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PERSPECTIVE

Non-invasive assessment of liver fibrosis in MASLD: the need of sex-adjusted scores

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ABSTRACT

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a spectrum of conditions from simple steatosis to advanced fibrosis that may represent the cradle for hepatocellular carcinoma. Thus, an accurate assessment of fibrosis is critical for patient management. Noninvasive tools, including serum biomarkers and imaging techniques, have emerged as practical alternatives to liver biopsy, which presents limitations for invasiveness, cost, and sampling variability.

Areas covered: In this review, we examined references from relevant articles on PubMed, to investigate the most used noninvasive scores, focusing on their specific applications in various pathological conditions, including those beyond the liver. The application of these tools is particularly vital in challenging subpopulations, where conventional metabolic risk factors may be absent, or to target new therapeutical approaches. Sex-specific differences in hormonal and metabolic profiles, however, influence fibrosis progression and the interpretation of noninvasive tools, necessitating further refinement to optimize their clinical utility.

Expert opinion: Despite these complexities, integrating noninvasive scores with imaging techniques has proven effective in stratifying risk, guiding treatment decisions, and improving long-term outcomes. As research continues to enhance these tools, their routine use in clinical practice represents a cornerstone for the early detection, monitoring, and personalized management of MASLD with sex-specific cutoffs.

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Noninvasive liver fibrosis scores; hepatic steatosis; metabolic syndrome; hepatocellular carcinoma; liver fibrosis

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a range of metabolic disorders in individuals with hepatic steatosis. The diagnosis requires evidence of liver fat accumulation alongside at least one cardiometabolic risk factor among the five criteria for Metabolic Syndrome diagnosis (overweight and obesity, hypertriglyceridemia, low HDL cholesterol, arterial hypertension, impaired fasting glycemia or glucose intolerance) [1]. MASLD varies in severity, from simple fat accumulation in the liver to metabolic dysfunction-associated steatohepatitis (MASH), a more severe form characterized by inflammation and liver cell damage. If left untreated, MASH can progress to advanced fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC). Liver fibrosis is the most critical determinant of adverse outcomes, including liver-related morbidity, mortality, and cardiovascular or neoplastic complications [2]. Therefore, the ability to accurately assess and monitor fibrosis is central to the management of MASLD patients.

While liver biopsy is the gold standard for fibrosis staging, its limitations – including invasiveness, cost, sampling variability, and potential complications – have driven the development of noninvasive methods that rely on serum biomarkers, clinical parameters, and advanced imaging techniques.

Thus, scores based on standard clinical and laboratory data have become essential tools in routine practice for assessing risk and monitoring patients with MASLD. Their main purpose is to identify individuals with hepatic steatosis and fibrosis who may require closer monitoring or therapeutic intervention, while sparing low-risk patients from unnecessary invasive procedures (Figure 1).

Indeed, an algorithmic approach combining noninvasive fibrosis scores with imaging techniques enhances diagnostic accuracy and minimizes the need for liver biopsy. Patients identified as high-risk based on noninvasive indices often undergo TE or MRE for confirmation, with those exhibiting discordant results that are considered for liver biopsy. This structured assessment facilitates early intervention, enabling the timely implementation of lifestyle modifications, pharmacotherapy, and cardiovascular risk management, ultimately reducing the burden of hepatic and extrahepatic complications in MASLD patients [3].

However, since MASLD encompasses a wide spectrum of different phenotypes, future research should focus on refining existing fibrosis scores to improve their predictive accuracy in diverse MASLD patients subgroups, capturing early fibrotic changes before structural liver damage becomes irreversible.

For instance, lean MASLD is increasingly recognized as a distinct disease phenotype, consisting of individuals with liver steatosis and at least one cardiometabolic risk factor other than overweight or obesity. Thus, such patients often making their disease progression less predictable

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Article highlights

- When liver biopsy can not be performed, diagnosing and assessing the grade of injury due to fat infarction (steatosis) and tissue inflammation (fibrosis) is mandatory to prevent evolution to cirrhosis and eventually hepatocellular carcinoma.
- Noninvasive scores, based on laboratory tests and the presence of specific conditions, have been validated to assess liver steatosis and fibrosis grades and have been shown also to predict development of liver and extra-hepatic cancers, cardiovascular diseases and a plethora of pathogenetic conditions.
- While metabolic and hormonal conditions differently affect liver steatosis and MASLD development in males and females, these scoring systems do not consider different cutoff in the two sexes. Thus, their reliability could be improved considering sex-differences.

and challenging to diagnose without the integration of noninvasive fibrosis scores with advanced imaging and direct fibrosis biomarkers [4].

Similarly, identifying patients with 'At-Risk MASH' – typically defined as MASH with a NAFLD Activity Score (NAS) of ≥ 4 and a fibrosis stage of ≥ F2—is of paramount clinical importance since these individuals are at the highest risk for progressing to cirrhosis, developing HCC [5]. Since, the clinical landscape is evolving, particularly with the advent of new pharmacotherapies (e.g. Resmetirom for MASH with moderate to advanced liver fibrosis [6] and the anticipated authorization of GLP-1 receptor agonists [7]), a crucial goal of noninvasive testing is not only to stage fibrosis but also to identify this high-risk subpopulation who would benefit most from therapeutic intervention. This challenge lies in accurately identifying At-Risk MASH without resorting to liver biopsy, choosing instead a multi-step approach that combines various noninvasive methods. This sequential, multi-modal approach is critical for efficiently identifying candidates for new therapies, monitoring treatment response, and ultimately mitigating the significant hepatic and extrahepatic complications associated with At-Risk MASH.

2. Noninvasive liver fibrosis scores: cutoffs, clinical applications, and prognostic implications

The latest clinical practice guidelines for MASLD [3] management advocate for the use of three widely adopted noninvasive fibrosis scores: Non-Alcoholic Fatty Liver Fibrosis Score (NFS), Fibrosis-4 Index (FIB-4), and Aspartate Aminotransferase to Platelet Ratio Index (APRI). Indeed, APRI and FIB-4 have demonstrated strong correlations with histological fibrosis stages [8].

Although not mentioned in the above-mentioned European Guidelines, other noninvasive scoring systems also play a significant role in clinical practice. AST/ALT Ratio (AAR) is another simple but less specific marker of fibrosis, which, when combined with platelet count as in the AST/ALT-to-Platelet Ratio Index (AARPRI), enhances predictive accuracy.

Additional scoring systems, including the Fatty Liver Index (FLI) [9] and the Forns Index [10], consider metabolic markers and lipid parameters to evaluate liver involvement and the likelihood of fibrosis. The Hepamet Fibrosis Score (HFS) also incorporates the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) [11] alongside the BARD score, which integrates Body Mass Index (BMI), the Aspartate Aminotransferase to Alanine Aminotransferase Ratio (AAR), and the presence of diabetes [12] (Figure 2). However, these scores have limitations, particularly in intermediate fibrosis stages, which necessitate further assessment with more specific biomarkers [13].

Since all the above-mentioned scores primarily provide indirect assessments of fibrosis through surrogate markers of liver damage and inflammation rather than fibrogenesis, noninvasive tests based on components of the extracellular matrix turnover have been developed. Including plasma markers such as hyaluronic acid, tissue inhibitor of metalloproteinases-1 (TIMP-1), procollagen III N-terminal peptide (PIIINP), and pro-peptide of type III collagen formation (pro-C3), indices such as the ADAPT and Enhanced Liver Fibrosis (ELF) scores provide a dynamic assessment of fibrotic activity and complement traditional clinical scores [14,15], particularly in patients with discordant results from routine noninvasive tests.

Moreover, by combining quantitative elastography data with metabolic and demographic parameters, some composite scores such as Agile 3, Agile 4 [16], and FibroScan-AST (FAST) score [17] have been proposed especially in intermediate-risk populations.

In parallel, a new class of metabolomics-based diagnostic tools has emerged, leveraging circulating lipid signatures to reflect hepatic lipotoxicity, mitochondrial dysfunction, and systemic metabolic stress. These tests, including the Metabolomics-Advanced StEatohepatitis Fibrosis (MASEF)

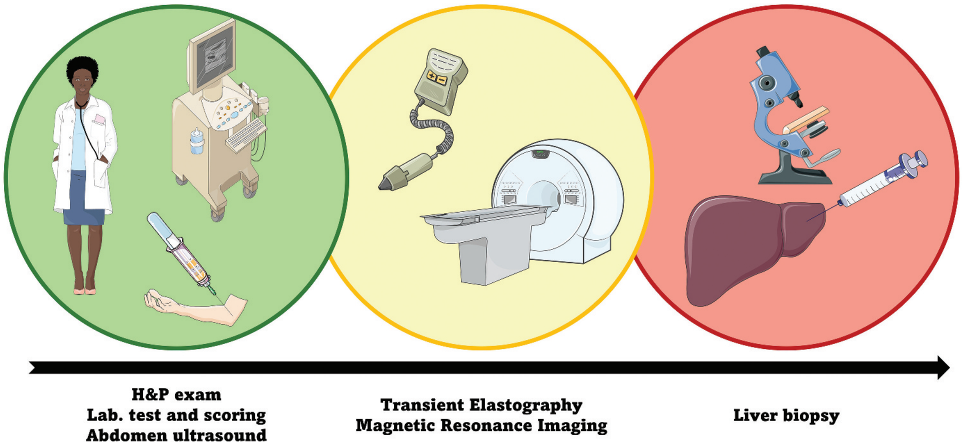

Figure 1. Diagnostic integrated approach in MASLD patients for detection of liver fibrosis.

