

From Aminotransferases to Target-Specific Testing: A Biochemical Framework for Non-Invasive Assessment of Metabolic Dysfunction-Associated Steatotic Liver Disease

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is common, clinically silent in many individuals and biochemically heterogeneous. The principal laboratory error is not simply choosing an imperfect biomarker; it is asking one measurement to answer the wrong biological question. Alanine aminotransferase and aspartate aminotransferase reflect hepatocellular stress, whereas FIB-4 combines age, aminotransferases and platelet count to triage advanced fibrosis; the Enhanced Liver Fibrosis (ELF) test and PRO-C3 interrogate extracellular-matrix turnover; and cytokeratin-18, NIS4 and related panels target cell death or fibrotic steatohepatitis. This critical review synthesizes international guidance, systematic reviews, validation studies and Indian evidence published through 3 September 2026. Evidence was organized by intended use: detection of steatosis, identification of metabolic dysfunction-associated steatohepatitis (MASH), exclusion or confirmation of advanced fibrosis, prediction of outcome and longitudinal monitoring. Routine aminotransferases cannot exclude clinically important disease, and the positive predictive value of any fibrosis test depends strongly on prevalence and referral setting. FIB-4 remains suitable as a low-cost first-line rule-out tool, but age, acute illness, thrombocytopenia and type 2 diabetes alter its performance. ELF and PRO-C3 add mechanistic information about matrix remodeling, yet thresholds, extrahepatic collagen turnover and pre-analytical effects require attention. CK-18 and emerging multi-omic panels are biologically attractive but are not interchangeable with validated fibrosis pathways. The review proposes TARGET-MASLD: Target the biological condition, Anchor interpretation to pre-test risk, Rule out with a validated routine score, Gate second-line testing, Examine discordance and analytical quality, and Trend results under comparable conditions. This framework converts biomarker selection from a catalogue of assays into a decision-linked biochemical strategy suited to resource-variable settings, including India.

Keywords: MASLD; MASH; non-invasive tests; FIB-4; Enhanced Liver Fibrosis test; PRO-C3; cytokeratin-18; clinical biochemistry

1. Introduction

The change from non-alcoholic fatty liver disease to metabolic dysfunction-associated steatotic liver disease is more than a revision of terminology. MASLD requires hepatic steatosis together with at least one cardiometabolic risk factor and sits within a broader steatotic liver disease classification that also recognizes alcohol-related and mixed metabolic-alcohol phenotypes. The new nomenclature removes a diagnosis defined primarily by exclusion and places metabolic biology at the centre of case definition (Rinella et al., 2023a). It also exposes a practical problem for clinical biochemistry: no single serum marker identifies the entire continuum from intracellular lipid accumulation to steatohepatitis, fibrosis, cirrhosis and clinical outcome.

The burden makes that distinction consequential. Global evidence shows that fatty liver disease has become one of the most common chronic liver conditions (Younossi et al., 2023). A systematic review of Indian studies estimated pooled adult prevalence at 38.6%, with higher estimates in groups carrying obesity, diabetes or cardiovascular risk (Shalimar et al., 2022). Those data pre-date the MASLD nomenclature and are heterogeneous, but they show why a biopsy-centred pathway cannot scale. Most affected individuals are encountered in primary care, diabetes clinics and general laboratories rather than specialist hepatology. The clinical task is therefore not to reproduce histology in everyone. It is to identify which biological question matters now, use an inexpensive test to remove sufficiently low-risk individuals from further work-up, and direct the remainder to more specific assessment.

Aminotransferases have historically acted as the gateway to investigation. That practice is attractive because alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are inexpensive and familiar. It is also unsafe when a normal result is treated as evidence that progressive disease is absent. Advanced fibrosis and even

cirrhosis can occur with aminotransferases within laboratory reference limits; conversely, increased values can reflect exercise, skeletal-muscle injury, alcohol exposure, drugs, acute illness or another liver disease. Gamma-glutamyl transferase (GGT) may track metabolic and oxidative stress but is similarly non-specific. The relevant lesson is not that liver enzymes lack value, but that they measure injury rather than architecture.

Fibrosis deserves priority because long-term cohorts and meta-analyses consistently associate increasing fibrosis stage with liver-related events and mortality more strongly than steatosis or necroinflammatory grade alone (Ekstedt et al., 2015; Angulo et al., 2015; Dulai et al., 2017). This prognostic observation explains the contemporary emphasis on non-invasive fibrosis tests (NITs). Current guidance recommends a multi-step strategy: a simple blood-based score such as FIB-4 first, followed in those with retained or uncertain risk by elastography or a collagen-related blood test such as ELF (Rinella et al., 2023b; EASL-EASD-EASO, 2024; Cusi et al., 2025). Yet the apparent simplicity of that pathway can conceal several biochemical assumptions about target condition, threshold, population and measurement stability.

This article addresses those assumptions through a structured critical review. Its central proposition is that a biomarker is useful only when its molecular signal, reference condition and decision threshold correspond to the clinical question. The same numerical result may have different meaning in a low-prevalence community sample, a diabetes clinic and a biopsy-enriched referral cohort. A panel that detects at-risk MASH is not automatically a prognostic test; a score that excludes advanced fibrosis is not a direct measurement of fibrogenesis; and a change in score may reflect biological or analytical variation rather than altered tissue fibrosis. The proposed TARGET-MASLD framework makes those distinctions explicit and links each test to a defensible decision.

