Emerging concepts in the detection of liver fibrosis in non-alcoholic fatty liver disease

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Review

Emerging concepts in the detection of liver fibrosis in non-alcoholic fatty liver disease

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ABSTRACT

Introduction

The non-invasive identification of liver fibrosis related to Non-Alcoholic Fatty Liver Disease is crucial for risk-stratification of patients. Currently, the reference standard to stage hepatic fibrosis relies on liver biopsy, but multiple approaches are developed to allow for non-invasive diagnosis and risk stratification. Non-invasive tests, including blood-based scores and vibration-controlled transient elastography, have been widely validated and represent a good surrogate for risk stratification according to recent European and American guidelines.

Areas covered

Novel approaches are based on 'liquid' biomarkers of liver fibrogenesis, including collagen-derived markers (PRO-C3 or PRO-C6), or 'multi-omics' technologies (e.g. proteomic-based molecules or miRNA testing), bearing the advantage of tailoring the intrahepatic disease activity. Alternative approaches are based on 'dry' biomarkers, including magnetic resonance-based tools (including proton density fat fraction, magnetic resonance elastography, or

corrected T1), which reach similar accuracy of liver histology and will potentially help identify the best candidates for pharmacological treatment of fibrosing non-alcoholic steatohepatitis.

Expert opinion

In the near future, the sequential use of non-invasive tests, as well as the complimentary use of liquid and dry biomarkers according to the clinical need (diagnosis, risk stratification, and prognosis, or treatment response) will guide and improve the management of this liver disease.

KEYWORDS

collagen-derived biomarkers x | liver fibrosis x | Non-Alcoholic Fatty Liver Disease x | non-invasive test x | magnetic resonance elastography x | PRO-C3 x | PRO-C6 x | proteomics x

1. Introduction

In the Non-Alcoholic Fatty Liver Disease (NAFLD) landscape, the identification of liver fibrosis stages represents a cornerstone of clinical practice. Liver fibrosis is the main determinant of prognosis in the NAFLD population, leading to increased incidence of overall mortality, liver-related events as well as extrahepatic outcomes (including cardiovascular disease). With a worldwide estimated 30% of NAFLD burden in the adult population, the non-invasive assessment of liver fibrosis for population screening purposes is one relevant unmet need, with liver histology still required for the identification and staging of fibrosis. Multiple surrogate scores using readily available clinical-biochemical variables, along with the use of elastography techniques, are the current strategies for first-line risk stratification, with the aim of a better selection of candidates for liver biopsy. More recently, blood-based fibrogenesis markers, raised from large proteomic studies, or magnetic resonance-based tools are being investigated in different cohorts to assess a potential role for either liver disease staging or longitudinal evaluation (including the prognostic value or treatment response).

In this review, we will discuss the current evidence of the most validated tools to non-invasively identify liver fibrosis, and we will focus on novel and most promising biomarkers that are currently being investigated.

2. Current paradigms in NAFLD

2.1. Prevalence and risk factors

NAFLD has emerged as one of the leading chronic liver diseases with an estimated prevalence of 25% globally [1]. More recent analyses report an increase in prevalence to more than 30%, with even higher numbers in Latin America and Middle-East-North Africa [2]. An elevated prevalence of NAFLD is also seen in patients with chronic diseases, such as an infection with human immunodeficiency virus (HIV) [3] or psoriasis [4]. Despite an increasing prevalence with higher age [5,6], rising numbers are already seen in children and adolescents [7].

The term NAFLD compromises two distinct disease stages histologically: non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is defined as the presence of > 5% hepatic steatosis within hepatocytes without signs of injury. In contrast, NASH marks an active inflammation

of liver tissue with infiltration of immune cells, hepatocyte damage (i.e. hepatocyte ballooning), and the development of liver fibrosis [8]. A major driver of disease progression in NAFLD/NASH is the degree of fibrosis. Disease progression subsequently increases the risk to develop end-stage liver disease, namely liver cirrhosis and hepatocellular carcinoma (HCC) [9]. With a decline in chronic liver disease due to chronic hepatitis B and C virus infection as a result of major improvements in the treatment, NAFLD as well as alcohol-associated liver disease (ALD) will become the predominant cause to develop liver cirrhosis within the next 20 years [10]. NASH along with ALD has already become the leading cause of liver transplantation in the United States [11].

Despite evidence of steatosis on histology or imaging for a diagnosis of NAFLD, alcohol consumption should be below a threshold of <30 g/day (male) and <20 g/day (female) according to current practice guidelines [12]. In addition, secondary causes of liver injury (i.e. chronic viral hepatitis or other chronic liver diseases) need to be ruled out [13]. However, to quantify the extent of histological changes in NASH, a liver biopsy is needed. In recent years, non-invasive tests (NITs) have become increasingly available to identify patients with steatosis and liver fibrosis or steatohepatitis without the need for a liver biopsy.

A sedentary lifestyle along with poor dietary habits have led to a dramatic rise in the prevalence of obesity and type 2 diabetes (T2DM) in the general population. In addition to visceral obesity and insulin resistance/T2DM, other metabolic risk factors including hyperlipidemia and arterial hypertension, which are all components of the metabolic syndrome (MetS), are known risk factors of NAFLD. In particular, NAFLD and T2DM are closely associated with each other with a prevalence of NAFLD and advanced fibrosis of more than 55% and 17%, respectively, in patients with T2DM [14]. As a result of the close association of NAFLD with metabolic risk factors, the term metabolic dysfunction-associated liver disease (MAFLD), primarily focusing on metabolic abnormalities without the strict exclusion of high alcohol intake and secondary causes, has been suggested [15]. In this context, a discussion around the use of metabolic dysfunction-associated liver disease (MAFLD) has emerged. While the broad field appreciates the positive definition and reduction of stigma, the lack of a clear definition of metabolic risks has more recently led to the context of steatotic liver disease (SLD) as an overarching term [16]. Moreover, NAFLD can have a significant impact on health-related quality of life [17]. If patients with NAFLD are not detected at an early stage, adverse outcomes may even impose a substantial economic burden on healthcare systems [18].

Nevertheless, the main prognostic factor in patients with NAFLD remains the stage of fibrosis. More importantly, fibrosis is also linked to extrahepatic comorbidities and is associated with overall higher mortality [19]. In the progression of NAFLD, NASH has become one of the highest risk factors to develop liver cirrhosis and HCC, especially in those patients with underlying T2DM. In the majority of patients with NAFLD, a higher incidence of cardiovascular disease is the main driver of mortality [20]. Especially the stage of fibrosis is closely associated with the occurrence of ischemic heart disease [21]. However, several other comorbidities have also been identified to be associated with NAFLD, including chronic renal disease [22], anxiety/depression [23], dementia [24], and extrahepatic cancer [25].

2.2. Pathophysiology of liver fibrogenesis

Liver fibrosis is characterized by progressive accumulation of extracellular matrix (ECM), resulting from the imbalance of pro-fibrogenic and anti-fibrogenic mechanisms on a protracted liver injury (Figure 1). ECM is mainly composed of fibrillar collagens, non-collagenous glycoproteins, glycosaminoglycans such as hyaluronic acid, and proteoglycans. In liver fibrosis, the ECM undergoes both qualitative and quantitative remodeling, with collagen being the principal component of the fibrotic scar, which mainly consists of fibrillar type I and type III collagen [26]. Several factors regulate ECM deposition and clearance, such as the balance between fibrolytic matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs).