Abbreviations: H&P exam, history and physical examination.

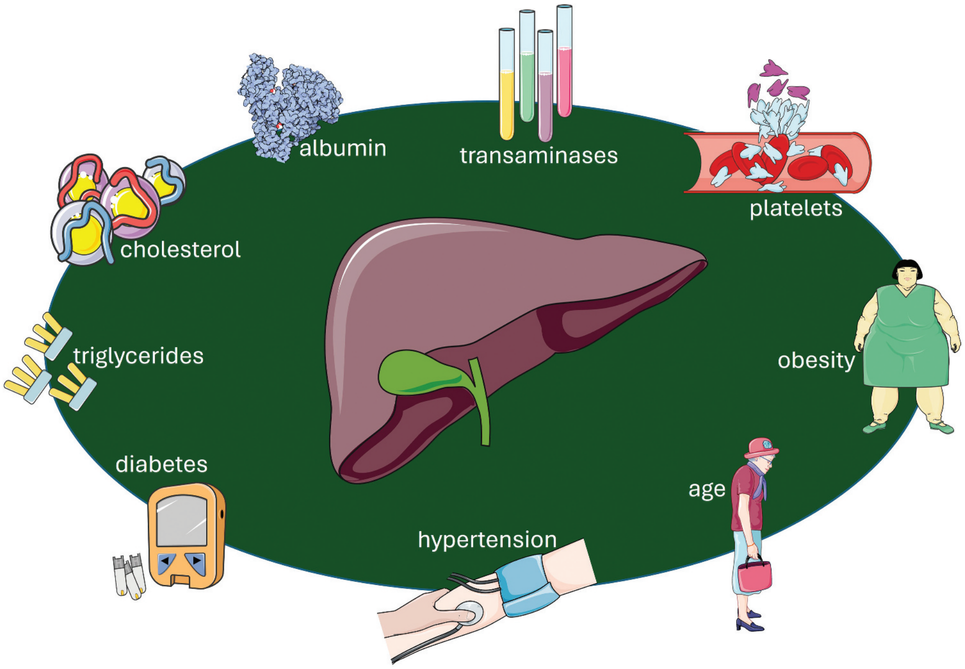

Figure 2. Laboratory test and clinical conditions considered in noninvasive liver fibrosis scores.

score [18] and the OWLiver panel [19], capture pathophysiological mechanisms that precede structural fibrosis, offering early and biologically grounded identification of patients at risk for progressive disease.

Additionally, research efforts continue to refine noninvasive tools by incorporating novel parameters and machine-learning algorithms. Emerging biomarkers, including keratin-18 fragments, cytokeratin-18 (CK18), and micro-ribonucleic acids (miRNAs), have shown promise in enhancing the diagnostic performance of existing scores and are considered in computation of NIS4 (Non-Invasive Steatohepatitis Score) [20] and MACK-3 Score (Metabolic, AST, Cytokeratin-18 Score) [21].

We will now analyze the noninvasive indexes, focusing on their respective cutoffs and their applications in clinical practice. Clinical studies for this review were identified by searches of PubMed, and references from relevant articles were found using the search terms 'noninvasive score,' 'liver fibrosis,' 'MASLD,' 'MASH,' 'liver steatosis.' Abstracts and reports from meetings were included only when they related directly to previously published work. Only articles and/or reviews published in English for the past 20 years were included, and we also reviewed the references of all included papers and relevant reviews. To avoid data entry errors and to establish inter-rater reliability data extraction was performed by at least two authors.

Table 1 provides a summary of all the scores along with their associated formulas.

2.1. Fibrosis-4 index (FIB-4)

Originally proposed by Sterling et al. in 2006 [22], FIB-4 was developed as a tool to assess liver fibrosis in individuals co-infected with hepatitis C virus (HCV) and human immunodeficiency virus (HIV), demonstrating a high negative predictive value in ruling out advanced fibrosis. Over time, it has been validated for use in other etiologies of liver fibrosis, including MASLD [23] and alcohol-associated liver disease [24]. The score incorporates age, AST, ALT, and platelet count to provide an easily calculable stratification of patients based on the likelihood of advanced fibrosis versus minimal or no fibrosis. A score < 1.3 indicates a low likelihood of significant fibrosis, effectively ruling out advanced disease in patients with hepatic steatosis. Conversely, a score > 2.67 strongly suggests advanced fibrosis, and warrants further investigation. In intermediate cases (1.3–2.67), additional testing with elastography or biopsy is recommended [3]. These thresholds have been confirmed as robust across diverse populations, with adjustments for age in older adults (> 65 years) to account for physiological changes in liver enzymes and platelets [25,26]. For Lean-MASLD, defined by the absence of obesity, a study compared the diagnostic efficacy of FIB-4 and NFS in Asian individuals with NAFLD, differentiating between lean and non-lean subjects. Findings revealed that NFS exhibited considerably lower sensitivity in lean patients compared to FIB-4 [27]. This implies that FIB-4 May serve as a more reliable screening tool for detecting advanced fibrosis in lean individuals, despite its overall diagnostic limitations.

FIB-4 has also been studied in the context of extrahepatic conditions, providing prognostic insights into various metabolic and systemic diseases. In patients with NAFLD, FIB-4 values ≥ 2.67 have been linked to an increased risk of cardiovascular (CV) events and mortality [28]. This threshold suggests that higher FIB-4 scores may serve as markers for elevated CV risk in this population. Another study identified a FIB-4 cutoff of 0.85 as a potential predictor of coronary artery disease (CAD) in patients with steatosis [29]. In the context of diabetes, higher FIB-4 scores have been found to be associated with an increased incidence of diabetic retinopathy, neuropathy, and nephropathy, suggesting that FIB-4 could serve as a potential marker for microvascular complications

Table 1. Non-invasive scores for MASLD and liver fibrosis.

Score	Formula
Fib-4	(Age × AST)/[Platelet count × (ALT)^0.5]
NFS	−1.675 + 0.037 × Age + 0.094 × BMI + 1.13 × IFG/Diabetes [yes = 1, no = 0] + 0.99 × AAR – 0.013 × Platelet count – 0.66 × Albumin
APRI	(AST/ULN AST) × 100/Platelet count
AAR	AST/ALT
AARPRI	AAR × 150/Platelet Count
HFS	1/{1 + e^[5.390–0.986 ×Age (45–64 years of age) – 1.719 ×Age (≥65 years of age) + 0.875 ×Male sex – 0.896 ×AST (35–69 IU/L) – 2.126 ×AST (≥70 IU/L) – 0.027 ×Albumin (4–4.49 g/dL) – 0.897 ×Albumin (< 4 g/dL) – 0.899 ×HOMA (2–3.99 with no Diabetes) – 1.497 ×HOMA (≥4 with no Diabetes) – 2.184 ×Diabetes –0.882 ×Platelet count ×1.000/μL (155–219)– 2.233 ×Platelet count ×1.000/μL (< 155)]}
FLI	e^[0.953 × log(Triglycerides) + 0.139 × BMI + 0.718 × log(GGT) + 0.053 × WC – 15.745)] x 100/{1 + e^[0.953 × log (Triglycerides) + 0.139 × BMI + 0.718 × log(GGT) + 0.053 × WC – 15.745]}
BARD	BMI ≥28: No = 0, Yes = 1; AAR ≥ 0.8: No = 0, Yes = 2; Diabetes: No = 0, Yes = 1
FORNS	7.811–3.131 × ln(Platelet count) + 0.781 × ln(GGT) + 3.467 × ln(Age) – 0.014 ×Total Cholesterol
ELF	2.278 + 0.851 × ln(HA) + 0.751 × ln(PIIINP) + 0.394 × ln(TIMP-1)
ADAPT	e^{log[(Age × PROC3)/(Platelet Count)^0.5]} + Diabetes [yes = 1, no = 0]
Agile 3	logit(p) = $\beta_0 + \beta_1 \times$LSM + $\beta_2 \times$Age + $\beta_3 \times$Sex [females = 1, males = 0] + $\beta_4 \times$Diabetes [yes = 1, no = 0] + $\beta_5 \times$AST + $\beta_6 \times$Platelet count
Agile 4	logit(p) = 7.50–15.42/(LSM)^0.5–0.01×Platelets −1.41/(AST/ALT) – 0.53×Sex [females = 1, males = 0] + 0.42×Diabetes [yes = 1, no = 0]
FAST	e^(0.024×LSM +0.0045×CAP −0.026×AST + intercept)/[1 + e^(0.024×LSM +0.0045×CAP −0.026×AST + intercept)]
MASEF	logit(p) = β_0 + Σ(βi × 12 lipid species) + β_BMI×BMI + β_ALT×ALT + β_AST×AST
OWLiver	Proprietary machine learning algorithm based on 11–13 serum lipid species → generates 3 outputs: MASLD (≥0.5), MASH (≥0.5), MASEF index (≥0.33)

Abbreviations: Fib-4, Fibrosis-4 index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NFS, Non-Alcoholic Fatty Liver Disease Fibrosis Score; BMI, Body Mass Index; IFG, impaired fasting glucose; AAR, AST to ALT Ratio; APRI, AST to Platelet Ratio Index ULN, upper limit of normal values; AARPRI, AAR to Platelet Ratio Index; HFS, Hepamet Fibrosis Score; HOMA, homeostatic model assessment; FLI, Fatty Liver Index; GGT, gamma-glutamyl transferase; WC, waist circumference; ELF, Enhanced Liver Fibrosis; HA, hyaluronic acid; PIIINP, procollagen III N-terminal peptide; TIMP-1, tissue inhibitor of metalloproteinases-1; PROC3, pro-peptide of type III collagen formation; LSM, liver stiffness measurement; FAST, FibroScan-AST; CAP, controlled attenuation parameter; MASEF, Metabolomics-Advanced StEatohepatitis Fibrosis; MASLD, Metabolic Dysfunction-Associated Steatotic Liver Disease; MASH, Metabolic Dysfunction-Associated Steatohepatitis.