2. Review Design and Analytical Scope

A structured critical-review method was used rather than a de novo diagnostic meta-analysis. PubMed-indexed reports, international clinical guidance and accessible full-text reviews published from January 2003 to 3 September 2026 were examined. Foundational earlier work was retained where it defined a test still used in current pathways. Search concepts combined MASLD, NAFLD or MASH with serum biomarker, aminotransferase, FIB-4, NAFLD Fibrosis Score, APRI, ELF, PRO-C3, ADAPT, cytokeratin-18, NIS4, fibrosis, diagnostic accuracy, prognosis, India, analytical variation and pre-analytical error. Reference lists of major guidelines and systematic reviews were used to identify derivation and external-validation studies.

Priority was given to adult human studies in which the biomarker had an explicit intended use and was evaluated against histology, elastography, magnetic resonance methods or clinical outcome. Paediatric pathways, treatment-response biomarkers and purely imaging-based methods were outside the main scope, although elastography is included when it forms the second stage of a biochemical triage pathway. Studies of viral, autoimmune or alcohol-related liver disease were used only when they clarified assay biology or analytical interference. Evidence was interpreted in the order of clinical guidelines, systematic reviews and participant-level meta-analyses, independent external validation, prospective cohorts and derivation studies.

The synthesis coded each test on six dimensions: biological process measured; intended target condition; variables or analytes; reference standard; validation setting; and principal source of misclassification. Numerical accuracy estimates are reported only when they illuminate a methodological point and are not treated as transportable constants. This is important because sensitivity and specificity can vary with spectrum and threshold, while positive and negative predictive values vary directly with disease prevalence. The review therefore emphasizes decision purpose and transportability over league-table ranking of area-under-the-curve values.

The terminology in the included literature changes across time. NAFLD and NASH are retained when referring to cohorts recruited under those definitions; MASLD and MASH are used for the contemporary clinical framework. These populations overlap substantially but are not perfectly identical. The change in case definition is therefore treated as a source of indirectness when older validation data are applied to present practice. No claim is made that a legacy threshold has been prospectively recalibrated for every MASLD subgroup or Indian laboratory setting.

Table 1. Evidence hierarchy used for the critical synthesis

Evidence type	Primary contribution	Interpretive caution
Guidelines and consensus	Current multi-step pathways and intended use	Broad evidence synthesis; recommendations may exceed locally validated data
Systematic reviews / meta-analyses	Average accuracy and heterogeneity	Threshold, spectrum and assay differences can be obscured by pooling
External validation	Transportability beyond derivation sample	Performance remains setting- and prevalence-dependent

Evidence type	Primary contribution	Interpretive caution
Prospective cohort	Workflow performance and outcome prediction	Referral and verification patterns can alter estimates
Derivation study	Biological promise and candidate thresholds	Optimism, overfitting and incomplete calibration are common
Analytical / pre-analytical study	Specimen stability, interference and platform effects	Does not establish clinical utility by itself

Note: The review is an evidence-mapped narrative synthesis. It does not report a PRISMA flow count or a new pooled diagnostic estimate.

3. One Disease Label, Several Biochemical Targets

3.1 Steatosis is a substrate state, not a fibrosis measurement

Hepatic steatosis develops when fatty-acid delivery and de novo lipogenesis exceed mitochondrial oxidation and very-low-density lipoprotein export. Insulin resistance increases adipose lipolysis and hepatic substrate flux; hyperinsulinaemia and carbohydrate excess promote lipogenesis. Triglyceride storage is partly protective because it packages potentially toxic lipid species. Serum triglycerides, glucose, insulin and HbA1c therefore describe the metabolic environment but do not directly quantify hepatocyte lipid or distinguish benign storage from lipotoxic injury. Ultrasonography, controlled attenuation parameter and MRI-proton-density fat fraction answer the steatosis question more directly than routine blood chemistry.

The diagnostic consequence is that a metabolic profile can establish risk without proving liver phenotype. A person with obesity and type 2 diabetes may have high pre-test probability of MASLD but little fibrosis, while a lean individual may carry substantial disease because of visceral adiposity, sarcopenia, diet or genetic susceptibility. Indian studies have reported associations of PNPLA3 and TM6SF2 variants with susceptibility and severity, including ethnic variation (De and Duseja, 2021). Genotype can refine biological understanding, but current guidelines do not support using a single variant as a stand-alone staging test because individual-level discrimination remains inadequate.

3.2 Injury and inflammation are dynamic

Lipotoxic fatty acids, oxidative stress, mitochondrial dysfunction, endoplasmic-reticulum stress and altered inter-organ signalling can trigger hepatocyte ballooning and death. Damage-associated molecular patterns then engage Kupffer cells and recruited immune cells, amplifying cytokine and chemokine signalling. ALT and AST may rise when membrane integrity is disturbed, but their circulating concentration depends on release, clearance and tissue mass. A low ALT value may therefore coexist with advanced fibrosis after loss of viable hepatocyte mass, and a high value may reflect transient injury without advanced scar.