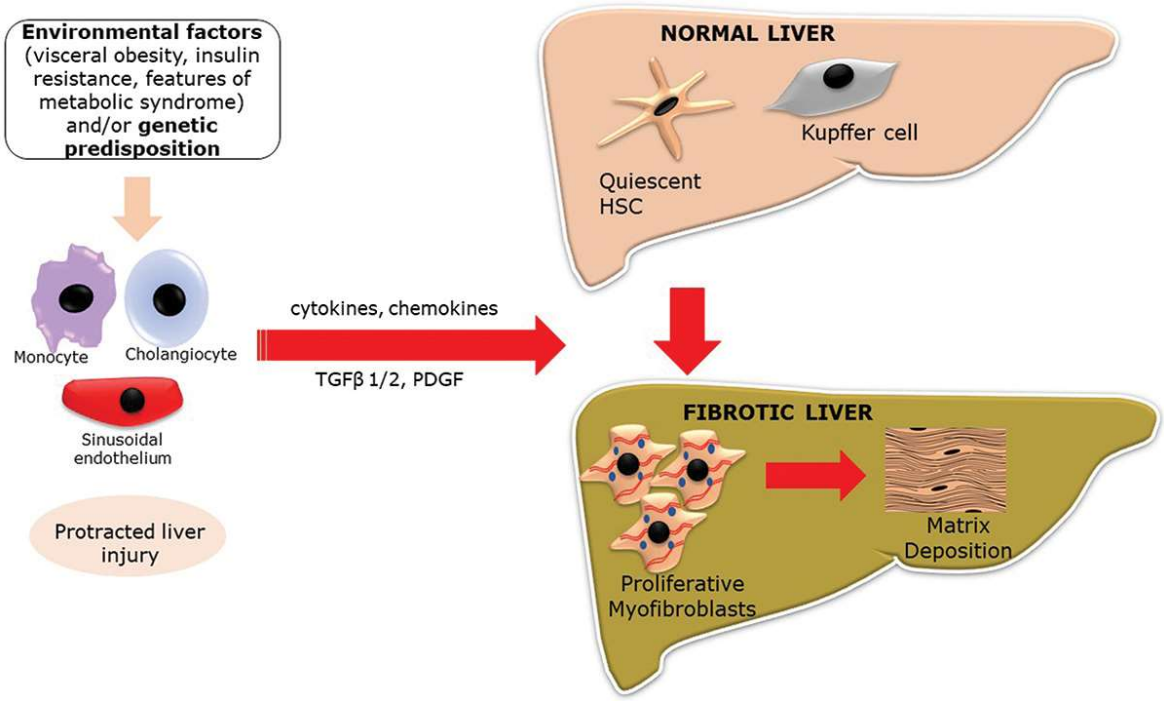

Figure 1. Simplified scheme of mechanisms leading to liver fibrogenesis. See text for details. Abbreviations. TGF: transforming growth factor; HSC: hepatic stellate cells; PDGF: platelet-derived growth factor.

During ECM turnover, which is notably increased in the fibrotic liver, a range of collagen-derived fragments resulting from fibrogenic and fibrolytic processes are released into the circulation and are involved in different signaling functions. For instance, during type III collagen formation the cleavage of the N-terminal propeptide of interstitial procollagen by specific proteases produces the Procollagen III N-terminal propeptide (PIIINP) and the N-terminal type III collagen propeptide (PRO-C3). Conversely, Matrix metalloproteinase-degraded C3 (C3M) is produced as a result of type III collagen degradation by MMPs [27].

Hepatic stellate cells (HSCs) are the main myofibroblast progenitor cells and their conversion from a quiescent phenotype into proliferative, contractile, and fibrogenic myofibroblasts represents a key event in fibrogenesis. The activation of HSCs is controlled by multiple soluble mediators, including PDGF (Platelet-Derived Growth Factor) and Transforming Growth Factor-β (TGFβ), released by inflammatory cells in response to hepatocyte stress and death, as in chronic liver diseases [28]. Among these cells, liver macrophages play a central role in the wound healing process, comprising resident Kupffer cells and monocyte-derived macrophages, mostly recruited by interactions

between C-C chemokine receptor types 2 (CCR2) and 5 (CCR5) and their ligands. While in the healthy liver, the majority of macrophages are resident Kupffer cells, in the setting of NASH, numerous studies show the presence of heterogeneous subtypes of macrophages, including the Ly-6Chi macrophages, which secrete profibrogenic molecules including TGFβ, and the Ly-6Clo population of restorative macrophages, and promoting HSC apoptosis and ECM degradation by increasing the production of matrix metalloproteinase (MMP) −9, −12, −13 [29].

Apparently, fibrogenesis in NASH does not follow a progressive and linear path from steatosis to fibrosis but appears to rather result from repetitive peaks of inflammation followed by an anti-inflammatory reparative immune response, reflecting the natural history of the disease. Macrophage activation at different stages of liver disease is an important feature in fibrosis development and progression. In acute bouts of inflammation, as in the early phase of liver injury, mostly pro-inflammatory and fibrolytic Ly-6Chi macrophages are recruited [30].

During the progression of the disease, characterized by repeat recovery phases alternated with peaks of inflammation, macrophages switch to a most anti-inflammatory profibrogenic Ly-6lo profile, as an attempt to resolve inflammation while repairing tissue injury, resulting in progressive fibrosis. Accordingly, during a long-term reparative phase, fibrolytic Ly 6Clo phenotype predominates, explaining the possibility of fibrosis regression in the fibrotic liver. Thus, it seems that the length of the recovery relative to the inflammatory periods may finally determine the extent of fibrosis progression, rather than linear disease progression [31]. In this context, a concept of inflammation-associated fibrogenesis (‘hot fibrosis’), as well as progressive fibrosis from interaction of activated stellate cells in the absence of tissue inflammation (‘cold fibrosis’), has been proposed.

Several studies suggest that the natural course of NAFLD is dynamic and characterized by increasing and decreasing disease activity over time, reflecting changes in histologic features of disease activity and fibrosis progression or regression [32]. NAFLD fibrosis progresses by approximately one stage every 14 years, with NASH fibrosis getting worse by one stage every 7 years. Among patients who develop progressive disease, 1 out of 5 are classified as ‘rapid progressors’ [33]. Besides, progression is not linear and can be different according to the baseline stage of the disease [31]. For instance, we know that fatty liver alone without ballooning or fibrosis, for a long time considered a nonprogressive liver disease, can progress to significant and advanced fibrosis. In those patients, diabetes was an independent predictor of fibrosis progression [34]. Apart from metabolic risk factors, multiple other factors, including age, comorbidities, and genetic and microbiome changes, have been identified as predictors of progression to cirrhosis, making the natural history of NASH less predictable than other chronic liver diseases [31].

Likewise, there is evidence of regression of the disease. In a large meta-analysis of 2015 including 411 biopsy-proven NAFLD patients, 22.3% had an improvement in the fibrosis stage without specific treatment intervention [33]. Moreover, prospective studies on patients who underwent weight loss through lifestyle changes or bariatric surgery have demonstrated not only that NASH is reversible, but that this regression is accompanied by fibrosis improvement [35].

2.3. Rationale for liver fibrosis detection in NAFLD

The need for proper identification and staging of liver fibrosis rises from its prognostic value in the NAFLD population. In fact, liver fibrosis of any stage has been associated with all-cause mortality and hard hepatic outcomes, including liver transplantation, and with hazard ratios (HR) ranging from 1.88 at fibrosis stage 1 to 10.9 at stage 4 following a stepwise increase [36]. Data extrapolated from the Simtuzumab trial, addressing either F3 patients to prevent progression to F4, or cirrhotic patients to prevent decompensation, found that about 20% of patients progress to cirrhosis within 2 years, with equal proportions of patients developing decompensation in the same time frame [37]. A large meta-analysis reported an increase in the incidence of liver-related mortality by fibrosis stages, with mortality rate ratios ranging reaching 42.3 for stage 4 [38], even after adjustment for confounding factors [39,40]. In the context of progressive dysmetabolic liver disease, the presence of NASH seems to have little impact on prognosis, when compared to fibrosis stages across a long-term follow-up [41,42]. This may have more pronounced evidence for advanced stages of fibrosis, where the histological features of liver injury are less represented due to the enhanced fibrogenesis process ('burnout' cirrhosis).

Since NAFLD is the hepatic expression of the metabolic syndrome, the burden of metabolic co-factors has a great impact on long-term prognosis, in particular, when considering cardiovascular (CV) and non-hepatic cancers. The incidence of CV events, either fatal or non-fatal, represents the most frequent cause of mortality in NAFLD, even in advanced fibrosis [43], while liver-related outcomes have higher incidence only in F4 patients [44], or restriction to severe (F3-F4) fibrosis [9]. A large Swedish population-based cohort study found that the risk for major CV outcomes was increased among NAFLD patients, with a higher incidence in cirrhotic individuals [45]. In the same cohort, the excess mortality was mainly due to extra-hepatic cancers, followed by liver-related outcomes, with a stepwise increase from simple NAFL to cirrhosis [46].

Taken together, these findings have highlighted the importance of fibrosis staging for patient management. Liver biopsy remains the reference standard for fibrosis staging, despite its 'imperfect' nature within this scope [47]. Liver biopsy is burdened by high costs, limited accessibility, and adverse events (including hemothorax) that can arise in up to 3% of cases. In addition, sampling variability and high inter-observer agreement, even among highly experienced pathologists, reduce its repeatability [48]. In fact, given a sampling variability of 30%, a study by Mehta et al. showed that the Area Under the Curve (AUROC) of surrogate markers cannot exceed 0.88. Removing the sample variability, most current NITs would have already achieved excellent AUROC above 0.9 [49].

Nevertheless, a great effort has been undertaken by the scientific community to explore multiple approaches to non-invasively stage liver fibrosis, exploiting both 'static' and 'dynamic' markers, alone or in combination with complex algorithms or as complimentary assessments.

