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ORIGINAL RESEARCH

# Serum LEAP2 Levels Across the Spectrum of Metabolic Dysfunction-Associated Fatty Liver Disease: A Potential Noninvasive Biomarker for Severity Stratification

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**Purpose:** To evaluate circulating liver-expressed antimicrobial peptide 2 (LEAP2) as a potential noninvasive biomarker for the presence of metabolic dysfunction-associated fatty liver disease (MAFLD) and its progression to metabolic dysfunction-associated steatohepatitis (MASH).

**Patients and Methods:** This prospective observational study enrolled obese patients with MAFLD, categorized into simple steatosis (SS) or MASH based on liver histopathology, along with healthy controls (HC). Serum levels LEAP2 were quantified using enzyme-linked immunosorbent assay (ELISA). Baseline characteristics were compared among groups, followed by univariable and multivariate ordinary logistic regression to identify MAFLD predictors. The diagnostic performance of LEAP2 was evaluated through receiver operating characteristic (ROC) curve analysis. Additionally, Hepatic LEAP2 transcriptomic data from public Gene Expression Omnibus (GEO) datasets (GSE126848, GSE135251) were analyzed for validation.

**Results:** Seventy-four participants (24 HC, 24 SS, 26 MASH) were analyzed. Serum LEAP2 levels significantly and progressively increased with MAFLD severity (median ng/mL: HC 11.54, SS 13.62, MASH 18.34; P<0.001), correlating positively with disease stage (Spearman's $\rho$=0.526, P<0.001). This pattern was validated using hepatic LEAP2 transcript data from GEO datasets (P<0.001; Spearman's $\rho$=0.317, P<0.001). Multivariate logistic regression identified serum LEAP2 as an independent factor associated with MAFLD presence (OR=1.14, 95% CI 1.03–1.26; P=0.014), alongside BMI and ALT, while HDL was protective. ROC analysis demonstrated good diagnostic performance for distinguishing MASH from HC (AUC=0.86) and moderate performance for adjacent stages (HC vs SS, AUC=0.70; SS vs MASH, AUC=0.70).

**Conclusion:** Serum LEAP2 levels progressively increase with MAFLD severity and are independently associated with the disease. LEAP2 demonstrates potential as a noninvasive biomarker for assessing MAFLD severity, particularly in distinguishing MASH from healthy individuals. These findings warrant further investigation into LEAP2's pathophysiological role and therapeutic potential.

**Keywords:** LEAP2, MAFLD, MASH, hepatic steatosis, biomarker

## Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) has emerged as a substantial global health burden, affecting approximately 25% of adults worldwide.[1] Its prevalence is increasing rapidly, especially in developing regions

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like China, where rising rates of obesity and metabolic disorders reflect similar trends in Western countries.[2] In China, a recent large-scale study of 22,970 participants undergoing routine health evaluations revealed that MAFLD affects 28.77% of the urban Chinese population, with 16.87% of MAFLD patients presenting with significant liver fibrosis, highlighting the substantial disease burden in Chinese communities.[3] Metabolic dysfunction-associated steatohepatitis (MASH) is the more severe form of MAFLD, which is characterized by progressive liver inflammation and is one of the most common chronic liver diseases globally.[4] MASH progression is closely associated with metabolic comorbidities including obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension.[5] Pathologically, MASH advances from initial hepatic steatosis, driven by fatty acid accumulation, to encompass hepatocyte injury, immune cell recruitment, chronic inflammation, and progressive liver fibrosis.[6] Without effective intervention, MASH can progress to cirrhosis and liver cancer. This progression results in annual all-cause and liver-specific mortality rates of 25.6 and 11.8 per 1000 individuals, respectively.[1] These considerable burdens underscore the urgent need for reliable biomarkers to enable better risk stratification, effective monitoring, and ultimately, improved therapeutic strategies for MAFLD.

Liver-expressed antimicrobial peptide 2 (LEAP2), originally identified as an antimicrobial peptide, has more recently been recognized as an endogenous antagonist of the growth hormone secretagogue receptor 1a (GHS-R1a) or ghrelin receptor.[7] By inhibiting ghrelin's action, LEAP2 counters its effects on appetite regulation and hormonal secretion pathways.[8] Circulating LEAP2 levels rise during caloric surplus and decreasing during energy deficit, opposite to ghrelin's dynamics.[9] Beyond metabolic regulation, LEAP2 modulates immune function through monocyte/macrophage behavior[10] and antimicrobial effects that inhibits microbial proliferation and inflammation.[11] These dual metabolic and immune roles make LEAP2 a promising biomarker candidate for MAFLD, but further investigation into whether its expression levels are clinically informative is required.

Despite the strong biological rationale of LEAP2's involvement in MAFLD, research in this area remains limited. Previous studies have mainly focused on the role of ghrelin in liver disease. For instance, Silveira et al demonstrated that bariatric surgery modulates ghrelin levels, significantly influences hepatic lipid metabolism, inflammation and fibrosis in obese rats.[12] This finding is supported by clinical data from Lassailly et al, which showed approximately 80% sustained MASH remission at 5 years after bariatric surgery.[13] While these findings underscore the relevance of ghrelin in MAFLD pathophysiology, the potential diagnostic and prognostic value of LEAP2 remains largely unexplored.

One of the major challenges in the management of MAFLD is the noninvasive differentiation between non-progressive simple steatosis (SS) and the more severe inflammatory stage of MASH. This distinction is essential for accurate disease staging, risk stratification, and therapeutic decision-making. Current noninvasive biomarkers lack sufficient specificity to reliably distinguish these conditions without liver biopsy. Although previous studies have investigated biomarkers including FIB-4, enhanced liver fibrosis (ELF), PRO-C3, and vibration controlled transient elastography (VCTE) for fibrosis staging, their potential for differentiating inflammatory activity between SS and MASH remains insufficiently explored.[14] Given LEAP2's biological functions in both the metabolic and inflammatory pathways, we hypothesized that it may serve as a valuable biomarker for the classification of MAFLD severity.

Thus, we aimed to determine whether LEAP2 levels differ across the MAFLD spectrum. Through a comprehensive analysis of clinical samples and validation in publicly available datasets, we seek to establish whether LEAP2 expression patterns can effectively stratify MAFLD severity and potentially reduce reliance on invasive diagnostic procedures.