Cytokeratin-18 (CK-18) provides a more mechanistically focused injury signal. M30 assays detect a caspase-cleaved epitope associated with apoptosis, whereas M65 assays capture both cleaved and uncleaved CK-18 and more broadly reflect cell death. The biological rationale is strong, yet threshold dispersion and imperfect standardization limit stand-alone classification. A 2020 meta-analysis of 41 studies and 5,815 participants found summary areas under the curve of 0.75 for M30 and 0.82 for M65 in detecting NASH, with weaker performance for fibrotic NASH and fibrosis (Lee et al., 2020). The result is best interpreted as evidence that cell-death markers can enrich a panel, not that one CK-18 value replaces histology.

3.3 Fibrogenesis, accumulated matrix and prognosis are related but distinct

Persistent injury activates hepatic stellate cells and other matrix-producing cells through transforming growth factor-beta, platelet-derived growth factor and inflammatory pathways. Collagen formation, cross-linking and degradation change the extracellular matrix long before decompensated cirrhosis appears (Friedman, 2008). Direct serum markers such as the amino-terminal propeptide of type III procollagen (PIIINP), hyaluronic acid, tissue inhibitor of metalloproteinase-1 (TIMP-1) and PRO-C3 interrogate matrix turnover. Elastography instead estimates mechanical consequences of accumulated tissue architecture. Histology samples matrix pattern directly but is invasive and vulnerable to sampling and reader variability.

A direct fibrogenesis marker can change more quickly than established scar, which is an advantage for biological monitoring but a challenge for interpretation. Conversely, a low-turnover cirrhotic liver may contain abundant cross-linked collagen without an equally large formation signal. Prognostic performance may arise from correlation with disease burden rather than a perfect one-to-one mapping to fibrosis stage. Laboratory reports and manuscripts should therefore state whether the target is active collagen formation, prevalent advanced fibrosis, future clinical events or response to intervention.

Table 2. Biological target determines the meaning of a biomarker

Target	Dominant process	Representative measurements	What the result cannot establish alone
Metabolic context	Insulin resistance, substrate excess	Glucose, HbA1c, triglycerides, insulin/HOMA-IR	Risk enrichment; not proof of liver stage
Hepatocyte injury	Membrane leakage and cellular stress	ALT, AST, GGT	Dynamic and non-specific; normal values do not exclude fibrosis
Cell death / MASH activity	Apoptosis and necrosis	CK-18 M30/M65; multi-analyte panels	Moderate stand-alone accuracy; assay and threshold variation
Active fibrogenesis	Collagen synthesis and matrix turnover	PRO-C3; PIINP, HA and TIMP-1 within ELF	Mechanistically direct, but extrahepatic turnover can contribute
Accumulated fibrosis	Scar architecture and portal effects	FIB-4/NFS as indirect scores; elastography as mechanical proxy	Best used in staged pathways rather than as isolated diagnoses
Clinical prognosis	Integrated disease reserve and event risk	Repeated FIB-4, ELF, liver stiffness and clinical variables	Prediction horizon and competing cardiometabolic risk must be specified

4. Routine Chemistry and Indirect Fibrosis Scores

4.1 Aminotransferases: useful context, unsafe exclusion

ALT is more liver-enriched than AST, whereas AST is also abundant in skeletal muscle, myocardium and erythrocytes. The AST/ALT ratio can increase with advanced fibrosis because ALT activity falls and mitochondrial injury contributes AST, but the ratio is not disease-specific. GGT responds to cholestasis, alcohol, medications and metabolic oxidative stress. Albumin, bilirubin and the international normalized ratio become important when synthetic or excretory function is impaired, but they are often preserved through compensated disease. A standard liver panel therefore describes injury and function; it is not a sensitive staging system.

Reference intervals deserve scrutiny. Population-derived upper limits can incorporate individuals with obesity or unrecognized fatty liver, making ‘normal’ less reassuring than it appears. Biological variability, time of sampling, recent strenuous exercise, intercurrent infection and medication exposure should be recorded when results are unexpected. Persistently increased aminotransferases require evaluation for alternative and coexisting liver diseases rather than automatic attribution to MASLD. The AASLD guidance explicitly warns that aminotransferases are frequently normal in advanced disease and should not be used alone to exclude clinically significant fibrosis (Rinella et al., 2023b).

4.2 FIB-4: a low-cost gatekeeper

FIB-4 combines age, AST, ALT and platelet count. It was originally developed in HIV/HCV coinfection and was later validated in other chronic liver diseases, including NAFLD (Sterling et al., 2006; Shah et al., 2009). Its enduring advantage is operational rather than molecular: the inputs are routinely available, the equation is transparent and low values have useful negative predictive value in appropriately selected populations. Contemporary pathways commonly treat values below 1.3 as low risk for advanced fibrosis, values from 1.3 to 2.67 as indeterminate, and values above 2.67 as high risk, with an age-adjusted lower threshold of 2.0 for individuals older than 65 years in several guidelines.

Those bands are triage thresholds, not histological boundaries. Age is included because fibrosis risk increases with time, but it can drive false-positive classification in older adults and false reassurance in those younger than 35 years. AST can rise in acute illness or muscle injury; ALT appears in the denominator; and platelet count can fall from immune, marrow, drug or haematological causes unrelated to portal hypertension. Type 2 diabetes also alters performance and concentrates risk within the indeterminate group. FIB-4 should not be calculated during an acute illness and should be followed by a second-line assessment when risk remains (EASL-EASD-EASO, 2024).

