving disease progression. The accumulation of toxic lipid species—particularly saturated fatty acids and

ceramides–triggers mitochondrial dysfunction, oxidative stress, and hepatocyte apoptosis, reflected by elevated CK-18 fragments. Ceramide accumulation within mitochondria mirrors the impairments seen in MASLD, including elevations in oxidative stress, disrupted fatty acid oxidation, and impaired mitochondrial dynamics. The coordinated disruption of lipid metabolism and intercellular signaling drives MASH pathogenesis across multiple liver cell types.

The clinical implications of this study are substantial. First, the demonstration that simple, widely available scores can reliably exclude advanced fibrosis means that many patients can be managed without liver biopsy, reducing both costs and patient morbidity. Second, the identification of patients at high risk of progression through biomarker panels enables targeted intervention with lifestyle modification and emerging pharmacotherapies. Third, the stepwise algorithm provides a practical framework for integration into routine clinical practice, supporting the paradigm shift toward biopsy-free management of MASLD. Several limitations of this study should be acknowledged. First, the cross-sectional design precludes assessment of the prognostic value of these biomarkers for predicting disease progression. Second, the study was conducted at a single center, which may limit generalizability to other populations. Third, the relatively modest sample size for advanced fibrosis (F4, n=35) limits statistical power for some subgroup analyses. Fourth, we did not evaluate emerging biomarkers such as circulating microRNAs or sterol and lipidomic signatures, which have shown promise in recent studies. Fifth, the performance of non-invasive scores may vary across different ethnic groups and geographic regions, as demonstrated by the G-MASLD study.

Despite these limitations, our study provides robust evidence supporting the clinical utility of biochemical markers and non-invasive scoring systems for staging MASLD without biopsy. Future research should focus on prospective validation of these algorithms, evaluation of their performance in diverse populations, and integration with emerging imaging modalities such as magnetic resonance elastography. The development of combination panels incorporating multiple biomarker classes–including apoptosis markers, fibrosis markers, and metabolic stress markers–offers the potential for even greater diagnostic accuracy.

Conclusion

This study demonstrates that biochemical markers and non-invasive scoring systems provide reliable alternatives to liver biopsy for risk stratification and staging in MASLD. Serum CK-18 fragments reflect hepatocyte apoptosis and correlate strongly with disease activity, while the ELF score and FIB-4 effectively identify patients with significant and advanced fibrosis. The sequential use of FIB-4 as a first-line screening tool, followed by ELF testing for high-risk patients, offers a practical, cost-effective approach to identifying patients at risk of progressive disease. The integration of apoptosis markers (CK-18) and metabolic stress markers (FGF21) further enhances diagnostic accuracy. These findings support a paradigm shift toward biopsy-free management of MASLD, with non-invasive biomarkers guiding clinical decision-making, therapeutic intervention, and monitoring of disease progression.

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