# Materials and Methods

## Study Design and Participants

This prospective, observational study recruited consecutive obesity patients scheduled for bariatric surgery at the Surgical Centre of Diabetes Mellitus, Capital Medical University Affiliated Beijing Shijitan Hospital, between August 2023 and November 2024. Healthy volunteers were recruited from the Physical Examination Center during the same period. Participants were classified into three groups: HC, SS, and MASH. After the initial enrollment, propensity score matching (PSM) was performed to balance age and gender among three groups with different disease severities. With the MASH group as the reference, nearest neighbor matching was performed at a 1:1:1 ratio to create balanced groups for the final analysis. All patients and healthy controls provided written informed consent before enrollment. This study was

approved by the Ethics Committee of Beijing Shijitan Hospital, Capital Medical University (No. SJTKY11-1X-2022-(098)), and was conducted in accordance with the Declaration of Helsinki.

## Inclusion and Exclusion Criteria

Patients with obesity were evaluated for study inclusion if they met the diagnostic criteria for metabolic syndrome based on international consensus guidelines.[15] These patients subsequently underwent liver biopsy during bariatric surgery to confirm MAFLD diagnosis and determine the disease severity (SS or MASH). Healthy controls were defined as individuals without any risk factors in the diagnostic criteria of metabolic syndrome and with no evidence of fatty liver on abdominal ultrasound. Participants were excluded for any malignancy, other liver diseases (eg autoimmune liver disease, alcohol-associated liver disease, and viral hepatitis), tuberculosis or HIV infection, use of steatogenic medications within the prior 6 months, or any condition deemed unsuitable by investigators.

## Sample Collection and Histological Analysis

Intraoperative liver tissue and peripheral blood were collected from MAFLD patients; HC blood samples were obtained during routine exams. Blood was processed within 2h (centrifuged at 3000g for 15 min), and resulting serum was aliquoted and stored at −80°C. Liver tissues were formalin-fixed, paraffin-embedded (FFPE), sectioned at 5μm, and stained with H&E. Two experienced pathologists independently reviewed and scored all slides according to the Nonalcoholic Fatty Liver Disease Activity Score (NAS) criteria. The NAS evaluates steatosis (0–3), lobular inflammation (0–3), and hepatocyte ballooning (0–2), with a total possible score of 0–8. Any diagnostic discrepancies were adjudicated by a third senior pathologist with over 10 years of experience in hepatopathology. Based on histological assessment, patients with NAS scores of 0–2 were classified as simple steatosis (SS), whereas those with scores of ≥4 were classified as metabolic dysfunction-associated steatohepatitis (MASH) only when hepatocyte ballooning and lobular inflammation were present according to Brunt criteria. Cases with NAS=3 were evaluated for the presence of hepatocyte ballooning to determine final classification.

## Measurement of Serum LEAP2 Levels

Serum LEAP2 concentrations were quantified using a commercial human LEAP2 ELISA kit (Phoenix Pharmaceuticals, Inc., CA, USA; Cat# EK-075-40) following the manufacturer's protocol. Briefly, 50 μL of samples or standards were incubated with primary antibody and biotinylated peptide for 2 hours at room temperature. After washing steps, wells were incubated with Streptavidin-Horseradish Peroxidase (SA-HRP) solution for 1 hour. Following further washes, TMB substrate was added and incubated for 1 hour before the reaction was terminated with 2N HCl stop solution. Optical density was measured spectrophotometrically at 450 nm, and concentrations were determined by interpolation against a standard curve.

## Statistical Analysis

All statistical analyses were conducted using Python 3.9.6 (Python Software Foundation, Wilmington, DE, USA) and R version 4.4.3 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were summarized as mean ± standard deviation (SD) for normally distributed data and median [interquartile range (IQR)] for non-normally distributed data. Categorical variables were reported as frequencies (n) and percentages (%). Categorical variables were compared using the chi-square test. Continuous variables were compared between two groups using Student's *t*-test (normal distribution) or Mann–Whitney *U*-test (non-normal distribution), and across more than two groups using ANOVA (normal distribution) or Kruskal-Walli's test (non-normal distribution). Univariate and multivariate analyses were performed via ordinary logistic regression, with significant factors from univariate analysis included in the multivariate model following stepwise selection and assessment of clinical relevance. Receiver operating characteristic (ROC) curve analysis was conducted to determine the area under the curve (AUC). Statistical significance was set at $P < 0.05$.

## External Dataset Validation

The external datasets (GSE126848 and GSE135251) were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The raw data underwent standardization using the preprocessCore package in R, followed by batch effect correction employing the limma package (version 3.62.2). LEAP2 expression profiles were then extracted from each experimental group for subsequent intergroup comparative analysis and receiver operating characteristic (ROC) curve evaluation.

# Results

## Baseline Characteristics

A total of 106 individuals with obesity and 200 healthy controls were initially screened. Of these, 74 participants met the eligibility criteria and were included in the final analysis (Figure 1). Participants were categorized into three groups: the MASH group (N=26, 35.1%), the SS group (N=24, 32.4%), and the HC group (N=24, 32.4%). Baseline demographic, clinical, and laboratory characteristics are presented in Table 1. Significant differences among the three groups were observed in body mass index (BMI), serum LEAP2 levels, fasting blood glucose (FBG), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol ($P<0.05$ for all). As expected, median BMI was significantly higher in the MASH (39.97 kg/m² [IQR, 38.86–46.42]) and SS (40.44 kg/m² [IQR, 37.02–46.17]) groups compared to the HC group (19.37 kg/m² [IQR, 18.78,20.80]; $P<0.001$). Regarding liver function indicators, the MASH group also exhibited higher median levels of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST) compared to the SS and HC groups. No significant differences ($P>0.05$) were observed among the groups for gender distribution, age, urea, total cholesterol