3. How to identify liver fibrosis?

3.1. Non-invasive tests: transient elastography, most validated scores, and their limitations

Non-invasive tests (NITs) have become valuable tools in the assessment of fibrosis in patients with NAFLD. These serve the purpose to replace the need for liver biopsy, especially in those patients requiring multiple biopsies to assess the outcomes of clinical trials or other interventions. Moreover,

NITs can be used to screen larger populations for severe liver disease, which becomes particularly valuable with an ever-rising prevalence of NAFLD. Whereas earlier NITs were particularly good in ruling out fibrosis, newer NITs have been recently developed to rule in those patients with high stages of fibrosis and even cirrhosis (F3 and F4). The utility of the newer and now most widely used NITs, as well as their limitations, will be discussed in the following sections.

The use of ultrasound-based techniques such as vibration-controlled transient elastography (VCTE, Fibroscan®) has become the standard of care in the non-invasive evaluation of patients with NAFLD. It is useful for both the diagnosis of de-novo NAFLD and follow-up assessment of already diagnosed NAFLD. In this context, the controlled attenuation parameter (CAP; dB/m) and the liver stiffness measurement (LSM; kPa) serve as surrogates of steatosis and fibrosis, respectively. To date, several studies have explored the sensitivity and specificity as well as the respective AUROCs with liver biopsy as the reference. A recent analysis showed that cutoffs used by practitioners vary, calling for more standardized cutoffs adopted for routine clinical practice [50]. The recently updated German and Italian NAFLD practice guidelines recommend setting a cutoff of < 8 kPa to rule out fibrosis in NAFLD [51,52]. To rule in advanced fibrosis, cutoff values of 9.7 kPa and 13.6 kPa for F3 (AUROC 0.80 (95% CI 0.75–0.84)) and F4 (AUROC 0.89 (95% CI 0.84–0.93)) fibrosis, respectively, have been identified [53]. In patients with advanced fibrosis (F3) and bridging fibrosis, an increase of > 5 kPa (or > 20%) in LSM was associated with a progression to cirrhosis and higher LSM values have been shown to predict the risk of liver-related adverse events [54,55]. More interestingly, LSM was an independent predictor of all-cause mortality, and values > 20 kPa were associated with a 5.2-fold higher mortality risk [56]. Overall, LSM values may not only serve as a parameter to rule out fibrosis but to identify those NAFLD patients with more advanced disease stages and a higher risk of mortality.

A compound score building on Fibroscan® is the Fibroscan-AST (FAST) score introduced by Newsome et al. in 2019, which combines CAP, LSM, and AST values into a specific equation [57]. The FAST score was specifically designed to identify patients with fibrotic NASH equivalent to a NAFLD activity score of at least ≥ 4 and fibrosis stage ≥ 2 on liver biopsy. These disease stages are mainly required for clinical trials in patients with NASH. In a meta-analysis of 12 studies with 5835 patients, the FAST score had an NPV and PPV of 92% to rule out (cutoff ≤ 0.35) and 65% to rule in (cutoff ≥ 0.67) fibrotic NASH, respectively [58]. Only recently, two newer scores, Agile 3+ and Agile 4, which are also based on LSM, have been validated and shown to effectively rule in advanced fibrosis and cirrhosis with a higher PPV in underlying NAFLD in comparison to LSM alone and the fibrosis-4 (FIB-4) index [59]. Both scores combine LSM, AST/ALT ratio, platelets, sex, diabetes status, and age for Agile 3+. Their scores show a greater AUROC (Agile 3+: 0.90, Agile 4: 0.92) in comparison to FIB-4 and LSM for diagnosing advanced fibrosis and cirrhosis [60]. Moreover, Agile 3+ had a higher AUROC (0.95) to predict liver-related events than LSM (0.93) [61]. Overall, NITs may serve the major purpose to identify those patients at particular risk of adverse events and monitor disease progression in serial use.

The FIB-4 index is a simple surrogate of fibrosis and compromises ALT/AST, age, and platelets. It has a high negative predictive value (98%) in ruling out advanced fibrosis, especially in primary care settings [62]. A persistently high cutoff above 2.67 was a strong predictor for progression to liver cirrhosis and HCC [63]. These results support the serial assessment of FIB-4 in patients with NAFLD.

Despite the widely accepted benefits of employing FIB-4, age imposes a major confounder with a low specificity in patients aged ≥65 years [64]. However, adding age, a high gGT, and impaired glucose to the FIB-4 score may improve diagnostic accuracy [65]. Besides FIB-4, the NAFLD fibrosis score, the APRI score, and the BARD score are of less importance.

Despite the major advantages of using NITs in the assessment of NAFLD, several limitations need careful consideration (Table 1). The population of interest and the respective prevalence are essential for the use of NITs. Thus, a population (patients with T2DM) with a higher prevalence of NAFLD shows a higher specificity but a lower sensitivity compared to a population with a lower prevalence [68]. In populations with a low prevalence, NITs should be rather used to rule out advanced fibrosis [69]. The diagnostic accuracy is overall lower in detecting NASH with significant fibrosis than in advanced fibrosis [70]. Higher sensitivity and PPV are rather seen in high-prevalence settings [71]. The sequential combination of NITs may increase sensitivity and specificity [62]. A FIB-4 cutoff at > 3.48 followed by an LSM cutoff >20 kPa showed a specificity of 90% to rule in cirrhosis [72]. Practice guidelines recommend risk stratification pathways in individuals at risk of advanced fibrosis [69]. These pathways include the FIB-4 as the initial risk stratification in patients with risk factors followed by VCTE in those with a FIB-4 > 1.3–2.67 (indeterminate risk) or > 2.67 (high risk), respectively. The limitation of this approach is in those patients with an indeterminate risk bearing a PPV of only 13% [73]. Thus, Udompap et al. suggest performing VCTE only in patients with T2DM and a FIB-4 > 1.3 which increases the PPV to 33% [73]. Despite the limitations of NITs in the assessment of fibrosis, the application and the sequential use of different NITs in high-risk populations may be valuable tools in non-invasive risk stratification.

Table 1. Characteristics of non-invasive tests for the identification of liver fibrosis.

	Utilization	Advantages	Disadvantages	Ref.
Liver biopsy	Tertiary care	Gold standard to identify, grade, and stage fibrosis, assessment of end points for clinical trials, possibility to rule out other differential diagnoses	Invasive procedure with significant bleeding risk, relevant interobserver variability, sampling error with misdiagnosis, and staging inaccuracies due to the heterogeneity in the histological distribution of fibrosis	[66,67]
Blood-based surrogate scores	Primary care	Low costs, no major harmful side effects	Test results may vary according to the clinical context	
FIB-4	First-line assessment of fibrosis in high-risk populations	High NPV (98%) in ruling out fibrosis	Low specificity with higher age (≥65 years)	[62,64]
VCTE-based tests and scores	Secondary and tertiary care	No harmful side effects	Not widely available, especially in primary	

	Utilization	Advantages	Disadvantages	Ref.
			care	
Fibroscan® (VCTE)	Second-line assessment in patients at high risk of fibrosis	Higher AUROC in ruling in F3 (0.80) and F4 (0.89) fibrosis	Lower accuracy in obese patients	[53]
FAST score	Surrogate of fibrotic NASH (equivalent to NAFLD activity score of at least ≥ 4 and fibrosis stage ≥ 2)	High NPV (92%) to rule out fibrotic NASH	Overestimation of FAST score in transiently elevated transaminases	[58]
Agile3+	Rule in advanced fibrosis (F3)	90% specificity to rule in F3	Adjustment of cutoffs in lower target prevalence	[59]

Abbreviations. AUROC: area under the curve; FAST: Fibroscan-AST score; FIB-4: fibrosis-4 score; NPV: negative predictive value; PPV: positive predictive value; VCTE: vibration-controlled transient elastography.

3.2. The evolution of 'dry' markers: magnetic resonance-based tools for liver fibrosis

Among the novel imaging tools, magnetic-resonance-based markers have shown a great potential in the identification of fibrosis stages. Magnetic resonance elastography (MRE) utilizes low-frequency mechanical shear waves to cause vibrations in the liver parenchyma. These can be detected by the use of modified phase contrast pulse sequences [74,75] and can be expanded to three-dimensional acquisitions by the use of additional spin-echo echo-planar- imaging [76], which allows the analysis of shear wave displacements over the whole liver instead of a singular region of interest (ROI). Standardization of the two-dimensional technique has been established for three different vendors; however, there is no consensus yet on the standards for the number, size, and shape of ROIs, which can lead to certain measurement variability [77]. In addition, MRE measurement failure rates can be influenced by liver iron deposition, even at mild stages [75,78].

