Non-invasive assessment of liver fibrosis

Laurent Castera, M.D. Ph.D. (1), Mary E. Rinella, M.D. (2), Emmanuel A. Tsochatzis, M.D. Ph.D. (3)

(1) Université de Paris–Cité, Hôpital Beaujon, Department of Hepatology, Assistance Publique–Hôpitaux de Paris, and INSERM Unité Mixte de Recherche 1149, Clichy, France.

(2) Division of Gastroenterology, Hepatology and Nutrition, University of Chicago, Pritzker School of Medicine, Chicago, USA.

(3) UCL Institute for Liver and Digestive Health, Royal Free Hospital and UCL, London, UK.

The three authors equally contributed to the manuscript.

Corresponding author:

Pr Laurent Castera, MD, PhD

Service d'hépatologie, Hôpital Beaujon,

100 Boulevard du Général Leclerc, 92110 Clichy, France

Tel: +33 1 40 87 57 64; Fax: +33 1 40 87 44 82

E-mail address: laurent.castera@bjn.aphp.fr

Word count: 3175

Number of table: 1

Number of figures: 4

INTRODUCTION

Chronic liver disease is a major public health problem, accounting for two million deaths annually and 4% of all deaths worldwide [1]. The prognosis and management of chronic liver disease is highly dependent on the extent and progression of liver fibrosis and the risk of developing cirrhosis and its complications.[2] Diagnosis of chronic liver disease and staging of fibrosis has relied on liver biopsy since the late 1950s,[3] an invasive procedure with rare but potentially life-threatening complications. [4] Patients with chronic liver disease can remain asymptomatic for decades and consequently are often diagnosed at a late stage once complications of cirrhosis have occurred and treatment is less effective (**Figure 1**).[5] Further complicating matters, standard liver blood tests often show normal, or only mildly elevated results, falsely leading care providers to be reassured.[5]

The limitations of liver biopsy have led to the development of non-invasive tests , which have revolutionised the practice of hepatology by allowing the identification of patients with advanced fibrosis or cirrhosis before they develop liver complications.[6] In addition, the increasing prevalence of steatotic liver disease in up to 30% of the adult population in western countries has made their use essential for risk stratification. [7,8] Although non-invasive tests have become the standard of care in liver clinics, they remain largely unknown and not commonly used outside of hepatology care (e.g., primary care, endocrinology, or cardiology), where most patients with advanced fibrosis are cared for. [9]

In this review, we discuss how the use of non-invasive tests can change clinical practice across all settings and prepare clinicians to manage the increasing burden of liver disease in the years to come. We provide an algorithm to identify asymptomatic patients with advanced fibrosis and provide an overview of the available noninvasive modalities with practical insight into their performance and limitations.

CURRENTLY AVAILABLE NON-INVASIVE TESTS

Several non-invasive methods for the assessment of liver fibrosis have been developed.[6,7] They can be classified into two distinct but complementary approaches: i) 'blood-based' including derived laboratory-based risk scores or biomarkers; and ii) 'imaging' based on liver stiffness measurement, using either ultrasound- or magnetic resonance-based elastography techniques (**Figure 2A**). Although complementary, these approaches are based on different rationales: Blood-based methods approximate the extent of liver fibrosis through laboratory parameters reflective of evolving or established fibrosis or portal hypertension or directly measure circulating by-products of the fibrogenic/fibrinolytic processes while imaging-based methods measure intrinsic physical properties of the liver parenchyma to provide a measurement of stiffness, which often reflects fibrosis burden.

BLOOD-BASED NON-INVASIVE TESTS

Blood-based non-invasive tests can be divided into 'indirect' and 'direct' markers. Indirect markers include scores or panels, derived from common parameters that reflect or correlate with liver injury, inflammation, or portal hypertension, e.g. the aspartate aminotransferase (AST)/platelet ratio index (APRI), the fibrosis-4 index (FIB-4) and the nonalcoholic fatty liver disease (NAFLD) fibrosis score (NFS). Given the simplicity of FIB-4 and how easily it can be automated, it is the easiest to incorporate into clinical practice.[10] Direct markers, include proprietary scores or panels, based on circulating components of fibrogenesis or extracellular matrix remodeling, such as Enhanced Liver Fibrosis score (ELF) (Siemens), FibroTest (Biopredictive, known as FibroSure [LabCorp] in the United States), and FibroMeter (Echosens) (**Figure 2A**).