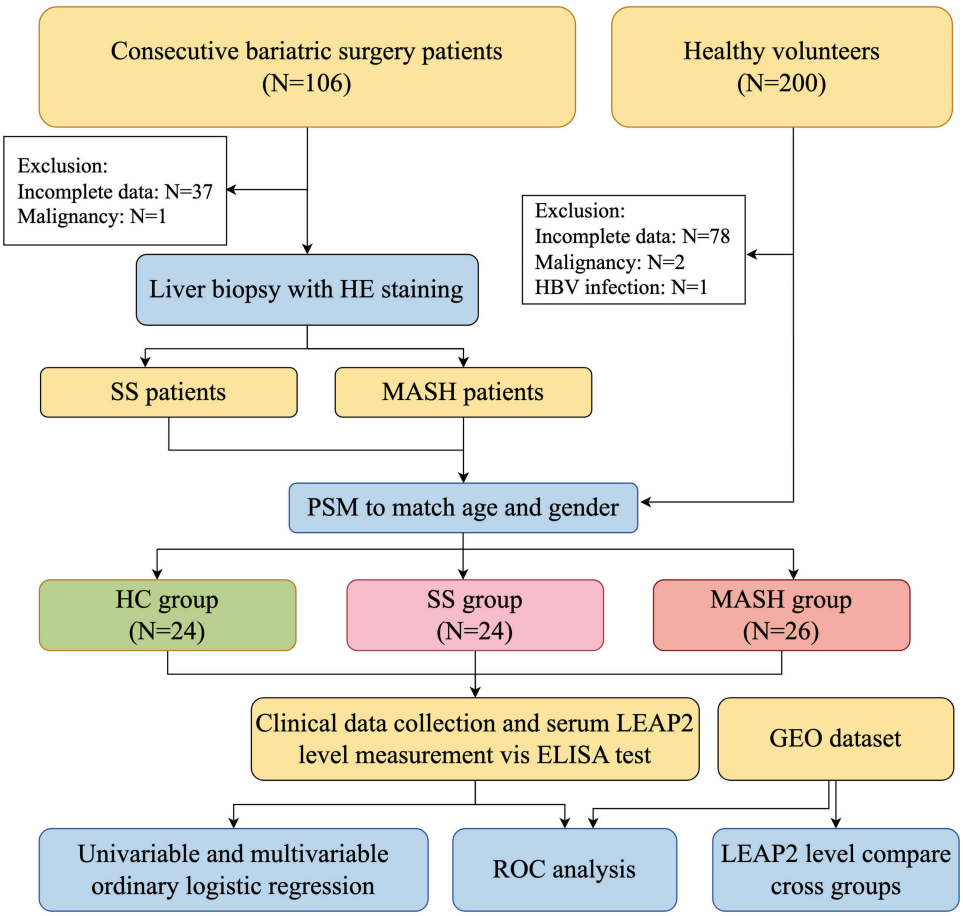


**Figure 1** Study Design Flowchart.

**Table 1** Clinical and Biochemical Characteristics of MASH, SS Patients and Healthy Controls

| Characteristics | Total (N = 74) | MASH (N = 26) | SS (N = 24) | HC (N = 24) | *P value* |
|---|---|---|---|---|---|
| **Basic Characteristics** | | | | | |
| Gender (Male), N (%) | 21 (28.4%) | 8 (30.8%) | 6 (25.0%) | 7 (29.2%) | 0.898 |
| Age, median (IQR) | 31.00 (24.25, 35.00) | 27.00 (24.00,34.00) | 29.50 (25.50,35.25) | 33.00 (29.25,36.00) | 0.369 |
| BMI, median (IQR) | 37.51 (20.92, 41.67) | 39.97 (38.86,46.42) | 40.44 (37.02,46.17) | 19.37 (18.78,20.80) | <0.001 |
| **Metabolic Parameters** | | | | | |
| LEAP2, Mean ± SD | 15.63 ± 6.62 | 19.46 ± 6.68 | 15.57 ± 6.71 | 11.54 ± 3.43 | <0.001 |
| FBG, median (IQR) | 5.17 (4.65, 6.16) | 6.12 (5.54,8.22) | 5.52 (4.98,6.12) | 4.51 (4.32,4.69) | <0.001 |
| HbA1c, median (IQR) | 5.95 (5.60, 6.97) | 6.45 (5.70,7.92) | 5.60 (5.57,6.03) | NA | 0.005 |
| GLO, median (IQR) | 30.50 (27.52, 33.08) | 31.50 (30.22,35.00) | 31.20 (30.25,33.20) | 26.90 (26.05,27.90) | <0.001 |
| **Lipid Profile** | | | | | |
| LDL, Mean ± SD | 2.99 ± 0.78 | 3.26 ± 0.88 | 3.12 ± 0.69 | 2.57 ± 0.58 | 0.004 |
| HDL, Mean ± SD | 1.27 ± 0.35 | 1.05 ± 0.27 | 1.17 ± 0.20 | 1.60 ± 0.32 | <0.001 |
| TG, median (IQR) | 1.14 (0.90, 1.66) | 1.52 (1.03,3.36) | 1.44 (1.01,1.66) | 0.91 (0.71,1.04) | <0.001 |
| TC, median (IQR) | 4.54 (4.09, 5.07) | 4.72 (4.13,5.50) | 4.62 (4.11,4.98) | 4.39 (4.03,4.64) | 0.097 |
| **Liver Function** | | | | | |
| ALT, median (IQR) | 25.50 (16.00, 44.00) | 42.00 (35.00,65.00) | 29.50 (20.00,42.00) | 12.50 (9.00,18.00) | <0.001 |
| AST, median (IQR) | 22.00 (18.00, 29.75) | 30.00 (20.25,34.50) | 21.00 (18.00,26.75) | 20.50 (18.00,23.00) | 0.004 |
| GGT, median (IQR) | 27.00 (15.25, 38.75) | 36.50 (25.50,70.75) | 35.00 (23.25,42.25) | 13.50 (10.00,18.25) | <0.001 |
| ALP, Mean ± SD | 68.53 ± 19.62 | 71.81 ± 23.21 | 73.33 ± 16.67 | 60.17 ± 15.78 | 0.036 |
| **Other Biomarkers** | | | | | |
| UA, Mean ± SD | 365.25 ± 129.05 | 390.59 ± 136.34 | 412.82 ± 137.77 | 290.23 ± 68.63 | 0.001 |
| CK, Mean ± SD | 102.08 ± 54.81 | 125.27 ± 70.83 | 97.12 ± 44.42 | 81.92 ± 32.41 | 0.015 |
| LDH, Mean ± SD | 187.65 ± 42.22 | 210.96 ± 56.77 | 185.42 ± 27.00 | 164.62 ± 15.93 | <0.001 |
| eGFR, median (IQR) | 119.00 (111.39, 127.50) | 124.50 (116.00,130.00) | 119.00 (116.00,128.25) | 113.79 (107.38,119.20) | 0.003 |
| **Others** | | | | | |
| UREA, median (IQR) | 4.52 (3.92, 5.45) | 4.67 (3.80,5.56) | 4.71 (4.09,5.52) | 4.38 (4.03,4.78) | 0.724 |
| Cr, median (IQR) | 58.50 (51.25, 64.75) | 55.00 (50.00,62.00) | 57.00 (49.75,62.25) | 61.50 (55.75,74.50) | 0.017 |
| TP, median (IQR) | 74.55 (71.50, 76.18) | 75.70 (71.95,77.75) | 73.80 (71.40,75.25) | 74.70 (71.30,76.30) | 0.393 |
| DBIL, median (IQR) | 3.40 (2.70, 4.70) | 3.50 (3.08,4.88) | 3.40 (3.05,4.33) | 3.05 (2.45,4.70) | 0.547 |
| TBIL, median (IQR) | 10.80 (8.22, 14.15) | 10.20 (8.27,14.62) | 12.10 (9.20,13.33) | 10.75 (8.02,14.72) | 0.849 |
| IBIL, median (IQR) | 7.55 (5.43, 9.65) | 7.05 (5.05,9.72) | 8.25 (6.55,8.90) | 7.60 (5.38,9.83) | 0.791 |