The advantages of MRE compared to ultrasound (US)-based techniques may rely on the lower measurement failure rate and better repeatability, which makes it a valuable tool for longitudinal investigations [79]. MRE techniques can be combined with the assessment of liver steatosis by other magnetic resonance-based techniques such as proton density fat fraction (PDFF), another quantitative magnetic resonance imaging biomarker derived from chemical shift imaging [80,81], or corrected T1 (cT1) maps, which offer a pixel-wise estimation of magnetic properties (T1 relaxation), which are influenced by the amount of water (inflammation), fat (steatosis) or extracellular matrix (fibrosis) [82].

The accuracy of MRE to detect early fibrosis is reported to be superior to transient elastography, and comparable to transient elastography in advanced fibrosis [74,83,84]. MRE achieved AUROC between 0.84 and 0.93, depending on the fibrosis stage, for the assessment of fibrosis, but had

lower accuracy to detect NASH (AUROC 0.58–0.73). In addition, the diagnostic accuracy of elastography-based methods is either equivalent or higher compared to cT1 measurements based on comparative studies in NAFLD-only cohorts [85,86]. A recent meta-analysis summarized 70 studies for a comparative review of non-invasive techniques to diagnose liver fibrosis [79]. While the diagnostic performance for the detection of any fibrosis (F0 vs. F1–4) was rather weak for all non-invasive tests (sAUROC VCTE: 0.82, pSWE [share wave elastography]: 0.77 and MRE: 0.87), the diagnostic ability for detecting significant (F0–1 vs F2–4) and advanced (F0–2 vs F3–4) fibrosis was higher (sAUROC VCTE: 0.83, 2D-SWE: 0.86 and MRE: 0.91, resp. sAUROC VCTE: 0.85, pSWE: 0.89, and MRE: 0.92). Only pSWE and MRE were meeting the minimum acceptable criteria for diagnostic accuracy to detect advanced fibrosis.

Recently, magnetic resonance-based scores have been suggested for the implementation of fibrosis and NASH detection in NAFLD cohorts. For instance, the MRI-AST (MAST) score, which includes PDFF and MRE in addition to AST, reached an AUROC of 0.93 and a negative predictive value > 90% for the identification of fibrosing NASH. In addition, compared to FAST and FIB-4, the MAST score gathered fewer patients with indeterminate scores, supporting the role of better identification of patients with progressive NASH [87]. In addition, in a retrospective, 30-month (median) follow-up longitudinal study, the MAST score could predict a higher incidence of major adverse liver outcomes with HR 2.01 and excellent C-index (0.9) [88]. Similarly, the combination of MRE with FIB-4 (MEFIB) provided an AUROC of 0.90 (MRE >3.3 kPa and FIB-4 > 1.6) for the identification of significant fibrosis with a positive predictive value of 97.1%, which was maintained above 90% in an external validation cohort [89]. Finally, a head-to-head comparison of MRE-based scores in a prospective NAFLD cohort has shown the superiority of MEFIB with regard to MAST and FAST for the identification of either significant fibrosis (AUROC 0.90, 0.77, 0.72 respectively) and ‘at risk’ NASH (AUROC 0.77, 0.72, 0.68), with potential implications in the future evaluation of NASH candidates to pharmacological treatment [90].

Hence, MRE is beneficial for identifying patients at risk for advanced fibrosis and may complement existing diagnostics to enable clinicians to diagnose, stratify, and monitor liver fibrosis in response to new and existing treatments and reduce the need for invasive liver biopsy for follow-up examinations. Currently, the use of MRE is limited to experimental studies and as a complimentary evaluation in clinical studies with investigational products. Future analyses on accessibility and cost-effectiveness will determine the inclusion of magnetic resonance-based tools in clinical practice.

3.3. Novel ‘liquid’ biomarkers: collagen-derived peptides, multi-omics approaches

The study of the hepatic ECM has provided remarkable evidence on the fibrogenesis process, with a particular focus on its dynamicity over time. In fact, since the course of NAFLD is constantly shaped according to the multiple, intermittent harmful factors, liver fibrosis occurs with great inter-individual variability. Metabolic signatures can mirror intrahepatic disease activity, underlying the cross-talk between peripheral tissues (mainly visceral adipose tissue) and Kupffer cells as the main driver of liver injury at a pathophysiological level [91]. On the contrary, collagen-derived serum biomarkers are more prone to picture the progression rate of liver fibrosis and therefore the severity of liver disease. For such reason, they are currently addressed as the most plausible biomarkers for liver fibrosis detection and monitoring.

Among these biomarkers, PRO-C3 and its derivative score ADAPT have provided strong evidence for the identification of advanced fibrosis [92,93]. In a study including two wide, independent NAFLD cohorts, the ADAPT score reached the best accuracy for advanced fibrosis, with an AUC of 0.86 in the derivation and 0.87 in the validation cohort [94]. In a meta-analysis of 1658 patients with NAFLD, PRO-C3 reached AUC 0.81 for significant fibrosis and 0.79 for advanced fibrosis [93,95]. In addition, evidence from population-based studies suggest a high negative predictive value of ADAPT with regard to advanced fibrosis (approximately 98%), and the combined use of LSM and ADAPT enhances the diagnostic accuracy to 93% [96,97]. In the broader picture of a tight relationship between fibrogenesis and inflammation, collagen-based biomarkers have raised intriguing evidence. Post-hoc data from the CENTAUR study (phase II study using Cenicriviroc for fibrosing NASH), PRO-C3 could either discriminate between NAFL and NASH and was independently associated with fibrosis progression. The use of ADAPT score could further improve diagnostic accuracy, which performed best among the most commonly used NITs [98]. Similarly, in a post hoc analysis evaluating the effect of tirzepatide (dual GIP/GLP-1 receptor agonist) on non-invasive markers of liver fibrosis in individuals with T2DM, PRO-C3 displayed a significant reduction after 26 weeks in the treatment arm, when compared to placebo [99]. Similar positive trends in PRO-C3 responsiveness were observed in patients treated with Aldafermin, an FGF19 analog, after 24 weeks in a phase 2b study [100]. This aspect links the cross-sectional to the longitudinal utility of collagen-based biomarkers. PRO-C3 shows significant reductions in severely obese patients undergoing bariatric surgery, following the metabolic improvements linked to weight loss, and suggesting a potential role for monitoring liver fibrogenesis [101].

Among other patented tests, ELF (enhanced liver fibrosis) test has undergone conspicuous validation across different cohorts. This test includes the evaluation of direct markers involved in the liver matrix/vascular remodeling: tissue inhibitor of metalloproteinases 1, amino-terminal propeptide of type III procollagen, and hyaluronic acid. In pioneering studies on patients with HCV-related hepatitis, ELF showed a good performance to discriminate significant fibrosis (69% sensitivity and 97% specificity for a cutoff of 9.8) and cirrhosis (83% sensitivity and 97% specificity for a cutoff of 11.3) [102]. In a systematic review of 36 studies including different etiologies of biopsied patients, the ELF test showed excellent performance to detect advanced fibrosis and cirrhosis in patients with NAFLD (AUROC 0.79 to 0.98 and AUROC 0.85 to 0.92, respectively) and alcohol-related liver disease (AUROC 0.92 to 0.94 and AUROC 0.93 to 0.94), with less accuracy in viral hepatitis [103]. Interestingly, one observational study of 300 patients with liver disease of multiple etiology, the 9.8 cutoff was associated with a significantly higher incidence of liver-related outcomes (with an additional 2–53-fold increase for any ELF unit increase) [104], providing a rationale for ELF longitudinal use. A recent meta-analysis of 11 studies including NAFLD patients showed that in a population with a low prevalence of significant fibrosis (e.g. primary care), the ELF test can reach a high negative predictive value (>90%) to rule out advanced fibrosis with low cutoff. However, in this setting the positive predictive value of ELF is low since to reach a value > 80%, a high prevalence of significant fibrosis is needed (>50%), which is most commonly observed in third referral settings [105]. This aspect would require careful use of the test according to the context in use and with different accuracy according to the etiology.

The epigenetic modulation of gene expression has provided relevant opportunities in either the diagnostic or therapeutic field. Serum microRNA (miRNA) displays significant changes across the NAFLD spectrum and has been suggested as a potential biomarker for liver disease severity and progression. In one case–control study, 275 miRNAs showed different serum levels between NAFLD patients and controls. In particular, miR-193a-5p was significantly increased in patients with NASH and clinically relevant fibrosis (stages 2–4), and this finding was confirmed in an external validation cohort [106]. In addition, a recent test has been suggested for the identification of 'at risk' NASH, including miR-34a-5p, alfa-2 macroglobulin, YKL-40, and glycated hemoglobin (NIS4 test). In the NAFLD cohorts undergoing Elafibranor for NASH resolution and/or fibrosis regression (using the phase 2b study as the derivation cohort and the phase 3 study as the validation cohort), the NIS4 algorithm reached an AUROC of 0.80 in the derivation cohort and 0.83 in the validation cohort [107].