The attraction of one universal threshold must be balanced against prevalence. In a low-risk community, most positive FIB-4 results will be false positives even when sensitivity and specificity are unchanged. In a diabetes clinic, a low result carries more residual concern because advanced fibrosis is more prevalent and FIB-4 sensitivity may be lower. The correct question is therefore whether a FIB-4 result changes the post-test probability enough to stop, repeat or escalate. It is not whether the score has crossed a number that can be copied directly into a diagnosis field.

4.3 NFS and APRI: alternatives with specific constraints

The NAFLD Fibrosis Score (NFS) combines age, body mass index, impaired fasting glucose or diabetes, AST/ALT ratio, platelet count and albumin. Its derivation and validation cohorts showed good discrimination and a low cutoff with high negative predictive value for advanced fibrosis (Angulo et al., 2007). Nevertheless, obesity and diabetes are embedded in the equation, creating classification pressure in the very populations now targeted for screening. Missing or non-standard albumin values also complicate automated use. EASL guidance estimates only moderate accuracy for FIB-4 and NFS for advanced fibrosis and notes poor performance in younger adults.

APRI relates AST, its laboratory upper reference limit and platelet count and was originally developed in chronic hepatitis C (Wai et al., 2003). It is simple but inherits variability from both the AST reference limit and non-hepatic thrombocytopenia. In MASLD, FIB-4 is generally preferred as the first-line routine score because its evidence base and pathway integration are stronger. Retaining APRI or NFS can be useful for sensitivity analysis or where existing local protocols have validated them, but calculating multiple correlated scores and treating agreement as independent confirmation exaggerates confidence because the same analytes recur across equations.

Table 3. Routine biochemical scores: intended use and failure modes

Test	Variables / formula	Primary target	Practical value	Main failure modes
FIB-4	$\text{Age} \times \text{AST} / [\text{platelets} \times \sqrt{\text{ALT}}]$	Advanced fibrosis triage	Available and transparent; strong rule-out role	Age, acute injury, muscle AST, non-hepatic thrombocytopenia, T2D
NFS	Age, BMI, glycaemia, AST/ALT, platelets, albumin	Advanced fibrosis	Validated legacy score; two-cutoff strategy	Obesity/diabetes built into score; younger age; albumin method
APRI	$\text{AST} / \text{upper limit} \times 100 / \text{platelets}$	Significant or advanced fibrosis	Very simple; uses routine tests	Laboratory-specific upper limit and non-hepatic platelet reduction
AST/ALT ratio	AST divided by ALT	Crude injury/fibrosis context	Immediate and familiar	Low stand-alone discrimination; alcohol and muscle sources

Thresholds should follow locally adopted guidance and the clinical setting. The equations are shown to clarify biological inputs, not to authorize diagnosis without clinical evaluation.

5. Direct and Multi-Analyte Biomarkers

5.1 ELF: a composite signal of matrix remodeling

The ELF test combines hyaluronic acid, PIIINP and TIMP-1 in a logarithmic algorithm. Hyaluronic acid reflects extracellular matrix production and sinusoidal clearance; PIIINP is released during type III collagen metabolism; and TIMP-1 inhibits matrix metalloproteinases, shifting the balance toward matrix accumulation. These analytes provide a more direct window on fibrogenesis than routine aminotransferases. Cohort and meta-analytic evidence supports clinically useful discrimination and prognosis across chronic liver diseases (Rosenberg et al., 2004; Vali et al., 2020). The 2024 European guidance cites areas under the curve near 0.81 for significant fibrosis, advanced fibrosis and cirrhosis, while AASLD positions ELF as a preferred secondary blood test when FIB-4 is elevated or indeterminate.

ELF is not liver-exclusive. Age, sex and conditions with altered collagen turnover can affect components. Platform-specific calibration matters because the published equation is tied to validated immunoassays. A cut-off chosen to rule out disease prioritizes sensitivity; a higher cut-off chosen for prognosis or cirrhosis prioritizes specificity. The ADA consensus for people with diabetes describes ELF above 9.8 as consistent with increased probability of advanced fibrosis and values above approximately 11.2 as associated with cirrhosis, while AASLD notes that ELF above 11.3 in advanced fibrosis is linked to decompensation risk (Rinella et al., 2023b; Cusi et al., 2025). These are decision-linked probabilities, not interchangeable universal labels.

Recent laboratory work adds an important pre-analytical caution. Bitar et al. (2026) found that haemolysis reduced measured PIIINP and that delayed centrifugation caused marked fluctuation in serum TIMP-1, although the integrated ELF score was comparatively robust in their experimental setting. The study does not invalidate ELF; it illustrates why component stability, specimen matrix, separation time and haemolysis indices belong in validation protocols. A composite algorithm can dampen one component's variability, but that should be demonstrated rather than assumed for each platform and workflow.

5.2 PRO-C3 and ADAPT: active collagen formation

PRO-C3 detects a neo-epitope generated during formation of type III collagen and is intended to reflect active fibrogenesis. Daniels et al. (2019) combined PRO-C3 with age, diabetes and platelet count to create ADAPT. In biopsy-characterized derivation and validation cohorts, ADAPT achieved areas under the receiver-operating curve of 0.86 and 0.87 for advanced fibrosis and generally outperformed APRI, FIB-4 and NFS. A later meta-analysis of PRO-C3 alone found summary areas under the curve of 0.81 for significant fibrosis and 0.79 for advanced fibrosis, with material threshold variation across studies (Mak et al., 2021).

