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Original Research

Diagnostic performance of non-invasive markers of fibrosis compared with liver biopsy in MASLD patients in tertiary care centre

Mukesh Kumar[1], Bikash Narayan Choudhury[2], Mallika Bhattacharyya[3], Sandeep Saini[4], Usha Sharma[5], U.J Deka[6], B.J Baruah[7]

[1]Senior Resident, Department of Gastroenterology, GMCH, Guwahati, India
[2]Professor, Department of Gastroenterology, GMCH, Guwahati, India
[3]Associate Professor, Department of Gastroenterology, GMCH, Guwahati, India
[4]Senior Resident, Department of Gastroenterology, GMCH, Guwahati, India
[5]Professor, Department of Pathology, GMCH, Guwahati, India
[6]Associate Professor, Department of Medicine, GMCH, Guwahati, India
[7]Associate Professor, Department of Gastroenterology, GMCH, Guwahati, India

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Corresponding author: Mukesh Kumar
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Abstract:

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects a significant proportion of the global population, with a subset progressing to advanced fibrosis carrying risks of portal hypertension and hepatocellular carcinoma. Non-invasive tests (NITs) including liver stiffness measurement (LSM), FIB-4, NAFLD Fibrosis Score (NFS), and APRI are increasingly used for fibrosis assessment, yet their diagnostic accuracy varies across populations and phenotypes, including lean MASLD.

Objectives: To evaluate the diagnostic accuracy of LSM, FIB-4, NFS, and APRI against liver biopsy for detecting advanced fibrosis (≥F3) in MASLD, determine optimal thresholds, assess performance for significant fibrosis (≥F2) and any fibrosis (≥F1), and characterise diagnostic performance in lean MASLD (BMI <23 kg/m²).

Methodology: This observational study analysed a prospectively maintained MASLD cohort with paired liver biopsy and contemporaneous non-invasive testing. Diagnostic accuracy was evaluated using receiver operating characteristic analysis with bootstrap confidence intervals. Performance was assessed at standard clinical cut-offs and cohort-derived Youden-optimised thresholds. Spearman correlation quantified associations between NITs and histological fibrosis stage. Subgroup analysis stratified patients by lean status.

Results: Among 80 patients, 13 (16.3%) had advanced fibrosis (≥F3) by liver biopsy. LSM demonstrated the highest discrimination for ≥F3 (AUC 0.934; 95% CI 0.859–0.986), followed by FIB-4 (AUC 0.772), NFS (AUC 0.767), and APRI (AUC 0.662). LSM showed strong correlation with fibrosis stage (ρ=0.841; p<0.001). At the Youden-optimised threshold of 13.4 kPa, LSM achieved sensitivity 0.85, specificity 0.93, and LR+ 11.34. FIB-4 at cut-off 2.4 yielded sensitivity 0.62, specificity 0.97, and LR+ 20.62. For ruling out, LSM <8 kPa classified 45% of patients with NPV 1.00 and zero missed ≥F3 cases; NFS <−1.455 ruled out 66.3% with NPV 0.91. For rule-in, FIB-4 >2.67 achieved PPV 1.00. For ≥F2, LSM maintained excellent accuracy (AUC 0.961). Lean MASLD (n=9; 11.3%) showed comparable ≥F3 prevalence to non-lean patients (11.1% vs 16.9%; p=1.000). Metabolic syndrome was more prevalent in ≥F3 (92.3% vs 69.1%).

Conclusion: LSM provides excellent diagnostic accuracy for advanced fibrosis in MASLD, outperforming serum-based indices. A sequential triage strategy using LSM <8 kPa for rule-out and FIB-4 >2.67 for rule-in optimises clinical decision-making. Lean MASLD patients harbour comparable fibrosis prevalence to non-lean counterparts, warranting equivalent surveillance. These locally derived thresholds offer actionable guidance for non-invasive fibrosis assessment.

Keywords: Non-alcoholic Fatty Liver Disease; Liver Cirrhosis; Elasticity Imaging Techniques; Biological Markers; Biopsy; Diagnostic Techniques and Procedures; Body Mass Index; Metabolic Syndrome

Introduction

Non-alcoholic fatty liver disease has been redefined as metabolic dysfunction–associated steatotic liver disease (MASLD), recognising its tight links to obesity, insulin resistance and other metabolic traits.[1,2] While most people with MASLD have indolent disease, a clinically important minority develop significant fibrosis and a smaller group progress to advanced fibrosis with attendant risks of portal hypertension and hepatocellular carcinoma.[3–6] Identifying advanced fibrosis early is the pivotal step that determines surveillance, treatment intensity and referral.[7–10] Liver biopsy remains the gold standard for staging, yet it is invasive, subject to sampling variability, and not practical for broad triage in routine care.[11]