**Abbreviations**: SD, Standard Deviation; IQR, Interquartile Range; HbA1c, Glycated Hemoglobin A1c; GLO, Globulin; TG, Triglyceride; ALP, Alkaline Phosphatase; UA, Uric Acid; CK, Creatine Kinase; LDH, Lactate Dehydrogenase; eGFR, estimated Glomerular Filtration Rate; Cr, Creatinine; TP, Total Protein; DBIL, Direct Bilirubin; TBIL, Total Bilirubin; IBIL, Indirect Bilirubin.

(TC), total protein (TP), or bilirubin levels (total, direct, and indirect). Overall, the three groups demonstrated distinct clinical and biochemical profiles across metabolic, hepatic function, and LEAP2 levels.

## Serum and Hepatic LEAP2 Levels Across Groups

Serum LEAP2 concentrations progressively increased across the disease spectrum from HC to patients with SS and MASH (P<0.001; Figure 2A). Median levels were lowest in the HC group (11.54 ng/mL [IQR, 4.92–19.69]), intermediate in the SS group (13.62 ng/mL [IQR, 8.13–36.54]), and highest in the MASH group (18.34 ng/mL [IQR, 9.71–34.21]). All pairwise comparisons between the groups were statistically significant (HC vs SS, P=0.02; SS vs MASH, P=0.018; HC vs MASH, P<0.001). While LEAP2 concentrations were relatively homogeneous within the HC group, notable heterogeneity was observed in both the SS and MASH groups.

A similar pattern was observed for hepatic LEAP2 expression, analyzed using available Gene Expression Omnibus (GEO) datasets (GSE126848 and GSE135251; HC: N=36, SS: N=66, MASH: N=171; Figure 2B). Hepatic LEAP2 transcript levels differed significantly across the three groups (overall P<0.001), mirroring the serum findings. Pairwise comparisons revealed the lowest expression in healthy controls (vs SS, P<0.001; vs MASH, P<0.001), intermediate

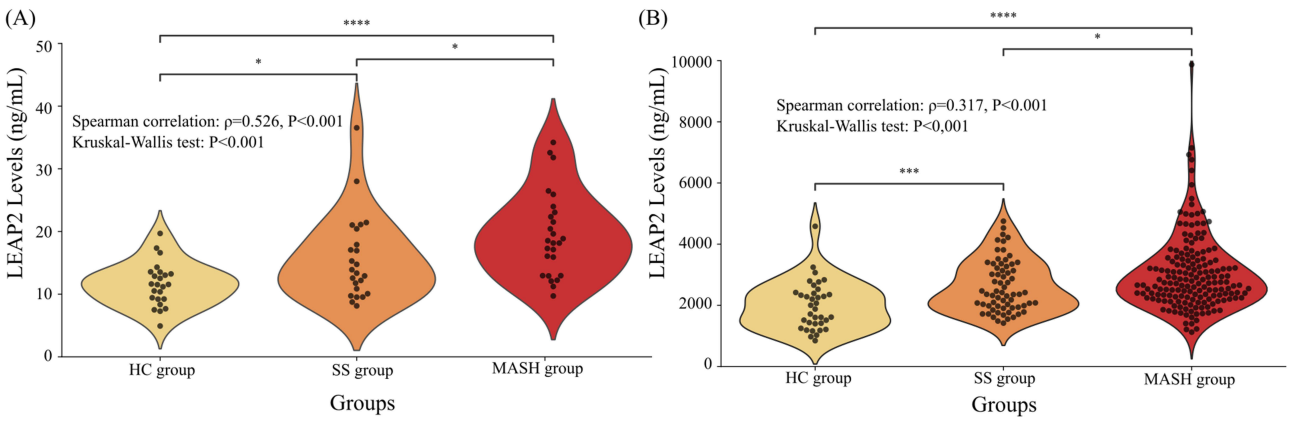


**Figure 2** Serum and hepatic LEAP2 levels across MAFLD stages. Comparison of LEAP2 levels across study groups. (**A**) Serum LEAP2 concentrations (ng/mL) by ELISA in the clinical cohort (HC N=24, SS N=24, MASH N=26). (**B**) Hepatic LEAP2 transcript levels from GEO datasets GSE126848 and GSE135251 (HC N=36, SS N=66, MASH N=171). Overall differences between groups were assessed by the Kruskal-Walli's test (P<0.001 for both (**A**) and (**B**)). Significance levels: *P<0.05; ***P<0.001; ****P<0.0001.

expression in the SS group, and the highest expression in the MASH group (SS vs MASH, P=0.016), suggesting a progressive increase in hepatic LEAP2 expression correlated with liver disease severity.