External evidence is encouraging but prevents overstatement. In 851 Asian patients with biopsy-proven metabolic fatty liver disease, ADAPT produced an area under the curve of 0.865 and a negative predictive value of 96.1% for advanced fibrosis (Tang et al., 2022). A 2024 multi-centre study found ADAPT only marginally better than FIB-4 for liver stiffness above 8 kPa and similar for higher stiffness, emphasizing that added assay cost must deliver added decision value in the intended setting (Hegmar et al., 2024). PRO-C3 and ADAPT are therefore promising second-line or trial-enrichment tools; they should not be represented as a universally superior first-line replacement without local cost-effectiveness and calibration data.

5.3 Cell-death and at-risk MASH panels

At-risk MASH, commonly defined in research as steatohepatitis with an activity score of at least 4 and fibrosis stage of at least 2, is a composite target. It requires information about both active injury and established fibrotic risk. MACK-3 combines HOMA-IR, AST and CK-18. The derivation study reported improved discrimination for fibrotic NASH compared with conventional fibrosis scores, but external validation identified a substantial indeterminate zone even when overall accuracy remained useful (Boursier et al., 2018; Chuah et al., 2019). The panel's logic is instructive: insulin resistance supplies metabolic context, AST reflects injury and CK-18 reflects cell death. Its limitation is equally instructive: no component directly stages accumulated collagen.

NIS4 combines miR-34a-5p, alpha-2-macroglobulin, YKL-40 and HbA1c. In a prospective derivation and global validation study, the panel achieved areas under the curve of 0.80 in derivation and 0.76 to 0.83 in external cohorts for at-risk NASH, using separate rule-out and rule-in thresholds (Harrison et al., 2020). The panel spans regulatory RNA, acute-phase/protease inhibition, tissue remodeling and chronic glycaemia. This biological breadth may improve classification, but it also increases dependence on assay standardization, proprietary algorithms and target-definition stability after nomenclature change.

No cell-death or multi-analyte panel should be evaluated only by discrimination. Calibration, indeterminate proportion, failure rate, specimen stability, batch effects and net benefit at a specified disease prevalence are necessary. A high area under the curve can coexist with poor positive predictive value in primary care or with too many missed cases if the first-stage gate is insensitive. Panels are most defensible when the result alters a defined next action: referral, elastography, biopsy consideration, treatment eligibility or research enrollment.

Table 4. Direct and multi-analyte biomarkers mapped to their intended target

Marker / panel	Biochemical components	Primary target	Strength	Principal limitation
CK-18 M30/M65	Caspase-cleaved or total CK-18	Apoptosis/cell death; MASH activity	Mechanistically close to injury	Threshold and assay heterogeneity; moderate stand-alone accuracy
ELF	HA, PIIINP, TIMP-1	Matrix remodeling; advanced fibrosis/prognosis	Validated second-line blood test	Extrahepatic turnover, age, platform and pre-analytics
PRO-C3	Type III collagen formation neo-epitope	Active fibrogenesis	Direct formation marker	Availability, threshold variation and limited low-risk validation
ADAPT	Age, diabetes, PRO-C3, platelets	Advanced fibrosis	Adds clinical context to a direct marker	Cost and incremental value vary by setting
MACK-3	HOMA-IR, AST, CK-18	Fibrotic MASH	Combines metabolism and injury	Large grey zone; insulin and CK-18 standardization
NIS4	miR-34a-5p, alpha-2-macroglobulin, YKL-40, HbA1c	At-risk MASH	Multi-pathway signal with external validation	Proprietary implementation and target-definition transfer

6. Why Diagnostic Performance Changes Across Settings

6.1 Spectrum, prevalence and verification

A biomarker validated in a hepatology cohort encounters a different spectrum from the same assay used in community screening. Referral cohorts contain more advanced fibrosis, more alternative liver disease and more individuals selected because existing tests were abnormal. This can increase apparent discrimination and positive predictive value. Community cohorts contain many low-risk individuals and a narrower biochemical range, making false positives operationally expensive. Predictive values must therefore be recalculated for the intended prevalence rather than copied from a publication.

Reference standards add another layer. Biopsy can classify ballooning, lobular inflammation and fibrosis pattern, but samples only a small tissue volume and has observer variability. Vibration-controlled transient elastography assesses stiffness, which can rise with inflammation, cholestasis and congestion as well as fibrosis. Magnetic resonance elastography samples more tissue but is less accessible. Clinical outcomes are highly relevant but require long follow-up and are influenced by competing cardiovascular and cancer risk. Apparent disagreement between a blood marker and reference method may reflect different biology rather than simple assay failure.

Verification bias occurs when only patients with high non-invasive scores undergo biopsy. Indeterminate and low-risk individuals then lack the reference standard, inflating or distorting accuracy estimates. Incorporation bias occurs when a component of the index test also influences referral or the comparator. Research reports should describe how the reference was assigned, the time interval between measurements and whether analysts were blinded. Clinical users should prefer externally validated pathways that resemble their own population and decision point.