Non-invasive tests (NITs) are used to fill this gap. [12–15] Serum scores such as FIB-4, the NAFLD Fibrosis Score (NFS) [16–18] and APRI [19] combine routinely available laboratory values, while transient elastography provides liver stiffness measurement (LSM) [20–22] as a physical correlate of fibrosis. Each test has known strengths and limitations: serum indices are inexpensive and scalable but may lack precision near decision thresholds; LSM is sensitive to fibrosis yet affected by inflammation and technical factors. Standard "rule-out" and "rule-in" cut-offs are often recommended, but their performance varies across settings and case-mix, and clinicians still need locally relevant numbers to guide decisions. [16–18,23] In particular, there is continuing uncertainty around the optimal thresholds for significant and advanced fibrosis in cohorts with differing metabolic profiles, and around the behaviour of these tests in lean MASLD.[18]

We undertook a biopsy-referenced evaluation of LSM, FIB-4, NFS and APRI in a prospectively maintained MASLD cohort. The primary objective was to quantify diagnostic accuracy for advanced fibrosis (≥F3) using receiver operating characteristic analysis and to report cohort-derived cut-offs alongside clinical thresholds. Secondary objectives were to assess performance for any fibrosis (≥F1) and significant fibrosis (≥F2), to provide decision-ready metrics at prespecified "rule-out" and "rule-in" values, and to characterise agreement between tests and histology across fibrosis stages. CAP was summarised descriptively but not used to assess fibrosis, as it indicates steatosis rather than stiffness.

Recognising clinical practice needs, we also modelled a practical triage approach by reporting how many patients could be safely ruled out or confidently ruled in for advanced fibrosis using simple thresholds, and how many would remain indeterminate. Finally, we examined performance in lean MASLD (BMI <23 kg/m²) given the distinct phenotype and the small but meaningful proportion of patients who meet this definition. The study is intended to provide clear, locally derived evidence on the use of NITs in MASLD, balancing statistical

accuracy with operational clarity for day-to-day care.

Methodology

Study design and setting: This observational analytical study was conducted at the Department of Gastroenterology, Gauhati Medical College and Hospital, Guwahati. The study period extended from April 2024 to October 2025. Both inpatients and outpatients attending the Department of Gastroenterology were included in the study.

Participants: Eligible individuals were adults aged 18 years or older with a clinical diagnosis of MASLD who had a liver biopsy and contemporaneous non-invasive assessments available in the dataset. Patients with significant alcohol intake, alternative chronic liver diseases, decompensated cirrhosis, or missing essential variables for a given analysis were handled by complete-case exclusion for that analysis. Total 150 patients were enrolled and after exclusion 80 patients undergo liver biopsy.

Measurements: Recorded variables included age, sex, body mass index, waist and hip circumferences, and the waist-to-hip ratio. Metabolic risk factors comprised hypertension, diabetes, metabolic syndrome, triglycerides, and HDL cholesterol. Laboratory tests were albumin, AST, ALT, and platelet count. Non-invasive indices included APRI, FIB-4, and the NAFLD Fibrosis Score. Transient elastography provided liver stiffness measurement (kPa) and controlled attenuation parameter (dB/m). Histopathology supplied the reference fibrosis stage recorded on a four-level ordinal scale (F0 to F4), the NAFLD Activity Score, and steatosis grade (0 to 3).

Case definitions and endpoints: Lean MASLD was defined as BMI <23 kg/m². The primary endpoint was advanced fibrosis, operationalised as biopsy stage ≥F3. Secondary endpoints were any fibrosis (≥F1) and significant fibrosis (≥F2). Controlled attenuation parameter was included in descriptive summaries but was not used to assess fibrosis discrimination because it captures steatosis rather than stiffness.

Statistical analysis: Analyses were two-sided with a significance threshold of 0.05. Continuous and ordinal variables were summarised as median with interquartile range after inspection of distributions. Categorical variables were summarised as counts and percentages. Baseline characteristics were compared between F0–F2 and ≥F3 groups. Continuous and ordinal variables were tested with the Mann–Whitney U test. Binary categorical variables were compared using Fisher's exact test. Prevalence of lean MASLD was reported overall and by fibrosis group.

To quantify the monotonic relationship between non-invasive measures and histology, Spearman's rank correlation coefficients were calculated for liver stiffness, FIB-4, NAFLD Fibrosis Score, and APRI against the ordinal biopsy stage (0–3). Uncertainty around correlation estimates was expressed using non-parametric bootstrap confidence intervals based on 5,000 resamples.