[30–32]. Recent studies have explored the role of FIB-4 in cancer risk stratification, particularly for hepatocellular carcinoma (HCC). Higher values have been associated with increased HCC incidence and mortality [33–36], as well as with an increased likelihood of HCC recurrence following surgical resection or ultrasound-guided microwave ablation (UGMWA) [37], highlighting its prognostic value in oncology. Emerging data also suggest a potential role for FIB-4 in predicting other systemic malignancies, such as colorectal, breast, and pancreatic cancer [38], including the eventual occurrence of metastasis [39].

2.2. Non-alcoholic fatty liver fibrosis score (NFS)

The NFS was developed by analyzing large cohorts of patients with biopsy-proven NAFLD, to derive a score that incorporates common clinical and biochemical parameters, including age, BMI, hyperglycemia, platelet count, albumin levels, and AAR [40]. Its creation was guided by the increasing prevalence of NAFLD, the metabolic nature of the disease, and the need for a practical tool to facilitate early diagnosis and management. The primary function of the NFS is to identify patients with advanced fibrosis (stages F3-F4) who may benefit from closer monitoring or therapeutic interventions. Studies show that NFS under the cutoff of − 1.455 exclude advanced fibrosis with high sensitivity, while scores above of 0.676 indicate a high probability of advanced fibrosis with specificity exceeding 90%. This dual threshold approach minimizes unnecessary referrals for advanced diagnostic tests and at the same time ensures high-risk patients are appropriately evaluated.

In the context of MASLD, the NFS has been recognized as a valuable tool for assessing fibrosis risk, although recent research suggests that refining its cutoff values based on population characteristics – such as ethnicity and metabolic conditions – could improve its accuracy. Since BMI and age are key components of its calculation, NFS has been observed to yield less reliable results in elderly and obese individuals [41,42]. Additionally, its predictive capability is reduced in patients with significant platelet count abnormalities, such as those with asplenia or a trans jugular intrahepatic portosystemic shunt [43].

Evidence indicates that elevated NFS values correlate with a heightened risk of liver-related complications. Notably, the NFS has been identified as a useful predictor of HCC. A long-term cohort study conducted by Peleg et al. in 2018 demonstrated that individuals with higher NFS values had a significantly greater likelihood of developing HCC over an average follow-up period of 10 years [44]. Specifically, NFS scores above 0.675 were linked to a 2.5-fold increased risk of HCC compared to lower values, underscoring its importance in identifying patients who may benefit from closer monitoring. Furthermore, Treeprasertsuk et al. [45] highlighted NFS prognostic value in the prediction of liver-related mortality; among 302 individuals affected by NAFLD followed for about 8 years, those with NFS values above the high-risk threshold experienced a significantly higher rate of liver-related deaths. This underscores the importance of the NFS as a tool for long-term risk stratification.

The NFS has also been linked to the prediction of extra-hepatic complications, particularly cardiovascular disease. Research conducted by Sanyal et al. (2021) [46] found that individuals with NFS levels exceeding 0.675 had a 3.2 times greater likelihood of developing CAD and 2.8 times higher risk of experiencing major adverse cardiovascular events (MACE), including myocardial infarction and stroke, with statistical significance maintained even after accounting for conventional cardiovascular risk factors such as hypertension and lipid abnormalities. Additionally, prior findings indicated that high NFS values were linked to a 2.5-fold increase (95% CI: 1.9–3.2) in the risk of heart failure when compared to individuals classified as low risk, suggesting the potential utility of this score to identify subjects in the general population who may have a higher likelihood of developing this condition [47].

The NFS has also been implicated in predicting the development of type 2 diabetes mellitus (T2DM). In a study by Chang et al., patients with an intermediate-to-high NFS (> -1.455) had a 4-fold higher risk of incident diabetes over a median follow-up of 5 years. The predictive value of the NFS in this context reflects the shared metabolic pathways driving both NAFLD and insulin resistance [48].

Higher NFS values have been associated with an increased risk of extrahepatic malignancies. A study found that NFS values > 0.675 conferred a 2.7-fold increased risk of colorectal cancer and a 3.1-fold increased risk of pancreatic cancer, highlighting the systemic nature of NAFLD and its metabolic complications.

2.3. Aspartate aminotransferase to platelet ratio index (APRI)

APRI combines readily available laboratory parameters capitalizing on the progressive increase in AST and decrease in platelet counts that occur with worsening fibrosis.

APRI was validated in 2003 in a cohort of patients with chronic hepatitis C who underwent liver biopsy for fibrosis staging [49].

The primary application of the APRI score is to assess the presence of significant (stage F2 or higher) and advanced (stage F3 or higher) fibrosis in patients with chronic liver diseases. A meta-analysis encompassing 40 studies determined that an APRI value exceeding 1.0 had a sensitivity of 76% and a specificity of 72% for identifying cirrhosis. Similarly, an APRI threshold above 0.7 was associated with a sensitivity of 77% and a specificity of 72% for detecting significant liver fibrosis. When using a cutoff of 2.0, specificity for cirrhosis increased to 91%, though sensitivity decreased to 46%. Lower APRI values (below 0.5) enhance the negative predictive value, making cirrhosis less likely, whereas higher scores (above 1.5) improve the positive predictive value, increasing the likelihood of cirrhosis. Intermediate values are less informative, as they do not effectively distinguish between different fibrosis stages [41]. Additional research indicates that combining multiple indices or employing algorithm-based approaches may enhance diagnostic accuracy beyond that of APRI alone [50], including HCC occurrence [51].

In MASLD, metabolic dysfunction significantly influences the parameters used in the APRI calculation. Conditions such as obesity and diabetes, which are prevalent in MASLD, can alter AST levels and platelet counts independently of fibrosis severity, and consequently APRI thresholds validated in other liver diseases may not directly be translated to MASLD population. For these reasons, higher thresholds have been proposed to reduce false positives but still require validation [42]. Conversely, in lean MASLD the metabolic confounders that affect APRI reliability are less frequent, so standard thresholds (> 1.5 for advanced fibrosis) are typically appropriate.

Beyond liver fibrosis, APRI has been evaluated in other pathological conditions. In a cross-sectional study involving 1225 subjects, our research group previously demonstrated the ability of APRI score to determine, when elevated, a significant increase in CV risk for both sexes, especially in females [52]. In a cohort of diabetic patients [53], APRI values were found to be significantly lower in the Sodium-Glucose Transport Protein 2 Inhibitors (SGLT2i) treatment group compared to the non-SGLT2i group, suggesting its reliability as a marker of fibrosis improvement following specific antidiabetic treatment. Additionally, logistic regression analyses identified APRI as an independent positive predictor of MetS in diabetic patients.

Although one study demonstrated that APRI score was not predictive of post-surgical outcomes in cholangiocarcinoma patients, emerging data suggest its potential role in oncological settings [54]. As a matter of fact, in the context of HCC, APRI values above 0.7 were observed to predict late recurrence of HCC after radiofrequency ablation [55] and values $>$ 1.5 are associated with worse prognosis and higher tumor burden, as elevated scores reflect advanced liver disease and portal hypertension. Furthermore, elevated APRI values have been linked to reduced overall survival (OS) and disease-free survival (DFS) in subjects affected by HCC [56]. Notably, in the setting of extrahepatic malignancies, our group previously demonstrated that pathologic APRI values were associated with an almost 7-fold higher risk of cancer (95% CI: 2.0–20.0) in a group of metabolic patients with thyroid nodules [57].

2.4. AST/ALT ratio (AAR) and AST/ALT-to-platelet ratio index (AARPRI)

AAR and its extension AARPRI are noninvasive scores developed to evaluate liver fibrosis. Initially, AAR was introduced in 1957 for the diagnosis of viral hepatitis [58] and was also validated in subsequent studies to assess progressive liver functional impairment [59]. Over time, its utility expanded to assess and stratify fibrosis severity in major chronic liver conditions. AARPRI, incorporating platelet count, was designed to improve sensitivity and specificity for advanced fibrosis detection.

AAR > 1.0 was associated with significant fibrosis, while values > 2.0 might suggest cirrhosis. However, the accuracy reported across studies showed considerable variability, with the positive predictive value (PPV) ranging between 0.64 and 1.00, and the negative predictive value (NPV) falling within 0.72 to 0.88. AARPRI levels exceeding 0.4 have been linked to advanced fibrosis, with research indicating a sensitivity of around 83% and a specificity of 72% at this threshold [60]. A cutoff of 0.2 is considered effective for ruling out significant fibrosis. This low threshold ensures a high NPV, making it a useful tool in screening populations. Moreover, in patients with chronic hepatitis B virus (HBV) and HCV infection, direct associations between AARPRI levels and METAVIR fibrosis stages were observed [61].