More recently, the paradigm of translational research in biomarkers has even shifted from genes to their products in the 'omics' landscape. In particular, proteomic-based molecules have raised as potential targets for the identification of progressive liver disease. These peptides – properly defined as 'liquid' biomarkers with elevated plausibility – have been tested in NAFLD cohorts and represent the most promising markers for risk stratification. A recent study extrapolated a panel of proteomic-based peptides from 4783 proteins tested in NAFLD patients of independent cohorts. The A disintegrin and metalloproteinase with thrombospondin motifs like 2 (ADAMTSL2) could distinguish individuals with 'at risk' NASH (AUROC 0.86) and NASH with significant fibrosis (AUROC 0.83). Similarly, an 8-protein panel could distinguish the two aforementioned clinical entities with AUROC 0.87 and 0.83, respectively. Both ADAMTSL2 and the 8-protein panel were superior to FIB-4 for the identification of advanced fibrosis [108].

These findings strongly support the potential role of the novel biomarkers exploiting recent technology advances for sparing liver biopsies and identifying novel therapeutic targets [109]. In fact, the study of the collagen matrix and the discovery of key disease drivers have critical implications for the understanding of disease progression mechanisms and novel strategies for either prognostic or therapeutic purposes. The use of 'liquid' markers would mostly serve for the proper identification of intrahepatic disease activity and long-term monitoring and treatment response. The use of 'dry' makers has a complimentary application to 'liquid' biomarkers, since a static, yet accurate picture of the disease has major implication in the risk stratification and optimal selection of future candidates for drug treatment.

4. Conclusions

The identification of liver fibrosis in patients with NAFLD is intensively studied and rapidly evolving. (Figure 2). In addition, prognostic biomarkers for patients at a higher risk of disease progression remain a largely unaddressed field. Multiple strategies have been suggested by guidelines mostly involving sequential use of NITS including non-invasive surrogates scores and elastography measures to better risk stratify the large group of patients. The pathophysiology of liver fibrogenesis is a highly dynamic process, influenced by environmental and genetic factors, along with individual responses to lifestyle changes. In this context, blood-based biomarkers raised from proteomics or 'multi-omics' approaches represent the most plausible targets to explore a true intrahepatic disease activity, with multiple implications with regard to natural history, prognosis,

and treatment response. Complimentary to this, the evolution of magnetic resonance technology has the potential to identify patients with fibrosing NASH or advanced fibrosis, which are the best candidates for drug treatment. In fact, many phase 2a trials have adopted non-invasive assessment of treatment response. The combined use of non-invasive scores, as well as liquid and dry biomarkers, will pave the way for a precise characterization of a 'patient's fibrogenesis process,' leading to tailored approaches for the diagnosis and treatment of NAFLD.

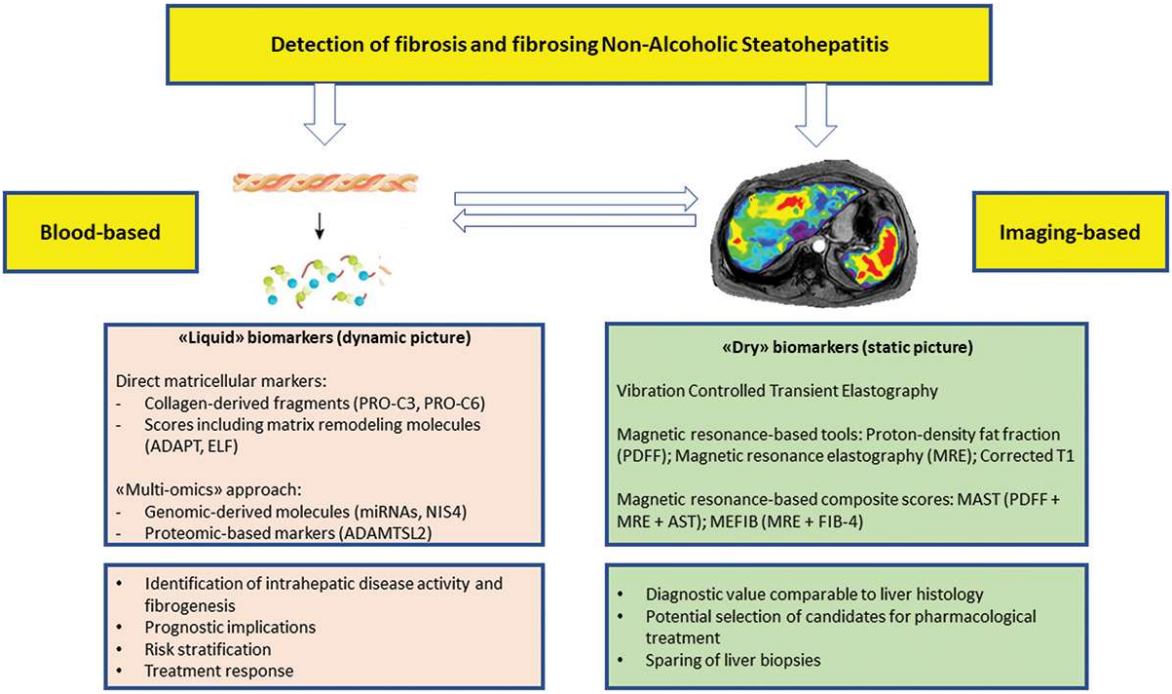

Figure 2. Overview of novel approaches to the identification of liver fibrosis, including 'liquid' and 'dry' biomarkers.Abbreviations. ADAMTSL2: A disintegrin and metalloproteinase with thrombospondin motifs like 2; ELF: enhanced liver fibrosis test; NIS4: PRO-C3: N-terminal type III collagen propeptide; PRO-C6: type 6 collagen propeptide.

5. Expert opinion

As the prevalence of NAFLD increases worldwide in parallel to the spread of metabolic derangements, the need for risk stratification at a population level becomes crucial. The evolution of fibrogenesis markers bears the advantage of a reliable picture of the ongoing intrahepatic disease process and can support medical decisions in different settings. Fibrosis prevalence differs across primary and secondary/tertiary settings, with different reliabilities in non-invasive tools and associated cutoffs. Current research approaches aim at large validations of non-invasive tests for fibrosis detection in all settings, providing useful information that will be used for healthcare pathways and inter-specialty referrals. The combined endpoint is to spare liver biopsies with acceptable accuracy, along with nearly imminent selection of the best candidates for pharmacological treatment of fibrosing NASH. In this context, readily available, cost-effective strategies will need to be implemented for a wide use of novel biomarkers, which are still restricted at the research area. The targeted 'multi-omics' approach is designed to picture the metabolic phenotype of NAFLD individuals, aiming at identifying common points as well as substantial differences. The comprehensive evaluation of the multiple features of dysmetabolism can highlight

key pathophysiology processes and suggest potential targets for either diagnosis or treatment strategies. In the near future, with more treatment for NAFLD (including more detailed lifestyle intervention strategies) or pharmacological (with increasing drugs accessing the phase 3 study), biopsy-free treatment endpoint will need a solid identification and validation. In this context, liquid biomarkers are the best candidates, which changes over time will potentially help identify either 'progressors' versus 'non-progressors,' or 'responders' versus 'non-responders.' The dynamic view of NAFLD as a disease continuum (recently highlighted by the suggested changes in the nomenclature into MAFLD) has great implications in this longitudinal evaluation. The static pictures of the disease, given by VCTE or novel MRE-based techniques, will benefit from the complimentary 'liquid' evaluation, for a deeper characterization of population for disease phenotype or even sub-phenotype. In addition, since the fibrosis stage is the strongest predictor of hepatic and overall events in NALFD population, the accurate identification of fibrosis through magnetic resonance is the best candidate for biopsy-free strategies in particular, for treatment endpoints. The accessibility and costs of the novel procedures represent now the major obstacle for large accessibility, and future paradigms will need to consider the economic aspects as well as the utility of the techniques. In conclusion, over the last decades great efforts from the scientific community have been undertaken to highlight the dynamics of liver fibrogenesis and promising advances have been obtained, from pathophysiology to diagnostic/treatment targets. In the near future, combined algorithms will provide a tailored picture of the protean phenotype of NAFLD individuals, leading to significant improvements in the diagnosis as well as management of this complex liver disease.

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Article highlights

- In the Non-Alcoholic Fatty Liver Disease (NAFLD) landscape, liver fibrosis detection still requires liver biopsy, but multiple non-invasive approaches are being investigated;
- Current paradigms are based on the sequential use of non-invasive tests, mainly FIB-4 score followed by vibration-controlled transient elastography (VCTE), which is the most accurate non-invasive tool for the identification of significant liver fibrosis;
- Novel 'liquid' biomarkers include direct collagen-derived peptides (e.g. PRO-C3 and PRO-C6), which are promising tools for either the risk stratification or the prediction of treatment response;
- The use of advanced 'dry' markers, especially magnetic resonance-based tools (e.g. PDFF, MRE, and cT1) can identify with high accuracy steatosis grade and fibrosis stage and will help identify the best candidates for pharmacological treatments and support the prognostication.

Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84. doi: 10.1002/hep.28431 AQ1
2. Henry L, Paik J, Younossi ZM. Review article the epidemiologic burden of non-alcoholic fatty liver disease across the world. Aliment Pharmacol Ther. 2022;56:942–956. doi: 10.1111/apt.17158
3. Michel M, Labenz C, Wahl A, et al. Prevalence and risk factors of nonalcoholic steatohepatitis with significant fibrosis in people with HIV. AIDS. 2022;36:1665–1674. DOI:10.1097/QAD.0000000000003312
4. Belinchón-Romero I, Bellot P, Romero-Pérez D, et al. Non-alcoholic fatty liver disease is associated with bacterial translocation and a higher inflammation response in psoriatic patients. Sci Rep. 2021;11:8593. doi: 10.1038/s41598-021-88043-8
5. Alqahtani SA, Schattenberg JM. NAFLD in the Elderly. CIA. 2021;16:1633–1649. doi: 10.2147/CIA.S295524
6. Golabi P, Paik J, Reddy R, et al. Prevalence and long-term outcomes of non-alcoholic fatty liver disease among elderly individuals from the United States. BMC Gastroenterol. 2019;19:56. doi: 10.1186/s12876-019-0972-6
7. Li J, Ha A, Rui F, et al. Meta-analysis: global prevalence, trend and forecasting of non-alcoholic fatty liver disease in children and adolescents, 2000–2021. Aliment Pharmacol Ther. 2022;56(3):396–406. doi: 10.1111/apt.17096

8. Diehl AM, Cause DC. Pathogenesis, and treatment of nonalcoholic steatohepatitis. N Engl J Med. 2017;377(21):2063–2072. doi: 10.1056/NEJMra1503519

9. Sanyal AJ, van Natta ML, Clark J, et al. Prospective study of outcomes in adults with nonalcoholic fatty liver disease. N Engl J Med. 2021:385;1559–1569. doi: 10.1056/NEJMoa2029349. .

•• Prospective study showing the negative effect of advanced fibrosis stages (F3, F4) in patients with NAFLD on mortality, hepatic and extrahepatic outcomes

10. Flemming JA, Djerboua M, Groome PA, et al. NAFLD and alcohol-associated liver disease will be responsible for almost all new diagnoses of Cirrhosis in Canada by 2040. Hepatology. 2021;74(6):3330–3344. doi: 10.1002/hep.32032

11. Wong RJ, Singal AK. Trends in liver disease etiology among adults awaiting liver transplantation in the United States, 2014-2019. JAMA Netw Open. 2020;3:e1920294. doi: 10.1001/jamanetworkopen.2019.20294

12. European Association for the Study of the Liver. EASL–EASD–EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388–1402. doi: 10.1016/j.jhep.2015.11.004

13. Liebe R, Esposito I, Bock HH, et al. Diagnosis and management of secondary causes of steatohepatitis. J Hepatol. 2021;74:1455–1471. DOI:10.1016/j.jhep.2021.01.045

14. Younossi ZM, Golabi P, de AL, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71:793–801. DOI:10.1016/j.jhep.2019.06.021

15. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol. 2020;73:202–209. DOI:10.1016/j.jhep.2020.03.039

16. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77(5):1797–1835. doi: 10.1097/HEP.0000000000000323.

• Updated and current AASLD guidelines on the management of patients with NAFLD.

17. Younossi Z, Aggarwal P, Shrestha I, et al. The burden of non-alcoholic steatohepatitis: A systematic review of health-related quality of life and patient-reported outcomes. JHEP Rep. 2022;4:100525. doi: 10.1016/j.jhepr.2022.100525

18. Schattenberg JM, Lazarus JV, Newsome PN, et al. Disease burden and economic impact of diagnosed non-alcoholic steatohepatitis in five European countries in 2018: A cost-of-illness analysis. Liver International 2021. 2021;41(6):1227–1242. doi: 10.1111/liv.14825.

•• This study shows that advanced disease stages in patients with NAFLD/NASH impose substantial economic costs highlighting the importance of early diagnosis and care of these patients

19. Ng CH, Lim WH, Hui Lim GE, et al. Mortality outcomes by fibrosis stage in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2023;21(4):931–939.e5. doi: 10.1016/j.cgh.2022.04.014

20. Adams LA, Anstee QM, Tilg H, et al. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut. 2017;66:1138–1153. doi: 10.1136/gutjnl-2017-313884

21. Labenz C, Huber Y, Michel M, et al. Impact of NAFLD on the incidence of cardiovascular diseases in a primary care population in germany. Dig Dis Sci. 2019;65(7):2112–2119. doi: 10.1007/s10620-019-05986-9

22. Kaps L, Labenz C, Galle PR, et al. Non-alcoholic fatty liver disease increases the risk of incident chronic kidney disease. United Eur Gastroenterol J. 2020;8(8):942–948. doi: 10.1177/2050640620944098

23. Labenz C, Huber Y, Michel M, et al. Nonalcoholic fatty liver disease increases the risk of anxiety and depression. Hepatol Commun. 2020;4(9):1293–1301. doi: 10.1002/hep4.1541

24. Kim G-A, Oh CH, Kim JW, et al. Association between non-alcoholic fatty liver disease and the risk of dementia: A nationwide cohort study. Liver Int. 2022;42:1027–1036. doi: 10.1111/liv.15244

25. Huber Y, Labenz C, Michel M, et al. Tumor Incidence in patients with non-alcoholic fatty liver disease. Dtsch Arztebl Int. 2020;117:719–724. DOI:10.3238/arztebl.2020.0719

26. Karsdal MA, Nielsen SH, Leeming DJ, et al. The good and the bad collagens of fibrosis - Their role in signaling and organ function. Adv Drug Delivery Rev. 2017;121:43–56. DOI:10.1016/j.addr.2017.07.014

27. Karsdal MA, Daniels SJ, Holm Nielsen S, et al. Collagen biology and non-invasive biomarkers of liver fibrosis. Liver Int. 2020;40:736–750. DOI:10.1111/liv.14390

28. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol. 2017;14:397–411. doi: 10.1038/nrgastro.2017.38

29. Karlmark KR, Weiskirchen R, Zimmermann HW, et al. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. Hepatology. 2009;50:261–274. DOI:10.1002/hep.22950

30. Ramachandran P, Pellicoro A, Vernon MA, et al. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A. 2012;109:E3186–95. DOI:10.1073/pnas.1119964109

31. Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression and regression in NASH. J Hepatol. 2018;68(2):238–250. doi: 10.1016/j.jhep.2017.11.012.
•• This paper provides a comprehensive view of the mechanistic pathways of fibrogenesis process.

32. Kleiner DE, Brunt EM, Wilson LA, et al. Association of histologic disease activity with progression of nonalcoholic fatty liver disease. JAMA Netw Open. 2019;2:e1912565.

DOI:10.1001/jamanetworkopen.2019.12565

33. Singh S, Allen AM, Wang Z, et al. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol. 2015;13(4):643-54.e1-9; quiz e39-40. doi: 10.1016/j.cgh.2014.04.014

34. McPherson S, Hardy T, Henderson E, et al. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015;62:1148–1155. doi: 10.1016/j.jhep.2014.11.034

35. Lassailly G, Caiazzo R, Ntandja-Wandji L-C, et al. Bariatric surgery provides long-term resolution of nonalcoholic steatohepatitis and regression of fibrosis. Gastroenterology. 2020;159(4):1290–1301.e5. doi: 10.1053/j.gastro.2020.06.006

36. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver Fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2015;149(2):389–97.e10. doi: 10.1053/j.gastro.2015.04.043

37. Loomba R, Adams LA. The 20% rule of Nash progression: the natural history of advanced fibrosis and cirrhosis caused by NASH. Hepatology. 2019;70(6):1885–1888. doi: 10.1002/hep.30946.
• **This paper highlights key elements of the NAFLD natural history.**

38. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology. 2017;65:1557–1565. DOI:10.1002/hep.29085

39. Henson JB, Simon TG, Kaplan A, et al. Advanced fibrosis is associated with incident cardiovascular disease in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2020;51:728–736. doi: 10.1111/apt.15660

40. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology. 2020;158(6):1611–1625.e12. doi: 10.1053/j.gastro.2020.01.043

41. Ampuero J, Aller R, Gallego-Durán R, et al. Definite and indeterminate nonalcoholic steatohepatitis share similar clinical features and prognosis: A longitudinal study of 1893 biopsy-proven nonalcoholic fatty liver disease subjects. Liver Int. 2021;41:2076–2086. DOI:10.1111/liv.14898

42. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67(6):1265–1273. doi: 10.1016/j.jhep.2017.07.027.
•• **A seminal study highlighting the relevance of fibrosis stage but not disease activity for prognostication.**

43. Mantovani A, Csermely A, Petracca G, et al. Non-alcoholic fatty liver disease and risk of fatal and non-fatal cardiovascular events: an updated systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2021;6:903–913. doi: 10.1016/S2468-1253(21)00308-3

44. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, et al. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: a multi-national cohort study. Gastroenterology. 2018;155(2):443–457.e17. doi: 10.1053/j.gastro.2018.04.034

45. Simon TG, Roelstraete B, Hagström H, et al. Non-alcoholic fatty liver disease and incident major adverse cardiovascular events: results from a nationwide histology cohort. Gut. 2022;71:1867–1875. doi: 10.1136/gutjnl-2021-325724

46. Simon TG, Roelstraete B, Khalili H, et al. Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. Gut. 2021;70:1375–1382. doi: 10.1136/gutjnl-2020-322786

47. Davison BA, Harrison SA, Cotter G, et al. Suboptimal reliability of liver biopsy evaluation has implications for randomized clinical trials. J Hepatol. 2020;73(6):1322–1332. doi: 10.1016/j.jhep.2020.06.025

48. Brunt EM, Clouston AD, Goodman Z, et al. Complexity of ballooned hepatocyte feature recognition: defining a training atlas for artificial intelligence-based imaging in NAFLD. J Hepatol. 2022;76:1030–1041. DOI:10.1016/j.jhep.2022.01.011

49. Mehta SH, Lau B, Afdhal NH, et al. Exceeding the limits of liver histology markers. J Hepatol. 2009;50:36–41. doi: 10.1016/j.jhep.2008.07.039

50. Lazarus JV, Castera L, Mark HE, et al. Real-world evidence on non-invasive tests and associated cut-offs used to assess fibrosis in routine clinical practice. JHEP Rep. 2022;5(1):100596. doi: 10.1016/j.jhepr.2022.100596

51. Marchesini G, Bugianesi E, Burra P, et al. Non-alcoholic fatty liver disease in adults 2021: A clinical practice guideline of the Italian association for the study of the liver (aisf), the Italian society of diabetology (sid) and the Italian society of obesity (SIO). Nutr Metab Cardiovasc Dis. 2022;32:1–16. DOI:10.1016/j.numecd.2021.04.028

52. Roeb E, Canbay A, Bantel H, et al. Aktualisierte S2k-Leitlinie nicht-alkoholische Fettlebererkrankung der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten (DGVS) – April 2022 – AWMF-Registernummer: 021–025. Z Gastroenterol. 2022;60:1346–1421. Not Available. doi: 10.1055/a-1880-2283

53. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2019;156:1717–1730. DOI:10.1053/j.gastro.2019.01.042

54. Loomba R, Huang DQ, Sanyal AJ, et al. Liver stiffness thresholds to predict disease progression and clinical outcomes in bridging fibrosis and cirrhosis. Gut. 2023;72:581–589. DOI:10.1136/gutjnl-2022-327777

55. Shili-Masmoudi S, Wong G-H, Hiriart J-B, et al. Liver stiffness measurement predicts long-term survival and complications in non-alcoholic fatty liver disease. Liver Int. 2020;40(3):581–589. doi: 10.1111/liv.14301

56. Vilar-Gomez E, Vuppalanchi R, Gawrieh S, et al. CAP and LSM as determined by VCTE are independent predictors of all-cause mortality in the U.S. adult population. Hepatology. 2023;77(4):1241–1252. doi: 10.1097/HEP.0000000000000023.
• This study shows that liver stiffness measurement (LSM) determined by VCTE can independently predict all-cause mortality.

57. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol. 2020;5(4):362–373. doi: 10.1016/S2468-1253(19)30383-8.
• This study investigated the use of the Fibroscan-AST (FAST) score to non-invasively identify patients with NASH.

58. Ravaioli F, Dajti E, Mantovani A, et al. Diagnostic accuracy of FibroScan-AST (FAST) score for the non-invasive identification of patients with fibrotic non-alcoholic steatohepatitis: a systematic review and meta-analysis. Gut. 2023;72(7):1399–1409. doi: 10.1136/gutjnl-2022-328689

59. Sanyal AJ, Foucquier J, Younossi ZM, et al. Enhanced diagnosis of advanced fibrosis and cirrhosis in individuals with NAFLD using FibroScan-based Agile scores. J Hepatol. 2022;78(2):247–259. doi: 10.1016/j.jhep.2022.10.034

60. Nakatsuka T, Tateishi R, Sato M, et al. Agile scores are a good predictor of liver-related events in patients with NAFLD. J Hepatol. 2023. doi:10.1016/j.jhep.2023.02.029.

61. Pennisi G, Enea M, Pandolfo A, et al. AGILE 3+ Score for the diagnosis of advanced fibrosis and for predicting liver-related events in nafLD. Clin Gastroenterol Hepatol. 2022;21(5):1293–1302.e5. doi: 10.1016/j.cgh.2022.06.013

62. Mahady SE, Macaskill P, Craig JC, et al. Diagnostic accuracy of noninvasive fibrosis scores in a population of individuals with a low prevalence of fibrosis. Clinical Gastroenterology And Hepatology. 2017;15(9):1453–1460.e1. doi: 10.1016/j.cgh.2017.02.031

63. Cholankeril G, Kramer JR, Chu J, et al. Longitudinal changes in fibrosis markers are associated with risk of cirrhosis and hepatocellular carcinoma in non-alcoholic fatty liver disease. Journal Of Hepatology. 2022;78(3):493–500. doi: 10.1016/j.jhep.2022.10.035

64. McPherson S, Hardy T, Dufour J-F, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol. 2017;112:740–751. DOI:10.1038/ajg.2016.453

65. Hagström H, Yan J, Talbäck M, et al. Improved prediction of 10-year risk of severe liver disease in the general population using commonly available biomarkers. Aliment Pharmacol Ther. 2022;57(4):418–425. doi: 10.1111/apt.17374.
• This study shows that using the FIB-4 in combination with other commonly available biomarkers (diabetes, impaired glucose or high GGT) improves its diagnostic accuracy.

66. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495–500. doi: 10.1056/NEJM200102153440706

67. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128:1898–1906. DOI:10.1053/j.gastro.2005.03.084

68. Usher-Smith JA, Sharp SJ, Griffin SJ. The spectrum effect in tests for risk prediction, screening, and diagnosis. BMJ (Clin Res Ed). 2016;353:i3139. doi: 10.1136/bmj.i3139

69. Berzigotti A, Tsochatzis E, Boursier J, et al. EASL Clinical practice guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. Journal Of Hepatology. 2021;75(3):659–689. doi: 10.1016/j.jhep.2021.05.025

70. Vali Y, Lee J, Boursier J, et al. Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study. Lancet Gastroenterol Hepatol. 2023;8(8):714–725. doi: 10.1016/S2468-1253(23)00017-1

71. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846–854. DOI:10.1002/hep.21496

72. Mózes FE, Lee JA, Selvaraj EA, et al. Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. Gut. 2022;71:1006–1019. DOI:10.1136/gutjnl-2021-324243

73. Udompap P, Therneau TM, Canning RE, et al. Performance of American gastroenterological association clinical care pathway for the risk stratification of patients with nonalcoholic fatty liver disease in the us population. Hepatology. 2022;77(3):931–941. doi: 10.1002/hep.32739

74. Imajo K, Kessoku T, Honda Y, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. Gastroenterology. 2016;150(3):626–637.e7. doi: 10.1053/j.gastro.2015.11.048

75. Wagner M, Corcuera-Solano I, Lo G, et al. Technical failure of mr elastography examinations of the liver: experience from a large single-center study. Radiology. 2017;284:401–412. doi: 10.1148/radiol.2016160863

76. Loomba R, Cui J, Wolfson T, et al. Novel 3D magnetic resonance elastography for the noninvasive diagnosis of advanced fibrosis in NAFLD: A prospective study. Am J Gastroenterol. 2016;111:986–994. DOI:10.1038/ajg.2016.65

77. Kim YS, Jang YN, Song JS. Comparison of gradient-recalled echo and spin-echo echo-planar imaging MR elastography in staging liver fibrosis: a meta-analysis. Eur Radiol. 2018;28:1709–1718. doi: 10.1007/s00330-017-5149-5

78. Cunha GM, Glaser KJ, Bergman A, et al. Feasibility and agreement of stiffness measurements using gradient-echo and spin-echo MR elastography sequences in unselected patients undergoing liver MRI. Br J Radiol. 2018;91:20180126. doi: 10.1259/bjr.20180126