Diagnostic accuracy was evaluated for the primary endpoint (≥F3) and for the secondary endpoints (≥F1 and ≥F2). For each marker, receiver operating characteristic curves were generated and the area under the curve was estimated using a non-parametric approach. Confidence intervals for the AUC were derived by bootstrap with 2,000 resamples. Performance was reported at two threshold strategies per marker. First, pre-specified standard clinical cut-offs were applied (LSM 12 kPa; FIB-4 2.67; NAFLD Fibrosis Score 0.67; APRI 1.5). Second, cohort-derived cut-offs were identified using the Youden index to balance sensitivity and specificity. For each threshold we calculated sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and the underlying contingency counts to support independent verification.

Subgroup analyses were performed by lean status. The cohort was stratified into lean (BMI <23 kg/m²) and non-lean (BMI ≥23 kg/m²) groups. Prevalence of ≥F2 and ≥F3 was reported within each stratum. ROC/AUC estimation and threshold performance were then repeated within each subgroup, including subgroup-specific Youden cut-offs. Estimates in the lean subgroup were interpreted with caution due to small event counts.

Software: All analyses were performed in SPSS v28.

Ethics: This work represents analysis of clinically acquired data from the patients. Ethical approval was obtained from the institutional ethical committee.

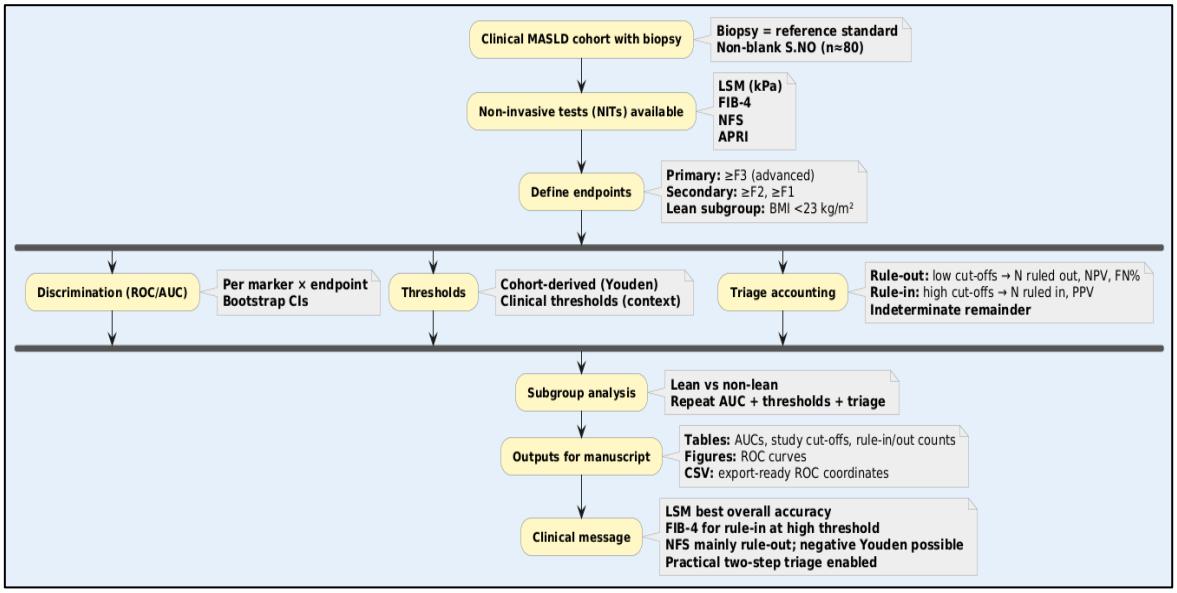

Figure 1 - Core methodology illustration for the MASLD NITs Study

Results

Baseline characteristics by fibrosis group (F0–F2 vs ≥F3): Among 80 participants, 13 (16.3%) had advanced fibrosis (≥F3). Biopsy staging showed F0 in 30 patients (37.5%), F1 in 20 (25.0%), F2 in 17 (21.2%) and F3 in 13 (16.2%). Age tended to be higher in the ≥F3 group (median 42 vs 37 years; p=0.051). Sex distribution did not differ significantly (male 38.5% vs 64.7%; p=0.120). Median BMI was comparable between groups, and the prevalence of lean MASLD was low and similar (7.7% vs 11.8%; p=1.000). There were no significant differences in waist, hip, waist-to-hip ratio, hypertension, diabetes or metabolic syndrome, although the latter was numerically more frequent with ≥F3 (92.3% vs 69.1%; p=0.102).