The most validated blood-based non-invasive tests are APRI,[11] FIB-4[12], and ELF.[13] Their practical advantages include: high applicability (>95%), good reproducibility, widespread availability, and low cost (APRI and FIB-4), since they are derived from common blood tests. However, they are not liver-specific (i.e. they are possibly influenced by extrahepatic fibroinflammatory change and other confounding factors) while ELF is a commercially available test that is relatively expensive (**Figure 2B**).[6]

LIVER STIFFNESS ASSESSMENT

Liver stiffness can be measured using various ultrasound-based elastography techniques or magnetic resonance elastography (MRE) (**Figure 2A**).[6] Vibration-controlled transient elastography (VCTE), using the FibroScan device (Echosens), is the best validated and most widely used technique worldwide.[7] Pulse-echo ultrasound acquisition is used to follow the propagation of a shear wave in the liver parenchyma and to measure its velocity, the latter being directly related to tissue stiffness – the stiffer the tissue, the faster the shear wave propagates.[14] Liver stiffness measurement results are expressed in kilopascals (kPa) and range from 2 to over 75 kPa, with normal values considered $\leq$ 5 kPa. Liver stiffness measurement more often yields false-positive than-false negative results. For a reading to be reliable, the interquartile range-to-median ratio must not exceed 30%, except in cases of values $\leq$ 7 kPa.[15] Practical advantages of VCTE include: point-of-care testing, easy learning, and reliability (>95% using XL probe in patients who are not morbidly obese). However, confounding factors should be carefully identified to avoid mischaracterization of fibrosis burden (**Figure 2B**). Other ultrasound elastography methods include point shear wave elastography (p-SWE) and two-dimensional shear wave elastography (2D-SWE). They have broadly similar diagnostic accuracies compared to VCTE, however differences across platforms, variations in diagnostic

cut-offs and somewhat limited validation have hampered their widespread use. Finally, conventional abdominal ultrasound may also suggest the presence of cirrhosis (liver nodularity or enlarged caudate lobe) but is not reliably accurate (low sensitivity).[4]

CONTEXT OF USE

Several critical issues should be considered when using non-invasive tests in practice: cost, availability, and the context of use (**Figure 2A**). The context of use refers to the population and clinical setting in which the non-invasive test will be used.[16] Indirect markers such as FIB-4 have a good sensitivity and therefore a negative predictive value (NPV) >90% to rule out advanced fibrosis when used in low-risk populations where the pretest probability (prevalence) of advanced fibrosis is low (<5%), but their specificity and subsequent positive predictive value (PPV) is modest. In patients seen in liver clinics where the pretest probability of advanced fibrosis is higher (20-30%),[10] the use of secondary tests with a higher specificity (e.g. VCTE or ELF), is recommended (**Figure 3**).

NON-INVASIVE STAGING OF FIBROSIS IN CHRONIC LIVER DISEASE

Liver fibrosis is scored by histopathologists on an ordinal scale, ranging from no fibrosis (stage 0) mild (F1), significant (F2), advanced fibrosis (F3) and cirrhosis (F4) (**Figure 1**). The readout of non-invasive tests is meant to reflect the histological stages of fibrosis, as liver biopsy is used as the reference standard to determine biomarker performance .[6] However, while non-invasive tests are useful to distinguish early from advanced fibrosis, they lack granularity and accuracy to distinguish individual fibrosis stages (F1-F4)**.**[7] The performance characteristics of the most used non-invasive tests are shown in **Table 1** [17-22]. While some non-invasive tests have been studied across etiologies of liver disease, others have been almost entirely studied within the

context of a specific liver disease, such as FibroTest, developed and validated in patients with chronic hepatitis C (HCV), and the ELF test in metabolic dysfunction-associated steatotic liver disease (MASLD), formerly NAFLD, and in alcohol-related liver disease (ALD).

INITIAL TESTING

Patients with risk factors for liver disease (hepatic steatosis, high cardiometabolic burden, excess alcohol consumption, viral hepatitis or chronic elevation in liver chemistries) should be screened for the presence of advanced fibrosis **(Figure 4).** Indirect non-invasive tests, such as FIB-4, have dual cut-offs: a low cut-off with high sensitivity (>90%) to rule out, a high cutoff with high specificity (>90%) to rule in fibrosis and an 'indeterminate' range, between these 2 values. However, patients with both indeterminate and high FIB-4 require further assessment with alternative non-invasive tests or biopsy.