Due to their simplicity and ease of calculation, AAR and AARPRI have been employed as noninvasive markers for investigating both hepatic and extrahepatic complications of liver fibrosis. In this line, AAR was analyzed as a predictor of left ventricular dysfunction in patients with heart failure with reduced ejection fraction [62] and, in patients with cirrhosis, AAR > 1.38 corresponded with an incidence of adverse outcomes of more than 20%, including mortality and liver transplantation [63]. Additionally, not only high AAR values were linked to a heightened risk of HCC, but previous studies also suggested AAR as a prognostic marker in patients undergoing thermal ablation combined with concurrent Transarterial Chemoembolization (TACE), with high preoperative AAR associated with poor overall survival [64]. AAR levels were notably higher in people presenting with dysphagia for either both solids and liquids or solids alone, compared to those with dysphagia limited to liquids in a retrospective cohort study involving 951 individuals diagnosed with esophageal carcinoma [65] further correlations included well-differentiated tumor grade, the presence of esophageal strictures on esophagogastroduodenoscopy, and a tumor appearance on computerized tomography (CT) characterized by both circumferential and mural involvement. Besides, AAR greater than 1.0, was found to be poor survival predictor in this population. Moreover, focusing on extrahepatic tumors, abnormal AAR values were associated with the incidence of colorectal [66] and thyroid cancer [57] and, as discussed in 8-years follow-up study conducted by our research group in a cohort of 653 women, AARPRI values exceeding 0.7 were linked to the highest risk of gynecological cancers development (OR = 6) among the other noninvasive scores for liver fibrosis considered [67].

2.5. *Hepamet fibrosis score (HFS)*

The ideation of HFS stems from the growing need for an accurate, reliable, and broadly applicable scoring system tailored to populations with metabolic disorders. Unlike traditional fibrosis scores, which often rely on parameters that are influenced by conditions such as inflammation or acute liver injury, HFS incorporates clinical and biochemical variables specifically relevant to metabolic liver disease. HFS includes age, presence of diabetes, BMI, and liver enzymes, as well as sex and platelet count, reflecting its comprehensive approach. The formula was derived through a large-scale, multi-center study conducted across Europe and Latin America which validated its utility in different populations. Nowadays, its primary use is to identify advanced liver fibrosis (stages F3-F4) in patients with MASLD. Studies have consistently demonstrated its superior accuracy compared to other noninvasive tools such as FIB-4 and NFS. In the pivotal study, HFS demonstrated superior performance in distinguishing patients with and without advanced fibrosis, achieving an AUROC significantly higher than both NFS and FIB-4. In the validation cohort, HFS values below 0.12 effectively ruled out advanced fibrosis, while levels exceeding 0.47 confirmed its presence, with a specificity of 97.2%, sensitivity of 74%, NPV of 92%, PPV of 76.3%, a positive likelihood ratio (PLR) of 13.22, and a negative likelihood ratio (NL) of 0.31, making it both a sensitive and specific tool [11].

Studies have demonstrated that HFS is not only effective in detecting significant fibrosis but also serves as a prognostic tool. Elevated HFS values have been associated with higher risk of liver-related events, extrahepatic conditions, and overall mortality in MASLD patients, underscoring its role in long-term risk prediction. In a study assessing the ability of noninvasive scoring systems in patients with metabolic disorders and hepatic steatosis, HFS performed well for the prediction of HCC and overall mortality [68]. Moreover, in a study involving 168 patients with CAD, a significant difference in HFS between patients with mono-vascular, bi-vascular, and tri-vascular CAD was observed. A linear correlation was also demonstrated between HFS and Syntax score, suggesting the use of HFS as noninvasive tool to be used in the assessment CV risk [69].

Furthermore, in 178 metabolically healthy subjects, without baseline T2DM, arterial hypertension, dyslipidemia, but with biopsy-proven NAFLD, a values of HFS > 0.12, but not abnormal NFS or FIB-4, predicted the occurrence of T2DM [70].

2.6. *Fatty liver index (FLI)*

FLI is a widely recognized noninvasive tool developed to estimate the presence of hepatic steatosis in the clinical setting. Introduced by Bedogni et al. in 2006 [9], the FLI was created by integrating four routinely available parameters [BMI, waist circumference (WC), triglycerides, and GGT], estimating the amount of fat present in the liver with values varying between 0 and 100. For hepatic steatosis, FLI cutoffs are well-established. A score below 30 has been found to effectively rule out steatosis, with sensitivity 87%, while scores above 60 confirm its presence with a specificity above 85%. Encompassing key features of MetS in its formula, FLI plays a pivotal role in stratifying risk for MASLD. In line with previous studies highlighting its accuracy [71–73], our group previously demonstrated that among noninvasive liver fibrosis scores, FLI exhibited the highest accuracy in identifying MASLD in a cohort of 1,069 individuals, with a cutoff value of 44 (AUROC = 0.82) [74]. Additionally, FLI was studied as marker for the assessment of the impact of the Mediterranean Diet on fatty liver and insulin resistance in subjects with NAFLD [75]. In addition, the effectiveness of FLI was demonstrated for other dysmetabolic conditions. Of interest, high levels of FLI were found to be a predictor for the incidence of prediabetes and T2DM in a cohort of 2,020 subjects over a 10 year-follow-up period, conferring a significantly increased risk of developing such conditions above the one conferred by obesity itself [76]. Furthermore, FLI levels > 60 have been associated with higher levels of visceral and cardiac fat, as well as an elevated cardiometabolic risk due to insulin resistance and other features of MetS, including hypertension and dyslipidemia. This highlights the potential benefit of reducing FLI components as a strategy to lower cardiometabolic risk [77]. Additionally, variations in FLI have been linked to fluctuations in the risk of heart failure (HF) and HF-related mortality, as observed in a study involving 240,301 individuals from the National Health Insurance Service database in Korea [78].

Due to the relationship between hepatic steatosis and hormonal disturbances, FLI was also studied in the assessment of many endocrine disorders. Lerchbaum et al. [79], for instance, reported that women with Polycystic Ovary Syndrome (PCOS) exhibited significantly higher FLI values compared to age-matched controls, while fibrosis indices remained comparable. Among women with PCOS, 88.7% of those with MetS had an elevated FLI, compared to 11.3% of those without MetS. In the control group, high FLI values were observed in 66.7% of women with MetS and 30.8% of those without MetS. Similarly, a study including 3,610 individuals identified FLI as the most significant risk factor for low testosterone levels in men, independent of insulin resistance, across various age groups [80].

To date, there are no studies highlighting the accuracy of FLI in the assessment of liver fibrosis; however, a nationwide cohort study in Korea described longitudinal changes in the score associated with risk of HCC [81]. Moreover, an association between pathologic FLI values and the incidence of extra-hepatic tumors such as breast and lung cancer was observed [82,83].

In summary, while other scores focus exclusively on fibrosis, the FLI encompasses steatosis and its metabolic correlates, making it more versatile in early disease stages. Anyways, although the FLI is a simple and cost-effective tool with strong diagnostic reproducibility for screening in clinical settings, its application in clinical practice is limited by its inability to accurately differentiate the severity of steatosis and predict its evolution.

2.7. BARD

The BARD score was first introduced in a study published in 2008 by Harrison et al., which aimed to create a simple, noninvasive scoring system to predict the presence of advanced fibrosis in NAFLD patients. It analyzed the associations between metabolic and biochemical parameters and histologically confirmed liver fibrosis [84].

The acronym BARD represents three variables: BMI, AAR, and the presence of diabetes mellitus. Each component contributes to the overall score, which ranges from 0 to 4.

The BARD score's primary utility lies in its ability to exclude advanced fibrosis, with a score of 0–1 having a high NPV across various studies, including cohorts from the United States, Europe, and Asia [85,86], also demonstrating effectiveness in detecting the absence of hepatic fibrosis in a bariatric population [12]. However, its PPV for advanced fibrosis is limited, necessitating further confirmatory testing for patients with scores of 2–4 [84].

To date, there are no studies validating BARD as an instrument for the stratification of risk for advanced fibrosis in patients specifically affected by MASLD according to the last definition. Moreover, despite its utility, its formula has several limitations. First, the score dichotomizes continuous variables (BMI, AAR), potentially losing important nuances in risk stratification. For instance, a patient with a BMI of 27.9 kg/sqm would score differently than one with a BMI of 28.1 kg/sqm. Secondly, the original study validated the score primarily in a U.S.-based NAFLD cohort and its applicability to diverse populations is less certain. Indeed, since obesity and diabetes are significant drivers of the score, it can be less reliable in populations with lower prevalence of such diseases.