Table 1 - Baseline characteristics by fibrosis group

Variable	F0–F2 (n=67)	≥F3 (n=13)	P value
Age (years)	37.0 [30.5–44.0]	42.0 [38.0–45.0]	0.051
Male sex	44/68 (64.7%)	5/13 (38.5%)	0.1199
BMI (kg/m²)	27.4 [26.7–29.1]	27.2 [26.4–28.2]	0.5482
Lean MASLD (BMI<23)	8/68 (11.8%)	1/13 (7.7%)	1
Waist (cm)	90.0 [85.5–92.0]	86.0 [82.0–92.0]	0.2955
Hip (cm)	94.0 [90.5–99.5]	91.0 [86.0–98.0]	0.1763
Waist:Hip ratio	0.9 [0.9–1.0]	0.9 [0.9–1.0]	0.9685
Hypertension	18/68 (26.5%)	3/13 (23.1%)	1

Diabetes	15/68 (22.1%)	4/13 (30.8%)	0.4909
Metabolic syndrome	47/68 (69.1%)	12/13 (92.3%)	0.1018
Albumin (g/dL)	4.6 [4.1–5.0]	4.5 [4.0–4.7]	0.5161
AST (U/L)	51.0 [38.5–68.5]	66.0 [51.0–109.0]	**0.0452**
ALT (U/L)	69.0 [44.0–106.0]	47.0 [39.0–90.0]	0.5838
Platelets (10^9/L)	160.0 [150.0–187.0]	150.0 [130.0–200.0]	0.4128
Triglycerides (mg/dL)	194.0 [159.0–235.5]	201.0 [174.0–261.0]	0.3929
HDL (mg/dL)	39.0 [36.0–46.0]	40.0 [38.0–42.0]	0.5829
LSM (kPa)	7.8 [6.9–10.6]	15.7 [14.6–16.0]	**< 0.001**
CAP (dB/m)	312.0 [288.5–340.0]	310.0 [299.0–345.0]	0.7296
NAS score	4.0 [3.8–5.0]	5.0 [5.0–6.0]	**0.0039**
Steatosis grade (0–3)	2.0 [1.0–2.5]	2.0 [2.0–3.0]	0.3953

Biochemistry showed higher AST in the ≥F3 group (66 vs 51 U/L; p=0.045), with no significant difference in ALT, albumin or platelet count. Triglycerides and HDL were comparable. Transient elastography values were substantially higher with ≥F3 (LSM 15.7 vs 7.8 kPa; p<0.001). CAP did not differ between groups. Histological activity was greater with advanced fibrosis (NAS 5.0 vs 4.0; p=0.0039). Steatosis grade distributions were similar.

Diagnostic accuracy for advanced fibrosis (≥F3): We evaluated the ability of four non-invasive markers—LSM, FIB-4, NFS and APRI—to discriminate advanced fibrosis (≥F3) using liver biopsy as the reference.

Table 2 - AUC for discrimination of advanced fibrosis (≥F3)

Marker	AUC	95% CI (lower)	95% CI (upper)
LSM (kPa)	0.934	0.859	0.986
FIB-4	0.772	0.589	0.937
NFS	0.767	0.608	0.906
APRI	0.662	0.493	0.813

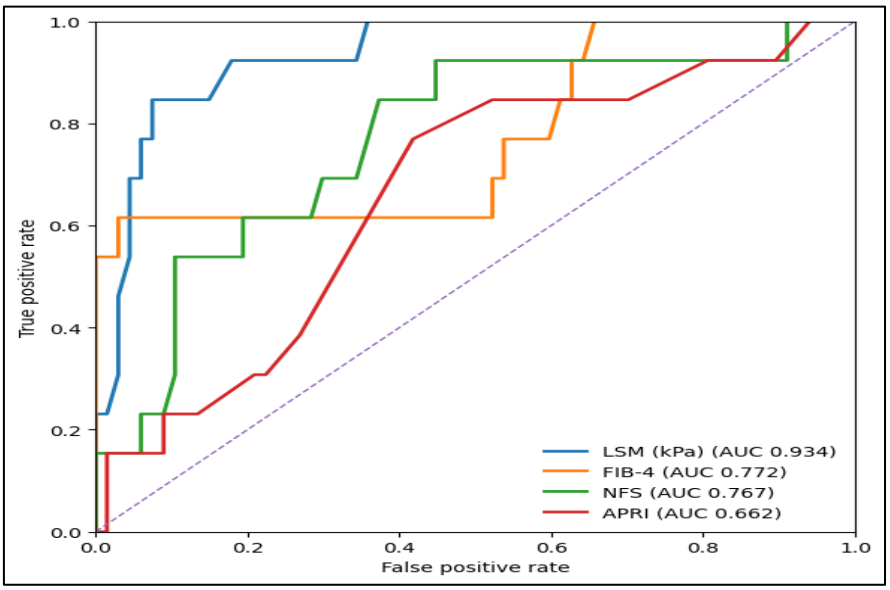

Figure 2 - ROC curves for discrimination of advanced fibrosis (≥F3) using biopsy as reference

Table 3 - Diagnostic performance at standard & Youden cut-offs (advanced fibrosis, ≥F3