6.2 Analytical and biological variation

Composite scores can appear stable because they are numerical, yet every input has uncertainty. AST and ALT vary within individuals and across analytical platforms; platelet counts are affected by anticoagulant, clumping and non-hepatic disease; albumin methods are not identical; insulin assays used in HOMA-IR lack full harmonization; and immunoassays for CK-18 or collagen fragments depend on antibody specificity and calibration. Rounding, unit conversion and silent equation changes introduce post-analytical error when scores are automated.

Longitudinal interpretation is especially vulnerable. A lower FIB-4 after weight loss may reflect reduced AST before any measurable regression of established fibrosis. A higher ELF value may reflect increased matrix turnover, ageing or another fibrotic condition. The 2024 European guidance recommends considering variability and using the same methods, devices and laboratories for individual follow-up when possible. A clinically meaningful change should exceed expected analytical and within-person biological variation and should be interpreted with changes in treatment, acute illness, weight, glycaemia and platelet count.

6.3 Population transportability and Indian priorities

India combines a high metabolic burden with heterogeneous ancestry, urbanization, diet, healthcare access and laboratory infrastructure. Asian populations may develop metabolic disease at lower body mass index, and lean MASLD can be missed when obesity is treated as the dominant entry criterion. The pooled Indian prevalence estimate of Shalimar et al. (2022) was derived from studies with substantial heterogeneity and older terminology, so it should guide service planning rather than serve as a uniform pre-test probability for every region.

A practical Indian validation programme should include community, diabetes-clinic and hepatology cohorts; urban and rural recruitment; lean and non-lean phenotypes; and prespecified age and sex analyses. Thresholds should be evaluated for clinical utility, not only area under the curve. Relevant outputs include the number referred per advanced-fibrosis case found, false reassurance among people with diabetes, assay failure, turnaround time, cost and loss to follow-up. The value of ELF, PRO-C3 or proprietary panels will depend on whether they reduce inaccessible elastography or biopsy without creating unaffordable repeat testing.

Table 5. Analytical and biological conditions that can alter interpretation

Measurement	Potential disturbance	Consequence	Minimum control
AST/ALT	Exercise, muscle injury, acute illness, alcohol, drugs	Spurious FIB-4 movement or non-MASLD enzyme elevation	Repeat when clinically stable; consider creatine kinase and alternative aetiology
Platelets	Clumping, marrow disease, immune thrombocytopenia, drugs	False elevation of FIB-4/NFS unrelated to portal hypertension	Verify count and smear flags; interpret clinical cause

Measurement	Potential disturbance	Consequence	Minimum control
Albumin	Inflammation, nutrition, renal loss, assay method	NFS change unrelated to hepatic synthetic reserve	Review method and non-hepatic loss/inflammation
Insulin/HOMA-IR	Fasting status and assay non-harmonization	MACK-3 variability across laboratories	Document fasting and platform; avoid cross-method trending
CK-18	Assay epitope, handling and threshold dispersion	Non-comparable rule-in/rule-out values	Use validated assay-specific thresholds
ELF components	Haemolysis, separation delay, age, extrahepatic matrix turnover	Component or composite shift unrelated to liver-stage change	Apply specimen-quality rules and investigate discordance

7. The TARGET-MASLD Framework

Core rule: Do not ask whether a biomarker is globally ‘good’. Ask whether its biochemical signal and threshold are fit for one defined decision in one defined population.

7.1 T — Target the biological condition

The request form, study protocol or clinical question should specify the target: presence of steatosis, active MASH, significant fibrosis, advanced fibrosis, cirrhosis, prognosis or longitudinal change. These are not synonyms. Routine chemistry may identify injury; FIB-4 may exclude advanced fibrosis; ELF may indicate matrix remodeling and prognosis; CK-18 may enrich for cell death; and imaging or biopsy may be required when morphology matters. A laboratory cannot select an optimal marker when the target is written merely as ‘fatty liver test’.

Target definition also determines the acceptable error. Primary-care triage prioritizes sensitivity and negative predictive value because the immediate purpose is to avoid missing advanced fibrosis. Treatment eligibility may require greater specificity or confirmation. Prognosis needs a defined time horizon and outcome. Monitoring requires evidence that within-person change is reproducible and related to outcome, not simply cross-sectional discrimination.

7.2 A — Anchor interpretation to pre-test risk

Pre-test risk should be anchored to age, type 2 diabetes, obesity or central adiposity, alcohol exposure, family history, known steatosis, persistent enzyme elevation and the care setting. Competing diagnoses must remain visible. A score calculated in acute hepatitis, sepsis or uncontrolled heart failure does not answer the stable MASLD question. The nomenclature also requires characterization of alcohol exposure and other causes rather than using metabolic risk as permission to stop aetiological evaluation.

Anchoring prevents the common mistake of applying referral-centre positive predictive values to screening. It also supports equity. A lean South Asian individual with diabetes or visceral adiposity should not be assigned a low pre-test probability solely because body mass index is below a Western obesity threshold. Conversely, widespread opportunistic testing in very low-risk young adults can produce many false positives and unnecessary downstream procedures.