2.8. Forns

The Forns Index is a noninvasive scoring system initially developed to predict the degree of liver fibrosis in patients with chronic HCV disease. It was introduced by Forns et al. in a seminal study published in 2002, which aimed to provide a reliable, simple, and cost-effective alternative to liver biopsy [87]. The index is calculated using four routine clinical parameters: age, GGT, cholesterol levels, and platelet count. The resulting score ranges from approximately 0 to 10, with higher scores indicating a greater likelihood of significant liver fibrosis. The cutoffs proposed in the original study were < 4.2, which reliably excluded significant fibrosis, and > 6.9, which indicated a high probability of significant fibrosis. Patients with scores in the intermediate range require additional testing for definitive diagnosis. Since its inception, the Forns Index has been validated in various populations and liver disease etiologies, demonstrating robust predictive performance in chronic hepatitis patients [88]. However, its application to other liver diseases is less well established, although since its formula encompasses cholesterol levels, emerging evidence suggests that the Forns Index can be a useful tool in the stratification of risk in MASLD patients, particularly for ruling out advanced fibrosis [89].

In terms of assessing complications of liver fibrosis, we previously demonstrated how Forns levels > 7.61 were accurate in the prediction of mortality and severity of COVID-19 in a cohort of hospitalized SARS-COV-2-infected-patients. Just like for the other noninvasive scores considered, in the same study, we observed a significant correlation between Forns levels and days of hospitalization in the whole population [90]. Moreover, a previous study analyzed the association between Forns and incidence of impaired-glicaemic conditions [91]. In the oncological setting, Forns was found to identify patients at low risk of developing HCC after sustained virologic response (SVR) to antiviral therapy for HCV [92], as well as to predict recurrence and overall survival after curative resection of HBV-related HCC [10].

2.9. Enhanced liver fibrosis (ELF) and ADAPT

The ELF and the ADAPT scores are emerging as pivotal tools in the noninvasive assessment of liver disease and related conditions, reflecting advancements in hepatology to improve patient stratification and reduce reliance on invasive methods.

The ELF score incorporates three serum biomarkers reflecting extracellular matrix turnover and fibrosis progression: hyaluronic acid, TIMP-1, and PIIINP. The ELF score was shown to correlate strongly with histological fibrosis staging [93]. The defined cutoffs categorize patients into low, intermediate, and high risk for advanced fibrosis, with values typically set around 7.7 to 9.8 for stratification. Additionally, longitudinal studies have indicated that changes in ELF score over time correlate with disease progression or regression [94], making it a useful tool for monitoring fibrosis dynamics in patients receiving lifestyle or pharmacological interventions.

The ADAPT score [95], developed more recently, combines age, diabetes status, AST, platelet count, and the biomarker PRO-C3, a direct measure of type III collagen formation. ADAPT was validated in the prospective LITMUS study and further refined in trials including the NIMBLE consortium [96]. The proposed cutoffs range from approximately 3.2 for ruling out advanced fibrosis to above 5.0 for high likelihood. Moreover, PRO-C3 as an individual biomarker has shown promise in correlating with grade of histological steatohepatitis and stage of fibrosis, gaining a role as a dynamic marker of disease progression [97].

Both ELF and ADAPT outperform conventional noninvasive fibrosis scores such as APRI, FIB-4, and NFS by incorporating direct markers of fibrogenesis rather than relying solely on surrogate indicators like liver enzymes and platelet count. Indeed, previous studies have highlighted higher sensitivity of these scores in differentiating advanced fibrosis stages, particularly in metabolic patients with hepatic steatosis [98–100]. Furthermore, these scores have been linked to liver-related outcomes, surpassing traditional scores in predicting disease progression and complications such as cirrhosis [101] and HCC [102]. Notably, real-world evidence has begun to support their use in routine clinical settings, with healthcare systems in Europe and America increasingly incorporating these biomarkers into clinical algorithms to guide patient stratification and referral for specialty care [3,103].

The ability of ELF and ADAPT to dynamically assess fibrosis also has implications for drug development, as these biomarkers could be considered for use in clinical trial endpoints for antifibrotic therapies [104]. This has led to growing interest in integrating these scores into precision medicine approaches, allowing individualized patient monitoring and treatment strategies. Studies analyzing cost-effectiveness have further suggested that using ELF and ADAPT in routine practice may reduce the overall economic burden of liver disease by decreasing the need for liver biopsies and improving early identification of high-risk patients. As research continues to validate and refine these biomarkers, their role in liver disease management is expected to expand, ultimately transforming the paradigm of fibrosis assessment and intervention.

While both scores excel in specific domains, their combined use is gaining traction. For example, the specificity of ELF score for advanced fibrosis complements the broader applicability of ADAPT in metabolic disorders, offering a comprehensive approach to risk stratification. As validation studies continue, these scores are poised to play a central role in the multidisciplinary management of liver and systemic diseases.

3. Innovative strategies and future directions

3.1. Imaging-based evaluation of liver fibrosis and integrated algorithms: AGILE 3, AGILE 4, AND FAST score

After the initial risk assessment using noninvasive scores, imaging modalities play a pivotal role in confirming the presence and the severity of liver injury and guiding clinical decision-making. Transient elastography (TE) provides quantitative liver stiffness measurement (LSM), with higher values indicating more advanced fibrosis, as well as quantification of liver steatosis through the controlled attenuation parameter (CAP). This technique is widely accessible and serves as a cornerstone in MASLD management [105]. Magnetic resonance elastography (MRE) offers superior accuracy in assessing fibrosis compared to TE, particularly in obese patients or those with significant hepatic inflammation [106]. MRE provides more detailed mapping of LSM, allowing for better detection of heterogeneous fibrosis distribution. Additionally, contrast-enhanced magnetic resonance imaging (MRI) and ultrasound-based techniques, including shear wave elastography (SWE), complement biochemical scores by providing structural insights into liver architecture and fibrosis distribution [107]. The combination of elastography with serum biomarkers improves diagnostic accuracy, particularly in cases where clinical suspicion remains high despite non-conclusive fibrosis scores.

The evolution of noninvasive diagnostics in MASLD has led to the development of composite algorithms that integrate imaging-based LSM with clinical and biochemical variables, offering enhanced accuracy in fibrosis staging and patient stratification. Among these, Agile 3, Agile 4, and the FAST score represent well-validated and clinically actionable tools that build upon TE by incorporating key demographic and laboratory markers.

Agile 3 is specifically designed to detect advanced fibrosis (≥F3) by combining LSM with age, sex, presence of type 2 diabetes, AST levels, and platelet count. This composite approach addresses major limitations of standalone elastography and traditional fibrosis scores, particularly in patients with intermediate LSM values (e.g. 8–12 kPa), where the risk of misclassification is elevated [108]. Validation studies have demonstrated AUROC values consistently above 0.85, with superior diagnostic performance compared to FIB-4 and NFS. Agile 3 uses a rule-out cutoff of ≤ 0.451—associated with high sensitivity and negative predictive value – and a rule-in cutoff of ≥ 0.679, optimized for high specificity [16]. Patients with scores in the intermediate range warrant further investigation or monitoring.

Agile 4 is based on the same input variables but recalibrated for detecting cirrhosis (F4); it is particularly useful for ruling in cirrhosis in patients under evaluation for HCC surveillance, endoscopic screening for varices, or specialist referral. Agile 4 employs a rule-out threshold of ≤ 0.251, yielding very high sensitivity, and a rule-in cutoff of ≥ 0.565, providing strong specificity [16]. Both Agile scores were developed using large, biopsy-confirmed MASLD cohorts from multicenter studies, ensuring broad external validity and clinical relevance [109]. Notably, the inclusion of metabolic (e.g. diabetes) and demographic (e.g. sex) variables enhances their accuracy in underrecognized phenotypes such as lean MASLD, where isolated LSM values may underestimate disease burden.

Complementing these fibrosis-centered models, the FAST score was specifically developed to identify patients with active fibrotic nonalcoholic steatohepatitis (NASH), integrating LSM, CAP, and serum AST levels to reflect both fibrosis and hepatic inflammation. This dual-pathophysiological focus distinguishes FAST from fibrosis-only scores and enables earlier identification of high-risk patients. In its pivotal validation cohort of biopsy-proven MASLD cases, FAST achieved a high AUROC for identifying NASH with significant fibrosis. FAST employs a three-zone interpretive strategy: < 0.35 effectively rules out fibrotic NASH with high NPV; > 0.67 rules in the condition with high specificity; 0.35–0.67 constitutes an indeterminate zone, where further evaluation (e.g. biopsy or longitudinal follow-up) is warranted [17,110]. FAST has demonstrated consistent applicability in both primary care and hepatology referral settings [111], and is particularly useful in patients with obesity or type 2 diabetes [112], where inflammation may not be reflected in transaminase levels alone. The inclusion of CAP improves estimation of hepatic steatosis, adding further clinical nuance. Because of its reliance on readily available variables (FibroScan and AST), FAST is also cost-effective and easy to implement in real-world practice. Moreover, the FAST score has gained considerable traction in clinical trial design, where it is employed as a noninvasive surrogate endpoint for selecting patients with active fibrotic NASH for investigational therapies. Regulatory agencies increasingly acknowledge its role in phase II/III antifibrotic trials as a screening and monitoring tool [113].

Beyond diagnosis, Agile scores and FAST have shown prognostic value, correlating with clinically meaningful major liver outcomes including hepatic decompensation, portal hypertension, transplant and hepatocellular carcinoma development [114–116], as well as extrahepatic conditions such as cardiovascular events and chronic kidney disease [117]. Their application in clinical trial enrichment and longitudinal disease monitoring is expanding, contributing to the ongoing shift toward precision hepatology.