Marker	Threshold type	Cut-off	Sensitivity	Specificity	PPV	NPV	LR+	LR-	TP	FP	TN	FN
LSM (kPa)	Standard	12	0.846	0.866	0.55	0.967	6.299	0.178	11	9	58	2
	Youden	13.4	0.846	0.925	0.688	0.969	11.338	0.166	11	5	62	2
FIB-4	Standard	2.67	0.538	1	1	0.918	NA	0.462	7	0	67	6
	Youden	2.4	0.615	0.97	0.8	0.929	20.615	0.396	8	2	65	5
NFS	Standard	0.67	0.154	0.985	0.667	0.857	10.308	0.859	2	1	66	11
	For NFS we did not report a cohort-derived Youden cut-off because the data-driven optimum lay in the negative (rule-out) range.											
APRI	Standard	1.5	0.231	0.881	0.273	0.855	1.933	0.874	3	8	59	10
	Youden	0.9	0.769	0.582	0.263	0.929	1.841	0.396	10	28	39	3

LSM provided the highest discrimination for advanced fibrosis with an AUC of 0.934 (95% CI 0.859–0.986). FIB-4 and NFS demonstrated moderate discrimination (AUC 0.772 and 0.767, respectively), while APRI performed modestly (AUC 0.662).

At the **standard** cut-off of 12 kPa, LSM prioritised sensitivity (1.00) at the expense of specificity (0.36). The **Youden-optimised** LSM threshold of **13.4 kPa** balanced performance with sensitivity 0.85 and specificity 0.93, yielding a high LR+ of 11.34 and low LR− of 0.17.

For **FIB-4**, the standard cut-off of 2.67 gave perfect specificity (1.00) but moderate sensitivity (0.54). The Youden-optimised cut-off (**2.4**) improved sensitivity to 0.62 with specificity 0.97 (LR+ 20.62; LR− 0.40).

For **NFS**, the standard cut-off of 0.67 produced high specificity (0.99) with low sensitivity (0.15).

For **APRI**, the standard cut-off of 1.5 showed limited sensitivity (0.23) and fair specificity (0.88). The Youden-optimised cut-off (**0.9**) improved sensitivity (0.77) with moderate specificity (0.58), but likelihood ratios remained modest. Overall the LSM was the most accurate single non-invasive marker for detecting ≥F3. FIB-4 at higher thresholds offered strong rule-in capability, whereas NFS at a lower, optimised threshold was useful for ruling out advanced fibrosis.

Rule-out and rule-in clinical thresholds for ≥F3: We applied prespecified clinical thresholds to model a practical triage pathway. Rule-out thresholds were set to spare biopsy when values were below FIB-4 1.3, APRI 0.5, NFS −1.455, or LSM 8 kPa. Rule-in thresholds prioritised advanced disease when values exceeded FIB-4 2.67, APRI 1.5, NFS 0.67, or were at least LSM 12 kPa.

Table 4 - Rule-out thresholds for advanced fibrosis (≥F3) with predictive value among classified patients

Marker	Decision/Cut-off	N decided	N indeterminate	TN	FN	NPV among decided	Missed advanced (FN%)
FIB-4	Rule-out < 1.3	28	52	26	2	0.929	7.10%
APRI	Rule-out < 0.5	14	66	13	1	0.929	7.10%
NFS	Rule-out < -1.455	53	27	48	5	0.906	9.40%
LSM (kPa)	Rule-out < 8.0	36	44	36	0	1	0.00%

Using low thresholds, LSM <8 kPa classified 36/80 (45.0%) as not having advanced fibrosis with NPV 1.00 and no missed ≥F3 cases among those ruled out. FIB-4 <1.3 and APRI <0.5 each yielded NPV 0.93 with a small miss rate (7.1%). NFS <−1.455 ruled out the largest proportion, 53/80 (66.3%), with NPV 0.91 and a 9.4% miss rate.

Table 5 - Rule-in thresholds for advanced fibrosis (≥F3) with predictive value among classified patients

Marker	Decision/Cut-off	N decided	N indeterminate	TP	FP	PPV among decided
FIB-4	Rule-in > 2.67	7	73	7	0	1
APRI	Rule-in > 1.5	9	71	3	6	0.333
NFS	Rule-in > 0.67	3	77	2	1	0.667
LSM (kPa)	Rule-in >= 12.0	20	60	11	9	0.55

At high thresholds, FIB-4 >2.67 identified 7/80 (8.8%) as likely advanced with PPV 1.00 (no false positives). LSM ≥12 kPa flagged 20/80 (25.0%) with PPV 0.55, supporting use as a sensitive screen when combined with a high-specificity serum index. NFS >0.67 classified few patients (PPV 0.67), and APRI >1.5 had a low PPV (0.33).

Correlation of non-invasive markers with histological fibrosis stage (0–3): We quantified the monotonic association between each non-invasive test and biopsy fibrosis stage.