79. Selvaraj EA, Mózes FE, Jayaswal ANA, et al. Diagnostic accuracy of elastography and magnetic resonance imaging in patients with NAFLD: A systematic review and meta-analysis. J Hepatol. 2021;75(4):770–785. doi: 10.1016/j.jhep.2021.04.044.
• **Current evidence of dry (imaging-based) markers for the identification of NAFLD severity.**

80. Reeder SB, McKenzie CA, Pineda AR, et al. Water–fat separation with IDEAL gradient-echo imaging. J Magn Reson Imaging. 2007;25(3):644–652. doi: 10.1002/jmri.20831

81. Reeder SB, Pineda AR, Wen Z, et al. Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL): application with fast spin-echo imaging. Magn Reson Med. 2005;54:636–644. DOI:10.1002/mrm.20624

82. Tunnicliffe EM, Banerjee R, Pavlides M, et al. A model for hepatic fibrosis: the competing effects of cell loss and iron on shortened modified Look-Locker inversion recovery T1 (shMOLLI-T1) in the liver. J Magn Reson Imaging. 2017;45:450–462. doi: 10.1002/jmri.25392

83. Huwart L, Sempoux C, Vicaut E, et al. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. Gastroenterology. 2008;135:32–40. DOI:10.1053/j.gastro.2008.03.076

84. Park CC, Nguyen P, Hernandez C, et al. Magnetic resonance elastography vs transient elastography in detection of fibrosis and noninvasive measurement of steatosis in patients with biopsy-proven nonalcoholic fatty liver disease. Gastroenterology. 2017;152(3):598–607.e2. doi: 10.1053/j.gastro.2016.10.026

85. Cunha GM, Villela-Nogueira CA, Bergman A, et al. Abbreviated mpMRI protocol for diffuse liver disease: a practical approach for evaluation and follow-up of NAFLD. Abdom Radiol (NY). 2018;43:2340–2350. doi: 10.1007/s00261-018-1504-5

86. Eddowes PJ, McDonald N, Davies N, et al. Utility and cost evaluation of multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2018;47:631–644. DOI:10.1111/apt.14469

87. Noureddin M, Truong E, Gornbein JA, et al. MRI-based (MAST) score accurately identifies patients with NASH and significant fibrosis. J Hepatol. 2022;76(4):781–787. doi: 10.1016/j.jhep.2021.11.012.
•• **MR-derived score for the identification of NASH and fibrosis.**

88. Truong E, Gornbein JA, Yang JD, et al. MRI-AST (MAST) score accurately predicts major adverse liver outcome, hepatocellular carcinoma, liver transplant, and liver-related death. Clin Gastroenterol Hepatol. 2023. doi:10.1016/j.cgh.2023.02.003.

89. Jung J, Loomba RR, Imajo K, et al. MRE combined with FIB-4 (MEFIB) index in detection of candidates for pharmacological treatment of NASH-related fibrosis. Gut. 2021;70:1946–1953. DOI:10.1136/gutjnl-2020-322976

90. Kim BK, Tamaki N, Imajo K, et al. Head-to-head comparison between MEFIB, MAST, and FAST for detecting stage 2 fibrosis or higher among patients with NAFLD. J Hepatol. 2022;77:1482–1490. DOI:10.1016/j.jhep.2022.07.020

91. Rosso C, Kazankov K, Younes R, et al. Crosstalk between adipose tissue insulin resistance and liver macrophages in non-alcoholic fatty liver disease. J Hepatol. 2019;71:1012–1021. DOI:10.1016/j.jhep.2019.06.031

92. Daniels SJ, Leeming DJ, Eslam M, et al. ADAPT: an algorithm incorporating pro-c3 accurately identifies patients with NAFLD and Advanced Fibrosis. Hepatology. 2019;69:1075–1086. DOI:10.1002/hep.30163

93. Erhardtsen E, Rasmussen DGK, Frederiksen P, et al. Determining a healthy reference range and factors potentially influencing PRO-C3 – a biomarker of liver fibrosis. JHEP Rep. 2021;3(4):100317. doi: 10.1016/j.jhepr.2021.100317

94. Karsdal MA, Hjuler ST, Luo Y, et al. Assessment of liver fibrosis progression and regression by a serological collagen turnover profile. Am J Physiol Gastrointest Liver Physiol. 2019;316:G25–G31. DOI:10.1152/ajpgi.00158.2018

95. Mak AL, Lee J, van Dijk A-M, et al. Systematic review with meta-analysis: diagnostic accuracy of pro-c3 for hepatic fibrosis in patients with non-alcoholic fatty liver disease. Biomedicines. 2021;9(12):1920. doi: 10.3390/biomedicines9121920

96. Eslam M, Wong G-H, Hashem AM, et al. A sequential algorithm combining adapt and liver stiffness can stage metabolic-associated fatty liver disease in hospital-based and primary care patients. Am J Gastroenterol. 2021;116(5):984–993. doi: 10.14309/ajg.0000000000001059

97. Tang L-J, Ma H-L, Eslam M, et al. Among simple non-invasive scores, Pro-C3 and ADAPT best exclude advanced fibrosis in Asian patients with MAFLD. Metabolism. 2022;128:154958. DOI:10.1016/j.metabol.2021.154958

98. Nielsen MJ, Leeming DJ, Goodman Z, et al. Comparison of ADAPT, FIB-4 and APRI as non-invasive predictors of liver fibrosis and NASH within the CENTAUR screening population. J Hepatol. 2021;75(6):1292–1300. doi: 10.1016/j.jhep.2021.08.016.
• This paper marks the greater accuracy of collagen-derived scores with the identification of fibrosis stage by histology.

99. Hartman ML, Sanyal AJ, Loomba R, et al. Effects of novel dual gip and glp-1 receptor agonist tirzepatide on biomarkers of nonalcoholic steatohepatitis in patients with type 2 diabetes. Diabetes Care. 2020;43:1352–1355. DOI:10.2337/dc19-1892

100. Harrison SA, Neff G, Guy CD, et al. Efficacy and safety of aldafermin, an engineered fgf19 analog, in a randomized, double-blind, placebo-controlled trial of patients with nonalcoholic steatohepatitis. Gastroenterology. 2021;160(1):219–231.e1. doi: 10.1053/j.gastro.2020.08.004

101. Bel Lassen P, Nori N, Bedossa P, et al. Fibrogenesis marker pro-c3 is higher in advanced liver fibrosis and improves in patients undergoing bariatric surgery. J Clin Endocrinol Metab. 2022;107:e1356–e1366. DOI:10.1210/clinem/dgab897

102. Lichtinghagen R, Pietsch D, Bantel H, et al. The Enhanced Liver Fibrosis (ELF) score: normal values, influence factors and proposed cut-off values. J Hepatol. 2013;59:236–242.

doi: 10.1016/j.jhep.2013.03.016

103. Sharma C, Cococcia S, Ellis N, et al. Systematic review: Accuracy of the enhanced liver fibrosis test for diagnosing advanced liver fibrosis and cirrhosis. J Gastroenterol Hepatol. 2021;36:1788–1802. doi: 10.1111/jgh.15482

104. Irvine KM, Wockner LF, Shanker M, et al. The Enhanced liver fibrosis score is associated with clinical outcomes and disease progression in patients with chronic liver disease. Liver Int. 2016;36:370–377. DOI:10.1111/liv.12896

105. Vali Y, Lee J, Boursier J, et al. Enhanced liver fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: A systematic review and meta-analysis. Journal of Hepatology 2020. doi:10.1016/j.jhep.2020.03.036.
• **This paper explores the role of ELF in the identification of advanced fibrosis.**

106. Johnson K, Leary PJ, Govaere O, et al. Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: Diagnostic and mechanistic relevance. JHEP Rep. 2022;4:100409. DOI:10.1016/j.jhepr.2021.100409

107. Harrison SA, Ratziu V, Boursier J, et al. A blood-based biomarker panel (NIS4) for non-invasive diagnosis of non-alcoholic steatohepatitis and liver fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol. 2020;5(11):970–985. doi: 10.1016/S2468-1253(20)30252-1.
•• **Novel exploratory approaches in the field of liquid biomarkers.**

108. Corey KE, Pitts R, Lai M, et al. ADAMTSL2 protein and a soluble biomarker signature identify at-risk non-alcoholic steatohepatitis and fibrosis in adults with NAFLD. J Hepatol. 2022;76(1):25–33. doi: 10.1016/j.jhep.2021.09.026.
•• **Novel exploratory approaches in the field of liquid biomarkers.**

109. Schuppan D, Ashfaq-Khan M, Yang AT, et al. Liver fibrosis: direct antifibrotic agents and targeted therapies. Matrix Biol. 2018;68-69:435–451. DOI:10.1016/j.matbio.2018.04.006

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