7.3 R — Rule out with a validated routine score

FIB-4 is the default first-line option because it is inexpensive, transparent and embedded in major pathways. Before calculation, the laboratory or clinician should confirm that age and units are correct, aminotransferases were obtained during a stable state and platelet results are analytically plausible. The report should describe low values as indicating low probability of advanced fibrosis in the validated context, not as excluding MASLD or MASH. Young age, diabetes and strong clinical suspicion justify caution despite a low result.

Automated FIB-4 reporting can improve reach but should carry interpretive rules. It should not be released for people outside a locally approved age range, during a flagged acute encounter, or with implausible inputs. The equation version, units and thresholds should be governed as a laboratory calculation with verification, change control and clinical oversight.

7.4 G — Gate second-line testing

Patients with indeterminate or high FIB-4, or retained concern despite a low value, should proceed to a second-line method. VCTE measures a different property and is a common choice. ELF provides a blood-based alternative where imaging access is limited. Hybrid tools such as the FibroScan-AST score combine stiffness, steatosis attenuation and AST for the narrower target of fibrotic steatohepatitis, illustrating that a second-line combination

must be judged against its stated purpose (Newsome et al., 2020). The second-line test should add independent information rather than repeat the same analytes in a different equation. Local resources, patient access, failure rates and intended action determine the preferred gate.

Sequential testing is efficient only if the first gate has adequate sensitivity. A missed case at stage one cannot be recovered by a perfect stage-two test that is never ordered. Pathway audits should therefore report stage-specific attrition and false-negative concentration in diabetes, younger adults and lean phenotypes. High-risk clinics may justify concurrent or lower-threshold strategies, but these require prospective validation because sensitivity gains bring more false positives.

7.5 E — Examine discordance and analytical quality

Discordance is information. A low FIB-4 with high ELF may indicate early matrix turnover, age effects, extrahepatic fibrosis or a false-negative routine score. A high FIB-4 with low stiffness may reflect non-hepatic thrombocytopenia or transient AST elevation. The response should be to verify specimen and inputs, repeat unstable measurements, review alternative diagnoses and choose a method that addresses the unresolved biological target. Averaging discordant scores or selecting the preferred answer is not valid.

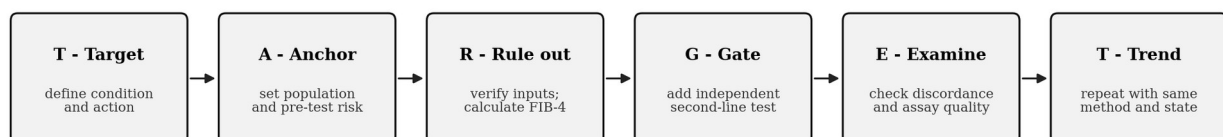
The laboratory contribution is central: haemolysis and lipaemia indices, specimen timing, analyser, lot, units, reference interval and calculation version should be traceable. Research reports should publish thresholds, indeterminate results and missingness. Proprietary panels should provide sufficient analytical documentation for independent verification even when the exact commercial algorithm is protected.

7.6 T — Trend under comparable conditions

Serial results should be compared using the same assay, laboratory and clinical state whenever possible. A change must be interpreted alongside weight, glycaemic control, medication, alcohol exposure, acute illness and platelet count. Rapid changes in injury markers are not equivalent to rapid changes in established fibrosis. Trend categories can inform reassessment, but current evidence is stronger for risk association than for using a specific percentage change as a surrogate endpoint in every setting.

The monitoring interval should reflect baseline risk. Current guidance commonly recommends repeating FIB-4 every one to three years, with more frequent assessment in people with diabetes or multiple metabolic risks. A trend that crosses a threshold should prompt confirmation rather than retrospective relabelling of previous results. Recording the full numerical history is preferable to storing only low/intermediate/high categories because threshold guidance may evolve.

Figure 1. TARGET-MASLD: a decision-linked biochemical assessment pathway



Source: Author's formulation. The framework separates the biological target from the operational sequence so that a result is not interpreted beyond what its analytes measure.

Table 6. Minimum documentation for TARGET-MASLD implementation

Step	Minimum record	Decision question
T — Target	Named condition and action threshold	What biological state must this test classify or predict?
A — Anchor	Risk setting, cardiometabolic factors and competing causes	What is the pre-test probability and what changes it?
R — Rule out	Verified routine chemistry and FIB-4	Is the residual risk low enough to stop or defer escalation?
G — Gate	ELF, elastography or another validated second-line test	Does this method add an independent signal that changes referral?
E — Examine	Specimen quality, confounders, discordance and alternatives	Can analytical or non-MASLD biology explain the pattern?
T — Trend	Same method, stable state and documented interval	Does change exceed expected variation and alter management?

8. Implementation in Clinical Biochemistry

Implementation should begin with laboratory governance rather than a new assay purchase. The laboratory information system can calculate FIB-4 from contemporaneous age, AST, ALT and platelet count, but the build must define eligible age, units, decimal precision, missing-data rules, suppression during acute care and interpretive comments. Verification should use manually calculated cases at low, intermediate and high values, including boundary and implausible inputs. Changes in formula or threshold require version control and clinical communication.

A reflex pathway can reduce loss to follow-up. Where policy and funding permit, an indeterminate FIB-4 could trigger clinician notification or a pre-agreed ELF order; a high value could prompt specialist referral. Reflex testing should not be automatic when the first-stage result may be distorted by acute illness or a non-hepatic platelet disorder. The laboratory should retain the individual analytes with the composite score so that discordance can be investigated.