Table 6 - Spearman correlation between markers and fibrosis stage

Marker	Spearman rho	95% CI (lower)	95% CI (upper)	P value
APRI	0.285	0.071	0.47	**0.01034**
FIB-4	0.536	0.353	0.687	**< 0.0001**
LSM (kPa)	0.841	0.764	0.892	**< 0.0001**
NFS	0.331	0.113	0.527	**0.002702**

Across 80 patients - LSM showed a strong positive correlation with biopsy fibrosis stage (ρ=0.841; 95% CI 0.764–0.892; p<0.001). FIB-4 correlated moderately with stage (ρ=0.536; 95% CI 0.353–0.687; p<0.001), as did NFS (ρ=0.331; 95% CI 0.113–0.527; p=0.0027). APRI showed a weaker but statistically significant

association (ρ=0.285; 95% CI 0.071–0.470; p=0.010). These data indicate that stiffness measurements track histological fibrosis most closely, while serum indices carry moderate ordinal information.

Secondary diagnostic accuracy for ≥F1 and ≥F2: We assessed diagnostic discrimination of LSM, FIB-4, NFS and APRI for any fibrosis (≥F1) and significant fibrosis (≥F2), using biopsy as the reference.

Table 7 - AUC for discrimination of ≥F1

Marker	AUC	95% CI (lower)	95% CI (upper)
LSM (kPa)	0.924	0.858	0.971
FIB-4	0.794	0.687	0.886
NFS	0.651	0.525	0.772
APRI	0.643	0.511	0.764

For any fibrosis, LSM showed excellent discrimination (AUC 0.924). FIB-4 performed well (AUC 0.794), while NFS and APRI were modest (AUCs 0.651 and 0.643).

Table 8 - Diagnostic performance at Youden cut-offs (≥F1)

Marker	Cut-off (Youden)	Sensitivity	Specificity	PPV	NPV	LR+	LR-	TP	FP	TN	FN
LSM (kPa)	8.7	0.78	0.967	0.975	0.725	23.4	0.228	39	1	29	11
FIB-4	1.74	0.56	0.967	0.966	0.569	16.8	0.455	28	1	29	22
NFS	For NFS we did not report a cohort-derived Youden cut-off because the data-driven optimum lay in the negative (rule-out)										
APRI	0.7	0.74	0.533	0.725	0.552	1.586	0.488	37	14	16	13

The Youden-optimised LSM threshold (8.7 kPa) gave high specificity (0.97) with sensitivity 0.78 (LR+ 23.4; LR− 0.23). FIB-4 at the Youden cut-off (1.74) balanced sensitivity 0.56 and specificity 0.97 (LR+ 16.8). NFS and APRI showed limited rule-in ability at their Youden thresholds.

Table 9 - AUC for discrimination of ≥F2

Marker	AUC	95% CI (lower)	95% CI (upper)
LSM (kPa)	0.961	0.921	0.99
FIB-4	0.764	0.641	0.868
NFS	0.658	0.528	0.783
APRI	0.65	0.527	0.773

Table 10 - Diagnostic performance at Youden cut-offs (≥F2)

Marker	Cut-off (Youden)	Sensitivity	Specificity	PPV	NPV	LR+	LR-	TP	FP	TN	FN
LSM (kPa)	9	0.967	0.84	0.784	0.977	6.042	0.04	29	8	42	1
FIB-4	1.83	0.567	0.86	0.708	0.768	4.048	0.504	17	7	43	13
NFS	For NFS we did not report a cohort-derived Youden cut-off because the data-driven optimum lay in the negative (rule-out)										
APRI	0.7	0.833	0.48	0.49	0.828	1.603	0.347	25	26	24	5

For significant fibrosis, LSM again showed very high discrimination (AUC 0.961). FIB-4 was moderate (AUC 0.764), with NFS and APRI modest (AUCs 0.658 and 0.650). The Youden-optimised LSM cut-off (9.0 kPa) yielded sensitivity 0.97 and specificity 0.84 (LR+ 6.04; LR− 0.04). At the Youden value (1.83) FIB-4 cut-off - balanced sensitivity 0.57 and specificity 0.86. Youden thresholds for NFS and APRI showed high specificity improved sensitivity but kept likelihood ratios modest.

Lean MASLD subgroup: We predefined lean MASLD as BMI <23 kg/m² and stratified the cohort into lean and non-lean groups. Within each stratum we reported the prevalence of F0, F1, F2 and F3 staging.