APRI is mainly used in patients with viral hepatitis, for the diagnosis of either significant fibrosis (rule-out and rule-in cutoffs of 0.5 and 1.5, respectively), or cirrhosis (rule-out and rule-in cutoffs of 1.0 and 2.0, respectively).[11] APRI >0.5 can identify most adults with chronic HBV and significant fibrosis. Consequently, despite a moderate specificity (65%), the 2024 World Health Organization (WHO) guidelines recommends the use of APRI to trigger antiviral therapy for chronic HBV in low- and middle-income countries.[21] This stresses the need to balance accuracy and pragmatism in the choice of non-invasive tests and their chosen cutoffs across different populations and healthcare systems characterizing the context of use.

FIB-4 was originally developed in a cohort of patients with HCV and human immunodeficiency virus (HIV) co-infection,[12] and has been extensively validated in MASLD and ALD.[23] In a primary care population with risk factors of chronic liver disease, using FIB-4 to screen for advanced

fibrosis is supported by several liver, gastroenterology and endocrine international societies.[24-27] In this population, FIB-4 <1.3 can reliably rule out advanced fibrosis (**Figure 4**).

SECONDARY TESTING

VCTE has been validated for all common causes of liver disease, with similar cutoffs for specific fibrosis stages across etiologies. Values <8 kPa rule out advanced fibrosis (≥F3) with >90% sensitivity, while values >12 kPa rule in advanced fibrosis with >90% specificity.[19] Although VCTE is widely available in hepatology settings, this is not the case in primary care, endocrinology, or cardiology settings.[9]

MRE outperforms ultrasound elastography to rule in earlier stages of fibrosis, (≥F2) but has similar diagnostic accuracy to rule-in advanced fibrosis (≥F3).[28] In all practice settings (primary care or specialized clinics), repeated measurements may be beneficial, especially in case of inconclusive results. Indeed, concordant results are frequently required [29] and sequential testing algorithms have been proposed in different etiologies of liver disease.[7]

DETECTION OF LIVER DISEASE IN ASYMPTOMATIC INDIVIDUALS

Although numerous international guidelines recommend FIB-4 as an initial risk stratification tool, followed by VCTE or ELF, in patients at risk of MASLD, [24-27] diagnosis is delayed in most patients, often until they are at a late stage, or they present with complications of cirrhosis. Therefore, alignment in messaging across societies and stakeholder groups is a priority to improve disease awareness and streamline risk stratification. Patients at risk of MASLD that would require such testing are those with type 2 diabetes, medically complicated obesity,

metabolic risk factors and concomitant alcohol misuse and those with a first degree relative with MASLD-related cirrhosis. [27]

A careful history and basic laboratory assessment will highlight risk factors for the most common causes of liver disease, including alcohol use, risk factors for viral hepatitis (history of intravenous drug use, birth in an endemic area, blood transfusions before the routine testing for HCV (varies by country), steatosis on imaging, hyperferritinemia, and the presence of cardiometabolic risk factors (**Figure 4**). Patients at risk for liver disease can be triaged in primary care or other settings, such as cardiology or endocrinology due to the wide availability of risk stratification tools to detect hepatic fibrosis and their easy applicability across practice settings. The fundamental goal of fibrosis risk stratification is to exclude the presence of advanced fibrosis, providing reassurance that a patient can continue to be followed in primary care, because no liver specific intervention is required. For that purpose, the low prevalence of advanced fibrosis in primary care make initial risk stratification tools, such as FIB-4, suitable for the exclusion of advanced fibrosis, as well as identify those more likely to have advanced fibrosis, who should be referred to a hepatologist **(Figure 4).**

Although steatotic liver disease is highly prevalent, only a small proportion of affected patients develop advanced fibrosis. Therefore, hierarchical testing is required for efficient risk stratification. In unselected patients with MASLD in primary care, the Camden and Islington care pathway (FIB-4 followed by ELF in those with an indeterminate score), resulted in a 5-fold increase in the identification of patients with advanced fibrosis as well as a reduction in inappropriate hepatology referrals.[30] Early diagnosis of fibrosis has been shown to result in sustained beneficial changes in alcohol consumption, diet, weight, and exercise in at-risk ALD and MASLD.[31]

While the prevalence of advanced fibrosis in primary care is generally low, patients such as those with type 2 diabetes, medically complicated obesity and/or concomitant alcohol use, have a higher prevalence of advanced fibrosis, which can impact the predictive value of NITs (**Figure 3**). Among patients with type 2 diabetes, the prevalence of advanced fibrosis may also differ between community practice and secondary or tertiary care diabetes clinics, where the overall metabolic burden is typically higher and thus more enriched with patients with advanced fibrosis, reducing the NPV of FIB-4.[32,33] In such scenarios, early re-testing with FIB-4 or additional testing with ELF or VCTE should be considered when there is clinical suspicion of fibrosis despite a low FIB-4 score.