Spearman correlation analyses were performed to quantify the association between LEAP2 and disease severity. These results confirmed significant positive correlations between LEAP2 levels and the stage of liver disease for both serum concentrations (Spearman's ρ=0.526, P<0.001; Figure 2A) and hepatic transcript levels (Spearman's ρ=0.317, P<0.001; Figure 2B). These findings suggest a stepwise elevation of LEAP2 levels along the spectrum of metabolic-associated fatty liver disease.

## Logistic Regression Analysis of Related Factors

Univariate logistic regression analysis revealed several factors significantly associated with the development of MAFLD (all P < 0.05, Table 2), notably including LEAP2, BMI, ALT, FBG, and markers of lipid metabolism (eg, HDL, LDL, and TG). In contrast, gender (P = 0.887), age (P = 0.476), albumin (P = 0.397), and total bilirubin (P = 0.446) were not statistically significantly associated. Odds ratios (ORs) and 95% confidence intervals (CIs) for all assessed factors are presented visually in the forest plot (Figure 3).

In the multivariate logistic regression model adjusted for potential confounders, several factors remained independently associated with the MAFLD status. Specifically, higher levels of LEAP2 (OR=1.14 [95% CI, 1.03–1.26]; P=0.014), BMI (OR=1.12 [95% CI, 1.04–1.21]; P=0.004), and ALT (OR=1.06 [95% CI, 1.01–1.11]; P=0.013) were associated with significantly increased odds of having MAFLD (Table 3). Conversely, higher HDL cholesterol levels demonstrated a strong independent protective association, correlating with substantially reduced odds of MAFLD (OR=0.01 [95% CI, 0.001–0.14]; P<0.001). To further support these independent associations, adjusted partial correlations of clinical features are presented in Figure S1. These findings highlight LEAP2 as a significant factor independently associated with the MAFLD disease spectrum.

## Diagnostic Performance of LEAP2 in Clinical Cohort

The diagnostic performance of serum LEAP2 levels (clinical cohort; Figure 4) and hepatic LEAP2 transcript levels (external GEO datasets GSE126848 and GSE135251; Figure 5) were evaluated using ROC curve analysis. In the clinical cohort, serum LEAP2 demonstrated the highest pairwise discriminatory accuracy between HC and MASH (AUC=0.86, 95% CI: 0.74–0.95), with moderate accuracy for HC vs SS (AUC=0.70, 95% CI: 0.53–0.84) and SS vs MASH (AUC=0.70, 95% CI: 0.54–0.83) (Figure 4A). One-vs-rest analysis showed good discrimination between HC (AUC=0.78, 95% CI: 0.66–0.88) and MASH (AUC=0.78, 95% CI: 0.72–0.84) but poor performance for SS (AUC=0.49, 95% CI: 0.37–0.65) (Figure 4B). The optimal LEAP2 threshold of 14.3 ng/mL for distinguishing healthy

**Table 2** Univariate Logistic Regression Analysis of Factors Associated with MAFLD

| Characteristics | Coefficient | β | OR with 95% CI | *P value* |
|---|---|---|---|---|
| **Demographics** | | | | |
| Gender | 0.068 | 0.031 | 1.07(0.419–2.733) | 0.887 |
| Age | −0.019 | −0.156 | 0.982(0.933–1.033) | 0.476 |
| BMI | 0.191 | 2.362 | 1.21(1.135–1.290) | <0.001 |
| **Metabolic Parameters** | | | | |
| LEAP2 | 0.171 | 1.124 | 1.186(1.088–1.294) | <0.001 |
| FBG | 0.892 | 2.193 | 2.441(1.559–3.821) | <0.001 |
| GLO | 0.260 | 1.105 | 1.297(1.136–1.480) | <0.001 |
| **Lipid Profile** | | | | |
| LDL | 0.956 | 0.739 | 2.6(1.388–4.871) | 0.003 |
| HDL | −5.551 | −1.946 | 0.004(0.000–0.033) | <0.001 |
| TG | 1.695 | 3.125 | 5.444(2.281–12.996) | <0.001 |
| TC | 0.649 | 0.704 | 1.914(1.138–3.219) | 0.014 |
| **Liver Function** | | | | |
| ALT | 0.099 | 3.970 | 1.104(1.061–1.148) | <0.001 |
| AST | 0.104 | 1.201 | 1.11(1.041–1.183) | 0.001 |
| GGT | 0.054 | 1.750 | 1.056(1.026–1.086) | <0.001 |
| ALP | 0.026 | 0.500 | 1.026(1.002–1.051) | 0.037 |
| **Other Biomarkers** | | | | |
| UA | 0.005 | 0.596 | 1.005(1.001–1.008) | 0.011 |
| CK | 0.013 | 0.732 | 1.014(1.004–1.024) | 0.007 |
| LDH | 0.033 | 1.371 | 1.033(1.016–1.051) | <0.001 |
| eGFR | 0.010 | 0.212 | 1.01(0.987–1.034) | 0.387 |
| **Others** | | | | |
| UREA | 0.073 | 0.074 | 1.075(0.710–1.629) | 0.731 |
| Cr | −0.061 | −0.769 | 0.941(0.905–0.979) | 0.002 |
| TP | 0.031 | 0.141 | 1.031(0.941–1.129) | 0.51 |
| DBIL | 0.189 | 0.371 | 1.208(0.936–1.558) | 0.146 |
| TBIL | 0.034 | 0.172 | 1.035(0.948–1.130) | 0.446 |
| IBIL | 0.015 | 0.053 | 1.015(0.897–1.149) | 0.816 |

**Abbreviations**: OR: Odds Ratio; CI: Confidence Interval.

controls from MASH patients demonstrates excellent clinical utility with high specificity (88%) and reasonable sensitivity (73%), making it suitable as a rule-in diagnostic test where values ≤14.3 ng/mL strongly suggest MASH. Additionally, the threshold of 15.9 ng/mL for MASH vs rest analysis showed balanced performance (sensitivity 73%, specificity 75%), while the threshold of 13.6 ng/mL for HC vs SS provided high specificity (83%) but limited sensitivity (50%). However, LEAP2 shows poor discriminatory ability for SS detection (optimal threshold 8.1 ng/mL with specificity of only 8%), limiting its clinical utility to MASH-specific applications rather than general fatty liver disease screening (Table S1). Validation using hepatic LEAP2 transcript levels from the GEO datasets revealed a similar pattern of diagnostic performance (Figure 5). The pairwise analysis again showed the highest accuracy between HC and MASH (AUC=0.79, 95% CI: 0.70–0.86), followed by HC vs SS (AUC=0.72, 95% CI: 0.59–0.81), and SS vs MASH (AUC=0.60, 95% CI: 0.52–0.68). Likewise, the one-vs.-rest analysis confirmed poor discrimination for SS (AUC=0.45, 95% CI: 0.47–0.62), while showing moderate-to-good performance for HC (AUC=0.77, 95% CI: 0.68–0.84) and MASH (AUC=0.67, 95% CI: 0.57–0.80) (Table S2).