Table 11 - Fibrosis stage distribution by lean status (BMI <23 vs ≥23 kg/m²)

Group	N	F0 n (%)	F1 n (%)	F2 n (%)	F3 n (%)
Lean (BMI<23)	9	5 (55.6%)	1 (11.1%)	2 (22.2%)	1 (11.1%)
Non-lean (BMI≥23)	71	25 (35.2%)	19 (26.8%)	15 (21.1%)	12 (16.9%)

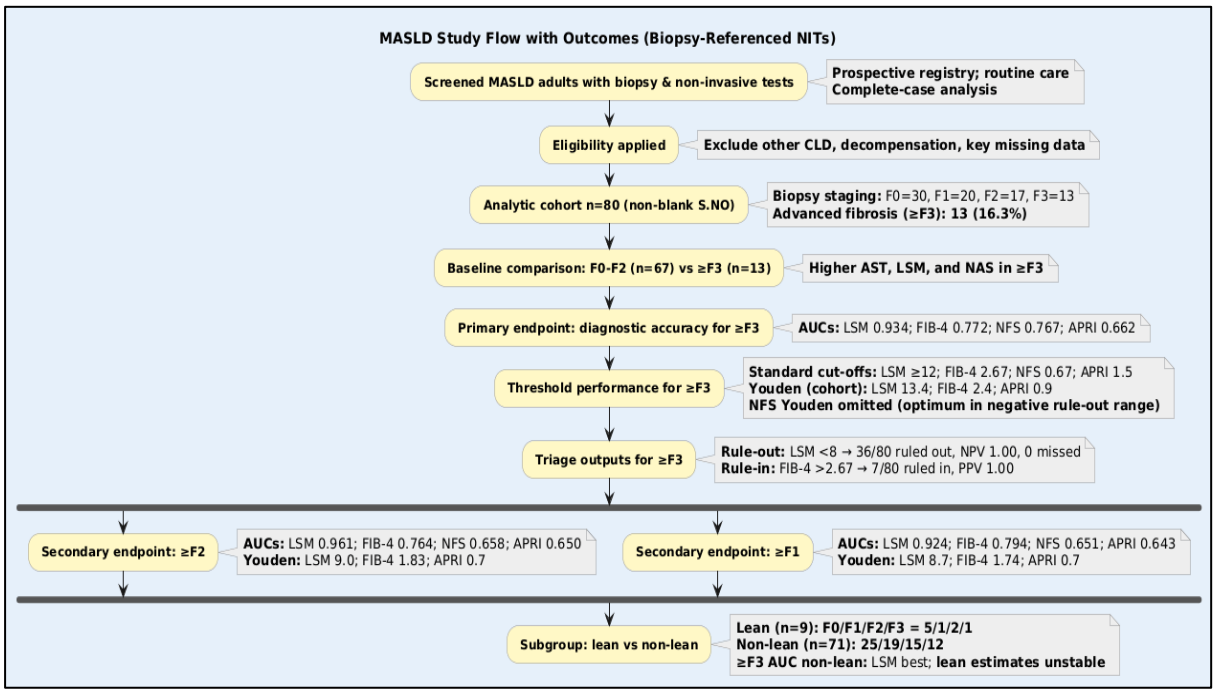

Figure 3 - MASLD Study Flow with Outcomes (Biopsy-Referenced Accuracy of NITs)

Discussion

Our study evaluated the diagnostic accuracy of non-invasive fibrosis tests against liver biopsy in a prospectively maintained MASLD cohort. The findings demonstrate that liver stiffness measurement by transient elastography provides the highest diagnostic accuracy for detecting advanced fibrosis (≥F3), with an AUC of 0.934, substantially outperforming serum-based indices. FIB-4 and NFS showed moderate discrimination (AUC 0.772 and 0.767, respectively), while APRI performed modestly (AUC 0.662). The strong correlation between LSM and histological fibrosis stage (ρ=0.841) confirms that stiffness measurements track fibrosis progression most closely among the markers evaluated. Importantly, the Youden-optimised LSM threshold of 13.4 kPa achieved an excellent balance of sensitivity (0.85) and specificity (0.93) with a high positive likelihood ratio (LR+ 11.34), making it particularly useful for clinical decision-making. For serum indices, FIB-4 at the Youden-optimised cut-

off of 2.4 demonstrated strong rule-in capability with specificity of 0.97 and LR+ of 20.62, while the standard cut-off of 2.67 achieved perfect specificity at the expense of sensitivity.

From a clinical triage perspective, our analysis of prespecified rule-out and rule-in thresholds provides actionable guidance for routine care. LSM <8 kPa successfully ruled out advanced fibrosis in 45% of patients with a perfect NPV of 1.00 and no missed cases, establishing it as the safest initial screening tool. NFS <−1.455 ruled out the largest proportion of patients (66.3%) with an acceptable NPV of 0.91, though with a small miss rate of 9.4%. For rule-in decisions, FIB-4 >2.67 identified patients with advanced fibrosis with 100% PPV, albeit classifying only a small proportion (8.8%). These findings support a sequential approach where LSM serves as the primary triage tool, with FIB-4 providing confirmatory specificity when needed. The lean MASLD subgroup, representing 11.3% of our cohort, showed a comparable prevalence of advanced fibrosis to non-lean patients (11.1% vs 16.9%), though interpretation was limited by small numbers. This observation aligns with emerging evidence that metabolic dysfunction rather than adiposity alone drives fibrosis progression.