In summary, Agile 3, Agile 4, and FAST exemplify the convergence of transient elastography with clinical and biochemical data to achieve high-performance, noninvasive diagnostics in MASLD. Their validated cutoffs, integration of clinically relevant variables, and demonstrated utility across diverse settings position them as central components of modern fibrosis stratification algorithms.

3.2. Omics-based scores: MASEF and OWLiver tests

In the expanding field of noninvasive diagnostics for MASLD, also metabolomic analysis have showed hallmark profile representing a promising field to analyze and interpret metabolic signatures in fatty liver disease [118]. For instance, lower plasma concentrations of long-chain fatty acids and higher concentrations of free carnitine, butyrylcarnitine, and methylbutyrylcarnitine were found in MASH patients compared to controls [119]. Furthermore,

lipidomics-based tools have gained attention for their ability to reflect the metabolic and molecular underpinnings of disease progression. The MASEF score is a logistic regression model developed to identify patients with at-risk MASH. It combines 12 serum lipid species – including sphingolipids, ceramides, and diacylglycerols – with BMI, AST, and ALT, capturing both hepatocellular stress and systemic metabolic dysfunction. MASEF was validated in a large, international, biopsy-proven MASLD cohort comprising 790 patients in the derivation set and 565 in the external validation cohort, achieving an AUROC of 0.79, supporting its use as a reliable rule-out tool in clinical settings [18].

Importantly, the MASEF algorithm was developed using the same lipidomic platform that underlies the commercial OWLiver test, created by OWL Metabolomics. OWLiver is a Conformité Européenne (CE)-marked, machine learning – based diagnostic panel that analyzes 11–13 plasma lipid species via ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). From a single serum sample, the OWLiver platform provides three distinct outputs, each associated with a specific disease component: OWLiver MASLD score (values ≥0.50 are consistent with the presence of MASLD); OWLiver MASH score (values ≥0.50 indicate likely steatohepatitis); MASEF at-risk MASH index (values ≥0.33 flag patients as at high risk for MASH and fibrosis ≥ F2) [19].

This structure makes MASEF both a stand-alone fibrosis score and an embedded diagnostic output within the OWLiver panel, offering clinicians a comprehensive, multidimensional molecular profile of liver disease. The three readouts – focused on steatosis, inflammation, and fibrosis – enable early risk stratification and facilitate decision-making regarding surveillance, lifestyle intervention, or eligibility for clinical trials.

By targeting disease-specific metabolic pathways rather than generic markers of liver injury, OWLiver tests support the growing paradigm of precision hepatology. Their ability to detect pathophysiological changes before histological fibrosis becomes irreversible is particularly valuable in lean-MASLD phenotypes [120], where traditional scores often fail to capture early disease activity. Moreover, the analytical reproducibility of lipidomic measurements, even in non-fasting states, enhances their suitability for outpatient workflows.

While access to mass spectrometry remains a current limitation to widespread clinical adoption, ongoing developments in analytical platforms and assay automation are likely to increase availability and reduce costs. In the interim, these tools hold substantial promise as secondary or confirmatory assessments for patients with inconclusive first-line results, and as trial enrichment tools in drug development programs targeting MASH and fibrosis.

4. Sex-specific mechanisms in liver fibrosis: hormonal, metabolic, and clinical implications for non-invasive assessment

4.1. Biological sex-differences in MASLD pathogenesis and assessment

Sex differences have a significant impact on the progression of MASLD and other chronic liver diseases, particularly in influencing the development of fibrotic progression. These differences profoundly affect the performance and interpretation of widely used noninvasive scores for liver fibrosis; thereby, by examining hormonal influences, metabolic patterns, and the impact of comorbidities, we can better understand the nuances of these scores and emphasize the need for future sex-specific applications.

Hormonal divergences are mediated through complex pathways involving nuclear receptors, metabolic signaling, immune modulation, and epigenetic regulation. Estrogens, acting predominantly through estrogen receptor-α (ERα), play a protective role in liver diseases by reducing inflammation, enhancing mitochondrial function, and modulating lipid metabolism. Studies have demonstrated that premenopausal women, who have higher circulating estrogen levels compared to postmenopausal women and men, exhibit slower fibrosis progression in MASLD and viral hepatitis [121]. Mechanistically, estrogens have been shown to inhibit hepatic stellate cell (HSC) activation [122] —a pivotal step in fibrogenesis – by attenuating transforming growth factor-β (TGF-β) signaling, reducing extracellular matrix (ECM) deposition, and promoting ECM degradation through matrix metalloproteinases (MMPs) [123].

Testosterone, on the other hand, plays a dichotomous role in hepatic fibrosis, exerting deleterious effects in men in case of deficiency while promoting fibrotic progression in women through distinct molecular mechanisms. In men, hypogonadism is associated with increased hepatic fibrosis due to the loss of antifibrotic actions, which are primarily mediated by the androgen receptor (AR) [124]. The enhancement of AR signaling in HSCs plays a crucial role in limiting their activation and fibrogenic potential by inhibiting the expression of key profibrotic mediators [125], including TGF-β1 and connective tissue growth factor (CTGF). Additionally, testosterone influences nuclear factor kappa B (NF-κB) pathway [126], leading to a decrease in the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), both of which contribute to HSC activation and ECM accumulation. Moreover, testosterone promotes the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) [127], maintaining HSCs in their quiescent state and preventing their transition into myofibroblasts. Conversely, in women, hyperandrogenism (like in the context of PCOS) promotes hepatic fibrosis through an alternative set of molecular pathways, involving induction of oxidative stress and reactive oxygen species (ROS) generation [128,129] through increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in hepatocytes and HSCs [130]. ROS not only sustain HSC activation but also trigger lipid peroxidation and mitochondrial dysfunction, further aggravating fibrotic progression.

Additionally, hyperandrogenism enhances the production of pro-inflammatory cytokines through activation of the c-Jun N-terminal kinase (JNK) pathway, sustaining a profibrotic microenvironment [131]. In female HSCs, androgen receptor overactivation has been linked to increased expression of collagen type I (COL1A1) and alpha-smooth muscle actin (α-SMA), key markers of fibrosis [132]. Moreover, hyperandrogenism interferes with estrogen-mediated hepatoprotective effects, further exacerbating liver injury [133].

In viral hepatitis, estrogens anti-inflammatory effects have been implicated in slower fibrosis progression. For instance, clinical studies have highlighted that women with HCV infection have lower rates of cirrhosis compared to men, an effect that diminishes after menopause [134]. Estrogens ability to suppress pro-inflammatory cytokine production, inhibit NF-κB activation, and reduce oxidative stress is a likely mechanism [135]. Additionally, estrogen enhances autophagy, a cellular process crucial for clearing viral particles and damaged organelles, thereby mitigating hepatocyte injury [133]. The impact of testosterone in viral hepatitis appears to be more complex. Some studies indicate that testosterone amplifies inflammatory responses and oxidative stress [136], potentially accelerating fibrosis, while others suggest a neutral or protective role under certain conditions [137]. The specific influence of testosterone may depend on the duration of infection, the viral genotype, and the presence of comorbid conditions such as obesity and diabetes.

The interplay between sex hormones and liver fibrosis is further modulated by genetic and epigenetic factors. Polymorphisms in genes encoding ERs or ARs can alter hormone signaling pathways, influencing individual susceptibility to fibrosis [138]. For example, genetic variants in the ESR1 gene, which encodes ERα, have been associated with differential fibrotic outcomes in chronic liver diseases [139]. Epigenetic modifications, such as DNA methylation and histone acetylation, are also influenced by hormonal states and contribute to the regulation of fibrogenic genes [140]. Hormones can modulate miRNA expression, affecting pathways involved in inflammation, ECM remodeling, and HSC activation [141,142]. Furthermore, emerging evidence highlights the role of sex-specific gut microbiota composition – shaped by estrogens and androgens – in modulating systemic inflammation and hepatic fibrogenesis [143,144]. The gut-liver axis, influenced by microbial metabolites such as short-chain fatty acids and bile acids, adds another layer of complexity to the hormonal regulation of liver disease evolution.

In the context of MASLD, different metabolic profiles in men and women further complicate the application of non-invasive scores. These disparities significantly influence the prevalence and pathophysiology of MetS, dyslipidemia, insulin resistance, and obesity, contributing to differential metabolic risk profiles. Women, due to higher estrogen levels before menopause, exhibit a more favorable lipid profile with increased high-density lipoprotein (HDL) cholesterol and lower low-density lipoprotein (LDL) cholesterol. Estrogen upregulates LDL receptor expression in hepatocytes via ERα activation, promoting LDL clearance and reducing circulating atherogenic lipoproteins [145]. Additionally, estrogens enhance PPARα activity, modulating fatty acid oxidation in hepatic and skeletal muscle tissues [146]. In contrast, androgens in men promote visceral adiposity, predisposing them to atherogenic dyslipidemia characterized by increased triglycerides, higher levels of small, dense LDL particles, and lower HDL cholesterol [147]. These variations play a role in the earlier development of cardiovascular disease in men compared to premenopausal women [148]. However, the decline in estrogens levels post-menopause shifts this advantage, leading to a more atherogenic lipid profile in women, with increased LDL cholesterol and triglyceride levels [149].