The consistent diagnostic performance patterns observed for both serum LEAP2 in our cohort and hepatic LEAP2 transcripts in the external datasets strengthened the association of LEAP2 with MAFLD progression. Both analyses indicated that the highest diagnostic utility was for differentiating MASH from HC, with moderate performance between adjacent disease stages. This supports the potential role of LEAP2, particularly its circulating levels, as a relevant biomarker.

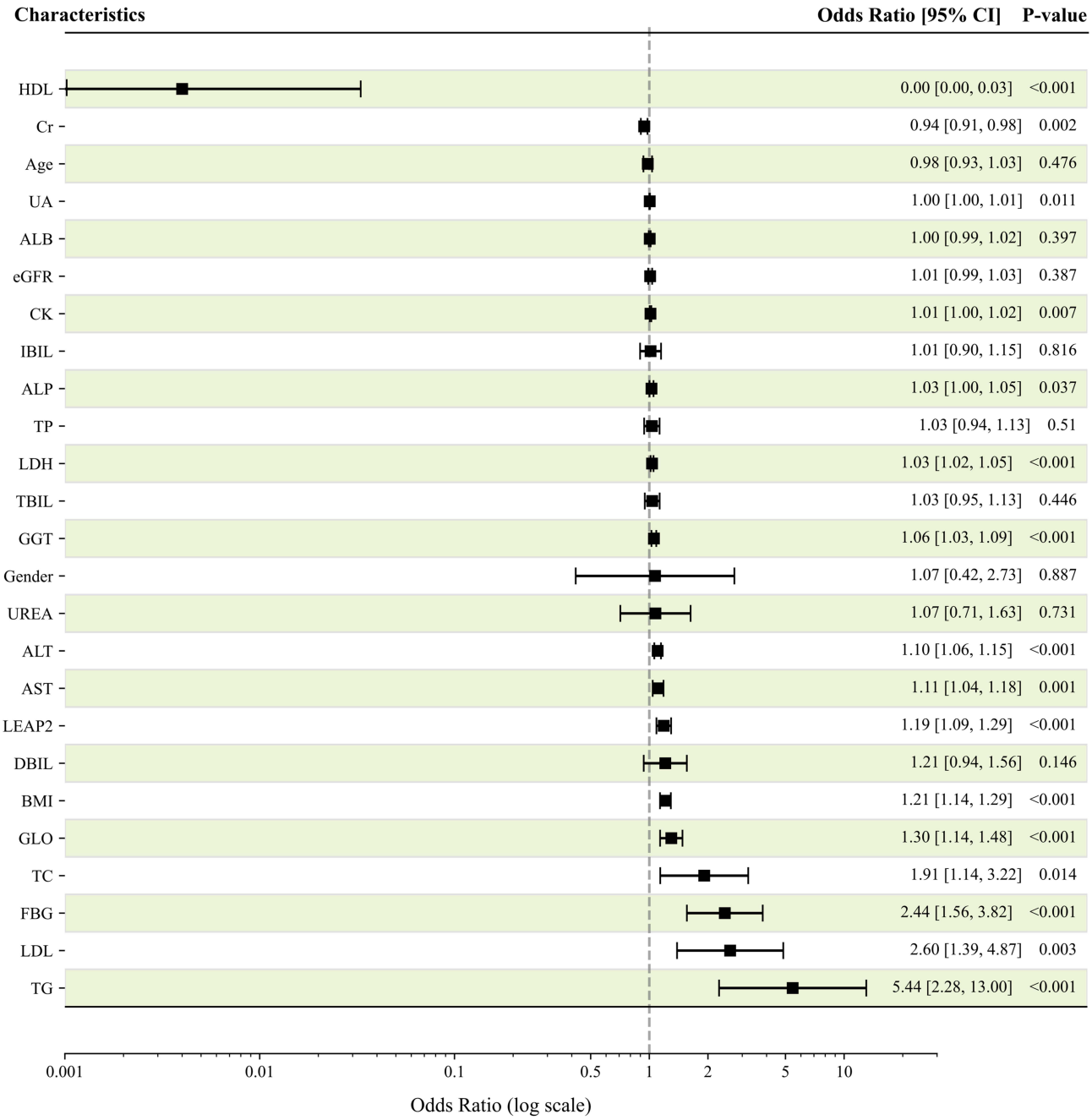


**Figure 3** Forest plot of univariate logistic regression analysis for factors associated with MAFLD. Forest plot illustrating the association between baseline characteristics and the presence of MAFLD based on univariate binary logistic regression analyses.

## Discussion

This study investigated the relationship between LEAP2 and MAFLD, and assessed its association with disease presence, severity, and diagnostic potential. Our results demonstrated a significant positive association between elevated circulating LEAP2 levels and both the presence and severity of MAFLD. Serum LEAP2 progressively elevated across the HC, SS, and MASH stages, correlating with the severity. This pattern corroborated by parallel findings for hepatic LEAP2 transcripts in external GEO datasets. Importantly, multivariate analysis confirmed serum LEAP2 as an independent risk factor for MAFLD status, alongside established factors like BMI and ALT, while HDL remained independently protective. Furthermore, the ROC analysis demonstrated the potential of serum LEAP2 as a biomarker for MAFLD. It effectively distinguished MASH from HC (AUC 0.86), although performance was moderate for differentiating adjacent

**Table 3** Multivariate Logistic Regression Analysis of Independent Risk Factors for MAFLD

| Characteristics | Coefficient | β | OR (95% CI) | P-value |
|---|---|---|---|---|
| LEAP2 | 0.128672 | 0.846660 | 1.137 (1.026–1.260) | 0.014 |
| BMI | 0.111705 | 1.384300 | 1.118 (1.036–1.207) | 0.004 |
| ALT | 0.059835 | 2.403156 | 1.062 (1.013–1.113) | 0.013 |
| HDL | −4.665723 | −1.636036 | 0.009 (0.001–0.136) | <0.001 |

stages (HC vs SS, AUC 0.70; SS vs MASH, AUC 0.70). Collectively, these findings established LEAP2 as an independent factor linked to the presence and severity of MAFLD.