Jaiswal et al. (2021) characterised the metabolic profile of lean NAFLD subjects in a cross-sectional study from Northeast India, proposing a spectrum of insulin resistance from non-obese controls through lean NAFLD to obese NAFLD with diabetes. Their study found that lean NAFLD subjects without diabetes had HOMA-IR values comparable to healthy controls, suggesting minimal insulin resistance in this phenotype. Our findings complement this metabolic characterisation by demonstrating that lean MASLD patients exhibit similar prevalence of advanced fibrosis (11.1%) compared to non-lean patients (16.9%), despite the reportedly lower metabolic derangement. Jaiswal et al. noted significantly higher FBG and HbA1c in lean NAFLD patients with diabetes compared to those without, paralleling the association we observed between metabolic syndrome and advanced fibrosis (92.3% vs 69.1%). However, while their study relied primarily on HOMA-IR for metabolic assessment, our study extends this by demonstrating that non-invasive fibrosis tools, particularly LSM, effectively identify advanced disease across BMI categories. Both studies underscore that lean NAFLD/MASLD represents a clinically important phenotype requiring active surveillance.[24]

Rahman et al. (2020) conducted a community-based study in rural Bangladesh and found that 18.5% of the population had NAFLD, with approximately one-quarter (23.55%) being non-obese. Notably, their study demonstrated that metabolic profiles were comparable between obese and non-obese NAFLD, apart from BMI itself—a finding that resonates with our observation that fibrosis severity was not significantly different between lean and non-lean MASLD groups. Rahman et al. identified age >40 years, metabolic syndrome, diabetes, and abdominal obesity as independent risk factors for NAFLD on multivariate analysis. Our cohort similarly showed a trend toward older age in the advanced fibrosis group (median 42 vs 37 years, p=0.051) and higher prevalence of metabolic syndrome (92.3% vs 69.1%). While Rahman et al. focused on prevalence and risk factors using ultrasonography, our biopsy-referenced study extends these findings by validating the diagnostic accuracy of NITs for detecting histologically confirmed fibrosis. The elevated ALT found in 22% of their NAFLD subjects aligns with the biochemical abnormalities observed in our cohort, though we additionally demonstrated that AST was significantly higher in the ≥F3 group (66 vs 51 U/L, p=0.045).[25]

Iritani et al. (2020) investigated prognostic factors in histopathologically-confirmed lean NAFLD in a Japanese cohort. Their multivariate analysis identified NFS ≥−1.455 as an independent predictor of

both severe fibrosis and mortality in lean NAFLD patients. This threshold aligns remarkably with the rule-out cut-off we applied in our study, where NFS <−1.455 ruled out advanced fibrosis with 90.6% NPV. Iritani et al. found that lean NAFLD patients had lower histological scores and less severe fibrosis at presentation compared to non-lean patients, yet prognosis was similar between groups—a paradox they attributed to potentially faster fibrosis progression in lean individuals. Our data partially support this concern, as lean patients showed comparable advanced fibrosis rates despite presumably lower metabolic burden.[14]

Kumar et al. (2013) similarly reported that lean NAFLD comprised 13.2% of their Indian cohort, with 89% being dyslipidaemic despite minimal insulin resistance. They found lower histological grades and fibrosis stages in lean compared to obese NAFLD, though NASH and advanced fibrosis prevalence were statistically similar across BMI categories. Our findings corroborate this pattern: the prevalence of ≥F3 fibrosis in lean MASLD (11.1%) was not significantly different from non-lean patients (16.9%), reinforcing that normal BMI does not preclude significant liver disease.[26]

Conclusion

In conclusion, our biopsy-referenced study demonstrates that liver stiffness measurement provides excellent diagnostic accuracy for detecting advanced fibrosis in MASLD, with an AUC of 0.934 that substantially exceeds serum-based indices. LSM at a threshold of 8 kPa offers a safe rule-out strategy with perfect negative predictive value, while FIB-4 >2.67 provides confirmatory specificity with 100% PPV. These findings support a practical sequential triage approach combining LSM for initial screening with FIB-4 for confirmation. Importantly, lean MASLD patients demonstrated comparable prevalence of advanced fibrosis to non-lean patients, consistent with prior South Asian studies showing that normal BMI does not preclude significant hepatic disease. The metabolic syndrome was highly prevalent in the advanced fibrosis group (92.3%), confirming its role as a key driver of disease progression regardless of adiposity. These locally derived thresholds and performance metrics provide clinicians with actionable guidance for non-invasive assessment of fibrosis in MASLD, potentially reducing unnecessary biopsies while ensuring appropriate identification of patients requiring specialist referral and surveillance.

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