Insulin sensitivity is another critical aspect where sex differences emerge. Women generally display greater peripheral insulin sensitivity compared to men, attributed to estrogen-mediated effects on glucose metabolism. Estrogens contribute to insulin receptor substrate-1 (IRS-1) phosphorylation [150], which supports insulin signaling, and glucose transporter 4 (GLUT4) translocation in both skeletal muscle and adipose tissue [151], thereby promoting glucose uptake. However, this advantage diminishes post-menopause, aligning the risk of type 2 diabetes with that observed in men [152]. Conversely, men exhibit higher hepatic insulin resistance and greater hepatic glucose production, predisposing them to earlier metabolic dysfunction. Androgens levels modulate insulin signaling pathways through interactions with the phosphoinositide 3-kinase (PI3K)/Akt pathway, influencing hepatic glucose output and lipid metabolism [153]. These discrepancies are also reflected in the prevalence of MetS, which tends to increase with age in both sexes but follows different trajectories, with men affected earlier and women showing a steep rise post-menopause [154]. The relative contributions of hyperinsulinemia, inflammation, and adipokine dysregulation further complicate these sex-specific patterns, highlighting the multifaceted nature of insulin resistance and glucose dysregulation. Body fat distribution is a major determinant of metabolic risk, with men predominantly accumulating adipose tissue in the abdominal (android) region, characterized by increased visceral fat, which is strongly associated with insulin resistance and systemic inflammation. Visceral adipose tissue exhibits greater endocrine activity and secretes pro-inflammatory cytokines, exacerbating chronic low-grade inflammation and further impairing insulin signaling. Women, conversely, store fat preferentially in the gluteofemoral (gynoid) region, a pattern linked to improved metabolic health due to reduced inflammation and enhanced lipid buffering capacity [155]. However, with aging and declining estrogen levels, postmenopausal women experience a shift toward central adiposity, aligning their metabolic risk with that of men [156]. Waist circumference, a surrogate marker of visceral fat, therefore holds different prognostic values across sexes, necessitating the use of sex-specific cutoffs for metabolic risk stratification [157]. In this context, although not proposed as a direct tool for liver fibrosis evaluation, bioelectrical impedance analysis (BIA) may represent a valuable complementary technique. BIA estimates body composition through the analysis of resistance and reactance to low-intensity electrical currents, providing indirect information on visceral adiposity, total fat mass, and skeletal muscle mass. These parameters are increasingly recognized as important modifiers of fibrosis progression [158,159]. Incorporating BIA into the routine assessment of MASLD patients may help identify high-risk phenotypes – such as sarcopenic obesity – who could benefit from more aggressive lifestyle or interventions. Although further validation is required, BIA may enhance the interpretation of fibrosis scores and imaging findings, offering a broader picture of the metabolic substrate driving disease progression.

The differential regulation of leptin and ghrelin, two key hormones involved in appetite control and energy homeostasis, also plays a role in shaping sex-specific metabolic outcomes. Women generally exhibit higher leptin levels, which contribute to enhanced energy expenditure and appetite suppression, while men display greater ghrelin sensitivity, potentially influencing energy intake and weight gain dynamics [160,161]. Leptin resistance, a hallmark of obesity, appears to develop differently in men and women, with variations in leptin receptor (LEPR) signaling contributing to sex-specific metabolic responses [162].

Sex differences also influence laboratory data used in the calculation of noninvasive fibrosis scores. First, several studies have established significant sex-based differences in platelet count and function, with women generally presenting with counts that are approximately 10–20% higher than those observed in men. This difference is particularly evident during the reproductive years, suggesting a potential role of estrogen and other sex hormones in thrombopoiesis [163]. Estrogens have been shown to upregulate thrombopoietin receptor expression on megakaryocytes, enhancing platelet production [164]. Conversely, testosterone is associated with a relative suppression of platelet count, potentially through its effects on bone marrow activity and erythropoiesis, which competes for hematopoietic progenitors [165]. These hormonal influences become less pronounced in post-menopause, where platelet counts in women tend to converge with those of men, indicating a substantial impact of ovarian hormone cycling on platelet physiology [166]. X-linked genes, such as those involved in the regulation of thrombopoiesis and platelet lifespan, may play a role in the observed disparity [167]. Additionally, miRNA expression differs between men and women, influencing platelet reactivity and turnover [168]. Women demonstrate increased platelet aggregation in response to agonists such as adenosine diphosphate (ADP), collagen, and thrombin [169]. This heightened reactivity may underlie the increased risk of thrombotic events observed in premenopausal women, particularly in the context of oral contraceptive use or pregnancy. In particular, the hypercoagulable state observed during pregnancy, characterized by elevated fibrinogen levels and increased platelet activation, serves as a protective mechanism to prevent postpartum hemorrhage but simultaneously increases the risk of venous thromboembolism [170].

Secondly, serum albumin, the most abundant plasma protein synthesized by the liver, demonstrates notable sex-based differences, with men generally exhibiting higher concentrations than women. This disparity is attributed to androgen-induced hepatic protein synthesis and differences in protein metabolism [171]. Estrogens, conversely, promote plasma volume expansion, leading to dilutional reductions in albumin levels [172], particularly in premenopausal women and those using oral contraceptives. During pregnancy, this effect is further exacerbated due to significant hemodilution. Furthermore, aging modulates albumin levels differently in men and women. While both sexes experience a decline in serum albumin with age, men exhibit a steeper reduction, resulting in a convergence of albumin levels in older populations. Postmenopausal women, in contrast, may show relative stabilization of albumin levels due to the cessation of estrogen-induced hemodilution [173]. Additional contributors to sex-related variations in albumin include differences in muscle mass, protein turnover, and nutritional intake, with men typically consuming higher dietary protein amounts. Malnutrition, a well-recognized cause of hypoalbuminemia, may disproportionately affect women in certain populations, further influencing sex-based differences in albumin concentrations [174].

Once again, noninvasive fibrosis scores encompassing laboratory parameters such as albumin and platelet count for their calculation should be adjusted to sex-specific cutoffs due to the inherent differences in these biomarkers between men and women. Applying uniform cutoff across sexes could distort fibrosis assessment, impact clinical decision-making, leading to inappropriate risk stratification and suboptimal patient management.

In summary, all the above-mentioned disparities highlight the necessity of sex-specific adjustments in noninvasive liver fibrosis assessment.

4.2. Sex-specific cut-offs for non-invasive methods: an unmet need

A comprehensive understanding of the above-mentioned sex-based disparities should enhance the use of sex-specific cut-offs for the noninvasive scores calculated starting from metabolic parameters, like BMI, WC, cholesterol levels, and insulin-resistance as well as direct fibrosis-related parameters. Indeed, using a single cutoff value for both sexes may lead to misclassification, potentially underestimating fibrosis severity in one group while overestimating it in the other. Incorporating specific thresholds into fibrosis assessment models would improve predictive accuracy, allowing for a more personalized approach to diagnosing and monitoring liver disease progression in both men and women.

Different thresholds for ELF and FiB-4 have already been proposed to achieve the same sensitivity, specificity and predictive values in two sexes [175]. Some differences between sexes were also found regarding FIB-4 capability of detecting MASLD-associated features by using same thresholds [176]. Indeed, FIB-4 (≥1.45) but not NFS (> 0.676) found liver fibrosis was more prevalent in men than in women [177], while when adopting FIB-4 ≥ 2.67 to diagnose advanced fibrosis among 1,935 patients with liver steatosis, an inverse difference between males and females was found (0.9% of men and 1.0% of women). However, such disparities could probably be explained by analyzing a population without considering potential sex-specific thresholds, and analyzing a population without considering potential sex-specific thresholds probably masks important observations.

Similarly, when testing FLI for MASLD detection based on sex, the cutoff value was notably lower in women (32; AUROC = 0.80) compared to men (60; AUROC = 0.80) [74]. The association between FLI-defined NAFLD and diabetes or hypertension was found significantly stronger in women than in men, possibly indicating that FLI captured the presence of a dysmetabolic state more effectively in females with regard to liver outcomes [178]. However, there is still no sex-stratified performance data (AUROC, sensitivity, specificity, or cutoffs) for the other previously mentioned scores, highlighting the need for further studies.

Sex-related differences deserving consideration extend to imaging-based techniques. While commonly perceived as objective and minimally operator-dependent, modalities such as ultrasound elastography, TE, and MRE may be affected by anatomical and hormonal differences between sexes, potentially influencing diagnostic accuracy. Indeed, higher liver stiffnes was independently associated with male sex [179] and Ramírez-Vélez et al. also proposed different references values for controlled attenuation parameter (CAP) and LSM in two sexes [180]. Furthermore, in ultrasound-based techniques like SWE, and Acoustic Radiation Force Impulse (ARFI) elastography, greater subcutaneous adipose tissue in women – especially in the lower abdominal wall – can interfere with shear wave propagation, leading to reduced measurement reliability or underestimation of LSM [181]. Despite these known limitations, sex-specific cutoffs are rarely implemented, and the potential for sex-related diagnostic misclassification remains largely unaddressed in clinical practice. Similarly, in TE the presence of increased subcutaneous fat in women, particularly in those with obesity or metabolic syndrome, may attenuate vibratory impulses and compromise measurement depth or accuracy [182]. Although the use of the XL probe has improved applicability in such populations, hormonal changes – particularly menopause – may independently affect intrahepatic vascular tone and extracellular matrix stiffness, altering liver stiffness values regardless of true histological fibrosis stage.