The confirmation of elevated BMI and ALT levels as risk factors, and HDL cholesterol as a protective factor, for MAFLD is consistent with extensive previous research linking these markers to metabolic health and liver disease pathogenesis.[16–18] Focusing on LEAP2, this study offers additional insights into the current understanding. The correlation observed between elevated LEAP2 levels and MAFLD severity aligns with the clinical findings of Ma et al, who reported lower serum LEAP2 levels in HC relative to SS patients; their complementary animal studies demonstrated that LEAP2 knockout mitigated hepatic steatosis in mice.[19] However, previous clinical comparisons between SS and MASH stages were lacking. In contrast, our results demonstrated a significant stepwise elevation in serum LEAP2 concentrations across the full disease spectrum (HC < SS < MASH). This graded relationship mirrors MAFLD progression, particularly the development of steatohepatitis and fibrosis characteristic of MASH advancement from SS.[20]

Furthermore, accumulating evidence implicates LEAP2 in hepatic fibrosis. Silvia et al observed that hepatic LEAP2 expression is upregulated in SS and correlates with profibrotic factors (eg, TGF-β1, COL1α1),[21] and Liu et al found that circulating LEAP2 levels correlate positively with the FIB-4 fibrosis index.[22] A preclinical study also suggested therapeutic administration of exogenous LEAP2 analogs may reduce liver fibrosis,[23] indicating potential complexities or context-dependent actions of endogenous versus exogenous LEAP2 pathways. Our findings, integrated with existing literature, strongly suggest LEAP2 is involved not only in hepatic fat accumulation but also in the inflammatory and fibrotic processes that drive the progression of MAFLD to MASH.

The circulating LEAP2 levels observed in our cohort correlated with MAFLD severity, suggesting that LEAP2 may promote MAFLD progression by antagonizing ghrelin's effects on hepatic lipid metabolism, fibrosis, and inflammation.[9,24] Mechanistically, LEAP2 exhibits dual effects on lipid metabolism. On one hand, LEAP2 can compete with ghrelin to reduce GH secretion, thereby inhibiting GH's lipolytic function and suppressing fatty acid synthesis.

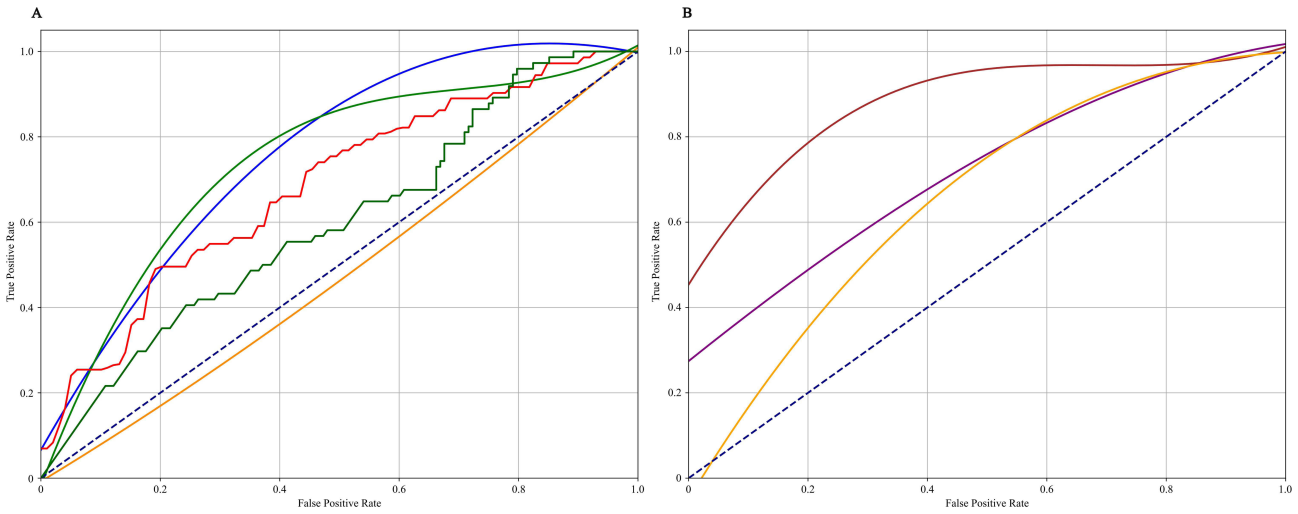


**Figure 4** Diagnostic performance of serum LEAP2 for differentiating MAFLD stages in the clinical cohort. Diagnostic performance of serum LEAP2 levels in the clinical cohort. (**A**) One-versus-rest classification analyses: HC vs Rest (blue line, AUC = 0.78), SS vs Rest (Orange line, AUC = 0.49), and MASH vs Rest (red line, AUC = 0.78). Multi-class performance is summarized by macro-average ROC (green line, AUC = 0.68) and micro-average ROC (dark green line, AUC = 0.60). (**B**) Direct pairwise comparisons: HC vs SS (red line, AUC = 0.70), HC vs MASH (purple line, AUC = 0.86), and SS vs MASH (orange line, AUC = 0.70).