Quality indicators should extend beyond analytical imprecision. A MASLD pathway should measure the proportion of eligible high-risk patients assessed, invalid calculations, second-line completion, time to referral, fibrosis yield, false-positive burden and subgroup performance. Results should be stratified by age, sex, diabetes and body-mass phenotype. Audit must also test whether people with normal aminotransferases are being systematically missed and whether rural or lower-income patients can complete the second stage.

Introducing ELF, CK-18, PRO-C3 or an omics panel requires a formal context-of-use statement. The laboratory should document matrix, collection tube, stability, centrifugation window, interference limits, calibration traceability, lot comparison, imprecision around clinical cut-offs and referral action. External quality assessment is desirable. When no programme exists, commutable controls and inter-laboratory comparison become especially important. An assay should not enter routine care merely because its derivation area under the curve exceeds that of a simple score.

Reports should communicate probability rather than certainty. Suggested wording for a low FIB-4 is 'low probability of advanced fibrosis in a stable adult within the validated age range; this result does not exclude steatosis or MASH.' For an intermediate result, the report should recommend second-line risk assessment according to the local pathway. For ELF or another direct panel, the comment should name the target and assay-specific threshold. This language prevents a biochemical triage output from being mistaken for a definitive histological diagnosis.

9. Research Agenda

The first priority is prospective pathway validation in India. Studies should recruit consecutive participants at the point where testing will occur, apply the reference standard independently of the index result, and report the full two-stage pathway. Separate estimates are needed for primary care, diabetes clinics and hepatology. Low, intermediate and high risk should be prespecified, and the proportion left indeterminate should be treated as an outcome rather than hidden.

The second priority is analytical harmonization. Multi-centre studies should separate biological from platform variation by using common pools, replicate testing and traceable specimen handling. CK-18 and emerging microRNA or proteomic panels require assay-specific transfer studies. ELF implementation should examine haemolysis, separation delay and extrahepatic fibrotic disease in the local workflow. Score automation requires independent software validation and monitoring for unit or interface errors.

The third priority is decision-curve and economic analysis. Incremental area under the curve does not reveal whether a new panel prevents biopsy, expands referral beyond capacity or improves clinical outcomes. Studies should estimate net benefit, cost per advanced-fibrosis case detected, patient travel and loss to follow-up. Resource-variable comparisons should include FIB-4 followed by VCTE, FIB-4 followed by ELF, concurrent approaches in high-risk diabetes populations and direct referral thresholds.

The fourth priority is longitudinal validity. Serial biomarker studies should measure within-person variation, define the smallest reliable change and relate trajectories to histology, stiffness and hard outcomes. Treatment-response research should distinguish rapid reductions in injury from slower changes in fibrosis. Biobanks should preserve serum, plasma and relevant metadata so that new assays can be evaluated against well-characterized trajectories rather than convenient cross-sectional samples.

Finally, fairness and transportability must be designed into validation. Lean MASLD, women across menopausal status, younger adults, older adults and different Indian ancestries require adequate representation. Algorithms should publish calibration and error distribution across subgroups. Genomic or machine-learning panels should demonstrate improvement beyond routine clinical data and be evaluated for accessibility, explainability and drift before routine deployment.

10. Limitations

This article is a structured critical review and does not provide a new pooled estimate. The search was purposive and focused on clinically influential biomarkers and guidance; less mature candidates may have been omitted. Many included cohorts used NAFLD/NASH definitions and biopsy-enriched samples, creating indirectness when findings are transferred to the MASLD/MASH framework. Diagnostic thresholds are method- and population-dependent and should not be applied without checking the current assay instructions and local policy.

The article concentrates on adult biochemical assessment and gives limited attention to paediatrics, treatment selection, imaging physics and competing chronic liver diseases. It also does not assess a specific Indian laboratory network or calculate cost-effectiveness. The TARGET-MASLD framework is a proposed synthesis that requires prospective evaluation against usual care. Its value should be judged by missed advanced fibrosis, unnecessary referral, patient completion and outcome, not by conceptual coherence alone.

11. Conclusion

MASLD is not represented by one biochemical signal. Steatosis reflects substrate handling; aminotransferases reflect injury; CK-18 reflects cell death; ELF and PRO-C3 reflect matrix turnover; indirect scores combine demographic and routine laboratory correlates; and elastography estimates mechanical tissue consequences. Confusing these targets produces both false reassurance and overdiagnosis.

The evidence supports a sequential strategy in which FIB-4 functions primarily as an accessible rule-out tool and retained risk is clarified with an independent second-line method. The strategy must be modified by age, diabetes, acute illness, platelet disorders, assay quality and pre-test prevalence. Direct and multi-analyte markers add valuable biology, but higher complexity does not remove the need for calibration, transparency and clinical utility.

TARGET-MASLD provides a practical discipline: Target the condition, Anchor the probability, Rule out with a validated routine score, Gate second-line assessment, Examine discordance and analytical quality, and Trend comparable results. For India, the most important advance is not immediate adoption of every emerging panel. It is construction of locally validated pathways in which each test answers a defined question and every result leads to an achievable next step.

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