These technical and biological sources of variability have practical consequences for composite algorithms that incorporate imaging-derived LSM, such as Agile 3, Agile 4, and the FAST score. Both Agile models explicitly include sex as a variable within their formulas, recognizing its pathophysiological relevance in fibrosis progression. However, and critically, their diagnostic cutoffs remain identical for men and women, with no current sex-specific thresholds implemented in clinical practice. This disconnect between model structure and interpretive thresholds may limit the benefit of including sex, particularly in borderline cases where misclassification risk is highest.

In contrast, the FAST score does not account for sex at all, relying exclusively on LSM, CAP, and AST levels – parameters that may themselves be modulated by sex-related anatomical or metabolic traits. As such, FAST may underestimate fibrosis risk in women with attenuated stiffness propagation or overestimate it in men with modest steatosis and transient inflammation.

Finally, also metabolomic and lipidomic tools introduce a biologically rich but interpretively complex dimension to non-invasive MASLD assessment. These platforms quantify specific serum molecules – such as ceramides, sphingolipids, and diacylglycerols – that reflect hepatic lipotoxicity, mitochondrial function, and systemic metabolic stress. However, the concentrations of these metabolites are not biologically fixed: they are profoundly modulated by sex, age, hormonal status, and energy demands, all of which influence biosynthetic pathways, organ distribution, and enzymatic activity.

Recent metabolomics literature has highlighted consistent sex-related divergence in metabolite distribution across serum, plasma, and urine, with numerous lipids, amino acids, and organic acids showing strong preferential abundance in one sex over the other [183,184]. These differences become even more pronounced during physiological transitions such as menopause, where the loss of estrogenic protection alters mitochondrial efficiency, hepatic substrate utilization, and oxidative stress responses. As a result, premenopausal women often exhibit metabolomic profiles marked by greater mitochondrial resilience and lower circulating lipotoxic intermediates, potentially leading to lower scores on tests like MASEF and OWLiver, despite histological disease. Conversely, postmenopausal women and men, who typically display increased visceral adiposity, sarcopenia, and systemic inflammation, may exhibit disproportionately elevated levels of harmful lipid species – thus amplifying metabolomic scores even in the absence of advanced fibrosis [184].

Despite these differences, most metabolomic algorithms have not yet been validated in sex-stratified cohorts and seldom incorporate menopausal status or hormonal therapy in their analysis. This represents a critical limitation in the context of precision hepatology, especially considering that tools like OWLiver tests may guide clinical trial and treatment eligibility or risk stratification in settings where liver biopsy is not feasible.

The integration of sex-specific cutoffs into clinical practice would require validation in large, diverse cohorts, ensuring that these adjustments are both sensitive and specific across populations. Additionally, further research is required to refine predictive models by incorporating sex-stratified thresholds or integrating additional metabolic parameters that capture the dynamic hormonal influence on liver health. Understanding these sex-based metabolic variations is crucial not only to improve the stratification of fibrosis risk and inform clinical decision-making, particularly in populations with significant hormonal or metabolic differences, but also to promote a personalized clinical management of chronic liver diseases and their consequences based on sex-specificity. Indeed, therapeutically, the differential roles of estrogens and testosterone in liver disease progression offer potential avenues for intervention. Hormone replacement therapy (HRT) in postmenopausal women has shown mixed results, with some studies suggesting benefits in reducing MASLD severity and fibrosis [121,185], while others caution against potential adverse effects such as increased thrombotic risk [186]. In men, testosterone replacement therapy (TRT) has demonstrated improvements in metabolic parameters, reductions in steatosis, and enhanced muscle mass, though its impact on fibrosis requires further investigation [187,188]. Combination therapies targeting both estrogen and androgen pathways may offer synergistic benefits. For instance, dual modulation of ERα and AR signaling could optimize anti-inflammatory and anti-fibrotic outcomes while minimizing adverse effects. The development of drugs targeting androgen and estrogen signaling pathways in a tissue-specific manner holds promise for addressing sex-related disparities in MASLD and liver disease outcomes.

5. Conclusions

Noninvasive liver fibrosis scores play a pivotal role in the management of patients with MASLD by providing a practical, cost-effective, and widely accessible alternative to liver biopsy. These tools enable early identification of fibrosis and stratification of patients at risk for severe outcomes, allowing timely interventions such as lifestyle modifications or targeted therapies. Their integration with imaging techniques further enhances diagnostic accuracy, reducing the need for invasive procedures while preserving resources and minimizing patient discomfort.

The integration of sex-specific differences in liver fibrosis progression represents a crucial step toward enhancing the accuracy of noninvasive diagnostic tools and improving patient outcomes. Hormonal, metabolic, and clinical disparities between men and women significantly influence disease development, response to treatment, and the performance of fibrosis scores. By adopting sex-adjusted cutoffs and incorporating additional sex-specific parameters into noninvasive assessments, healthcare providers can ensure more precise risk stratification and tailored management strategies.

Future research should prioritize the validation of sex-specific thresholds and further explore the complex interplay of hormones, metabolism, and genetics in liver disease. This personalized approach holds the potential to reduce diagnostic errors, optimize therapeutic interventions, and advance equity in the clinical management of MASLD and liver fibrosis across diverse patient populations.

5.1. *Expert opinion section*

The research on noninvasive liver fibrosis assessment in MASLD holds significant implications for clinical practice, particularly in diagnosis, treatment, and healthcare economics. While liver biopsy remains the gold standard, it has notable limitations, including invasiveness, high costs, and variability in results. The emergence of noninvasive tools, such as FIB-4, APRI, ELF, ADAPT, and the Hepamet Fibrosis Score, provides a practical alternative that can facilitate earlier detection and better risk stratification.

The use of these tools in routine clinical practice could improve patient outcomes by enabling earlier interventions and reducing the burden of liver-related complications. Moreover, combining noninvasive scores with imaging techniques like TE and MRE can enhance diagnostic precision and help tailor treatment strategies (Agile3, Agile 4, FAST score). However, despite these advancements, noninvasive methods have not yet achieved universal adoption. Barriers to implementation include variability in cutoff values, limited validation across diverse populations, and the need for further refinement of sex-specific considerations in fibrosis progression.

Despite the promise of noninvasive scores, several challenges persist. One primary concern is the accuracy of differentiating fibrosis stages, especially in intermediate cases. Many existing scores rely on surrogate markers of liver damage rather than direct indicators of fibrogenesis, which can lead to misclassification.

Furthermore, sex-specific metabolic and hormonal differences play a significant role in fibrosis progression, but most noninvasive scores do not account for these variations. Studies show that estrogen provides a protective effect against fibrosis in premenopausal women, while androgens may accelerate fibrosis in some conditions. Adjusting fibrosis cutoffs based on sex-specific metabolic profiles could improve the predictive accuracy of these tools.

Technological and methodological limitations also impede progress. While machine learning algorithms and emerging biomarkers, such as cytokeratin-18 and micro-RNAs that are considered in computation of NIS4 (Non-Invasive Steatohepatitis Score) and MACK-3 Score (Metabolic, AST, Cytokeratin-18 Score), offer promising improvements, they require extensive validation before widespread adoption. Additionally, the cost-effectiveness of combining multiple noninvasive modalities needs further exploration to optimize clinical decision-making.

The field of noninvasive fibrosis assessment is rapidly evolving, and further research holds substantial potential for refining current methodologies. Large-scale, multi-center validation studies are necessary to establish standardized cutoffs for different patient populations. Integrating artificial intelligence (AI) into fibrosis assessment could enhance predictive accuracy by analyzing complex datasets, including genetic, metabolic, and imaging parameters.

Future research should also address the dynamic nature of fibrosis. Moving beyond static cutoffs, we need tools that can track disease progression and regression over time. Including noninvasive markers in clinical trial endpoints for antifibrotic therapies could accelerate drug development and improve patient outcomes.

While noninvasive fibrosis assessment remains a cornerstone in MASLD management, research should also explore novel therapeutic targets to halt or reverse fibrosis progression. Areas like the gut-liver axis, epigenetic modifications, and immune-mediated pathways offer exciting avenues for potential intervention.

Moreover, refining noninvasive methods for lean MASLD patients – who often lack traditional metabolic risk factors – is crucial for ensuring early detection in this challenging subpopulation. Integrating fibrosis scores with metabolic health indicators, including insulin resistance markers and lipid profiles, could enhance risk stratification for these patients.

Looking ahead, noninvasive fibrosis assessment is likely to become the standard of care in MASLD management, potentially replacing liver biopsy in most cases. In five to ten years, we anticipate a more personalized approach, with fibrosis scores tailored to sex, age, and metabolic profile. AI-driven decision support tools could further refine risk stratification and treatment recommendations.

Additionally, the widespread adoption of digital health platforms may facilitate remote fibrosis monitoring, enable earlier interventions and reduce the need for hospital-based assessments. The shift toward precision medicine, incorporating genetic and biomarker-driven stratification, will likely redefine MASLD management, improving long-term patient outcomes while optimizing healthcare resource utilization.

The continuous refinement of noninvasive fibrosis assessment tools represents a transformative shift in MASLD diagnosis and management. By addressing current limitations, embracing emerging technologies, and integrating personalized approaches, the field is moving toward a more efficient, accurate, and patient-focused paradigm. In the coming years, integrating sex-specific algorithms, AI-driven predictive models, and novel biomarkers will likely redefine fibrosis assessment, ensuring better outcomes for patients worldwide.

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