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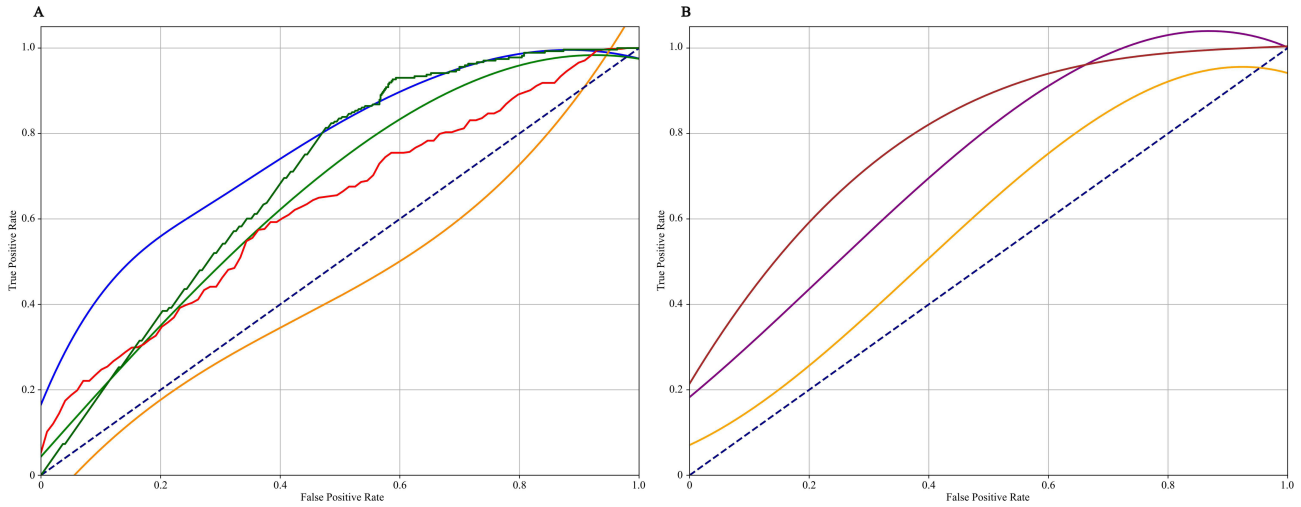


**Figure 5** Diagnostic performance of hepatic LEAP2 transcript levels for differentiating MAFLD stages using external GEO datasets. Diagnostic performance of hepatic LEAP2 transcript levels in the GEO datasets. (**A**) One-versus-rest classification analyses: HC vs Rest (blue line, AUC = 0.77), SS vs Rest (Orange line, AUC = 0.45), and MASH vs Rest (red line, AUC = 0.67). Multi-class performance is summarized by macro-average ROC (green line, AUC = 0.63) and micro-average ROC (dark green line, AUC = 0.70). (**B**) Direct pairwise comparisons: HC vs SS (red line, AUC = 0.72), HC vs MASH (purple line, AUC = 0.79), and SS vs MASH (orange line, AUC = 0.60).

Animal experiments have demonstrated that LEAP2 knockout can alleviate hepatic steatosis by reducing GH levels.[9,25]. Conversely, other studies indicate that LEAP2 can diminish ghrelin's effects on Gαs-cAMP-PKA and mTOR-PPARγ signaling pathways, consequently influencing feeding behavior and reducing hepatocyte lipogenesis and storage.[9,26,27] Furthermore, LEAP2's antagonism of ghrelin may promote hepatic fibrosis through multiple pathways. Specifically, ghrelin can regulate hepatic TGF-β1 and other profibrotic factors via the PI3K/Akt/mTOR signaling axis and its downstream pathways,[28] while also limiting HSC fibrotic characteristics and hepatic collagen deposition.[21] Similarly, LEAP2 may exacerbate hepatic inflammation by inhibiting ghrelin's protective effects. Indeed, animal experiments demonstrate that ghrelin can reduce oxidative stress and inflammation in rat hepatocytes by limiting the secretion of proinflammatory factors including IL-2, IL-6, IL-1β, TNF-α, and INF-γ,[29] thereby protecting rats from acute liver injury.[30]

Beyond LEAP2's potential impact on MAFLD progression, the disease itself may drive increased LEAP2 production through a bidirectional mechanism. Patients with advanced MAFLD exhibit progressive hepatic steatosis,[31] which can promote blood glucose elevation through impaired ketogenesis and increased acetyl-CoA oxidation.[31] Consequently, both hyperglycemia and hepatic fat accumulation can lead to insulin resistance and compensatory hyperinsulinemia.[32] This hyperinsulinemic state, in turn, can activate upstream transcription factors of the LEAP2 gene within hepatocytes, including SP1, FOXO1, FOXA, and HNF, thereby upregulating LEAP2 expression and secretion.[33] Additionally, studies suggest that LEAP2 positively correlates with estrogen/androgen ratios[34] and levels of numerous inflammatory factors (CRP, IL-6, IL-8, MCP-1, etc).[35] These factors, which are closely associated with MAFLD-induced hepatic dysfunction and inflammation, may have potential reciprocal interactions with LEAP2.

This study has several limitations. First, the relatively modest sample size (n=74) may affect the statistical power and require validation in larger cohorts, the substantial exclusion of participants which mainly due to incomplete data further limit generalizability to broader MAFLD population. Additionally, the substantial BMI differences between groups may not be fully addressed with our modest sample size, potentially confounding the observed LEAP2-MAFLD associations. Second, while PSM achieved balanced baseline characteristics, this approach may introduce selection bias. Third, differing diagnostic methodologies were employed due to ethical considerations (ultrasound screening for the HC group versus liver biopsy for MAFLD cases), which potentially introduced bias in group allocation. Fourth, while LEAP2 shows promise as a noninvasive biomarker, it is currently not part of routine clinical testing panels and involves relatively higher costs compared to standard liver function tests, though it presents no significant technical barriers for clinical adoption. Furthermore, our external validation utilized hepatic LEAP2 transcript data from the GEO database

rather than serum samples matching our primary analysis. This approach was chosen because suitable databases of peripheral tissue samples were unavailable, and analysis of the Human Protein Atlas (HPA) confirmed the liver as the predominant site of LEAP2 expression. Future studies should aim to expand sample sizes, conduct experimental research to further explore LEAP2's mechanistic role in MAFLD pathogenesis, and include longitudinal designs to establish causality and explore its potential as a therapeutic target.

## Conclusion

Serum LEAP2 levels were significantly and progressively higher with MAFLD severity (HC<SS<MASH) and were independently associated with disease status in this cohort. ROC analysis demonstrated good accuracy for serum LEAP2 in differentiating MASH from HC and reasonable effectiveness in distinguishing between other groups. These results position serum LEAP2 as a potential noninvasive biomarker reflecting MAFLD severity, warranting further exploration of its pathophysiological role and therapeutic relevance.

## Declaration of Generative AI and Other Tools Usage

Claude 3.7 sonnet was utilized for manuscript proofreading to enhance clarity and refine language during preparation. Figure 1 was created using Figdraw.

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## Disclosure

The authors report no conflicts of interest in this work.

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