NONINVASIVE TESTS FOR RISK STRATIFICATION IN CIRRHOSIS

Cirrhosis is the most severe form of liver disease though in itself, it represents a broad clinical spectrum of disease severity (**Figure 1**). It is characterized by a long asymptomatic phase, termed compensated cirrhosis, that through worsening portal hypertension can lead to cirrhosis complications.[34] The development of clinically significant portal hypertension is prognostically important in cirrhosis, as it is associated with a higher risk of cirrhosis complications.[34] Patients with clinically significant portal hypertension and/or oesophageal varices should receive primary prophylaxis with carvedilol to reduce the risk of decompensation and bleeding.[35] The gold standard for the assessment of clinically significant portal hypertension is the hepatic vein pressure gradient measurement (HVPG), >10 mmHg, which is invasive and not widely available.[34]

Non-invasive tests, particularly VCTE, are increasingly used for risk stratification in cirrhosis and may inform treatment decisions.[35] LSM <15 KPa combined with platelets >150.000/mm^3 can rule out clinically significant portal hypertension with >90% sensitivity. Conversely, LSM

>25 kPa is highly specific for clinically significant portal hypertension in cirrhosis and such patients may be considered for treatment with carvedilol (Figure 1). For patients with intermediate LSM (15-25 Kpa) the ANTICIPATE model, which combines LSM values and platelet count, can be used for risk stratification.[36]

Typically, patients with cirrhosis require an upper endoscopy for the assessment of eosophageal and gastric varices and if present, primary prophylaxis for portal hypertensive bleeding. The Baveno VI consensus conference proposed that patients with Child Pugh A cirrhosis, platelets >150.000/mm^3 and LSM<20 kPa, can safely avoid endoscopic screening for high-risk varices.[37] Using these criteria, over 20% of screening endoscopies can be avoided.[38]

Importantly, repeated measurements of non-invasive test can refine prognosis in cirrhosis. For example, a 20% LSM increase at any time was associated with a 50% increased risk of decompensation and liver-related death, although the exact magnitude of change requires further validation.[39] The current recommendation is to repeat LSM annually in such patients.[35]

Finally, non-invasive tests may also be useful to assess treatment response. In patients with cirrhosis and HCV cure, a post-treatment LSM of <20 kPa is associated with a negligible risk of decompensation.[40]

NONINVASIVE TESTS AS PROGNOSTIC INDICATORS FOR LIVER-RELATED EVENTS AND MORTALITY

Since the prognosis of chronic liver disease depends on the severity of fibrosis, it is logical that non-invasive tests reflective of fibrosis burden, may have a role in predicting long-term outcomes, including liver-related events (LRE) (hepatic decompensation or HCC), and mortality.

GENERAL POPULATION

FIB-4, APRI, LiverRisk score (LRS) and VCTE have been found to be predictive of mortality in the general population[41-44] (**Table 1**). However, the utility of noninvasive tests in population screening has not been validated. Most of these data has been derived from retrospective analyses of the US NHANES cohort or the UK biobank where VCTE was done only in selected groups who were considered at risk for hepatic fibrosis, and not systematically.

LIVER CLINICS

FIB-4 has been shown to perform as well as liver biopsy in predicting liver related events in MASLD[45] and better than APRI in predicting liver related events and mortality[46-48] (**Table 1**). Most of these studies were retrospective, with short follow-up and variable outcomes. The ability of ELF score or FibroTest to predict LRE has been less extensively reported than with FIB4.[49-53] Patients with advanced fibrosis and an ELF score >9.8 are more likely to progress to cirrhosis and in those with compensated cirrhosis, an ELF score >11.3 predicts the likelihood of hepatic decompensation.[51] Accordingly, ELF is the only test that has been approved by the US Food and Drug Administration as a prognostic biomarker for patients with MASLD and advanced fibrosis.[51]

VCTE is the elastography technique with the highest level of evidence for the identification of advanced fibrosis. Since the initial proof-of-concept study [54], the ability of LSM by VCTE to predict liver-related outcomes, including cirrhosis decompensation and mortality, has been extensively reported across etiologies but the majority of the data stem from the MASLD population.[46,55-62] For example, at LSM > 20 kPa, VCTE predicted liver outcomes in MASLD with an adjusted HR: 10.65 (95% CI, 6·53–17·35)[45] (**Table 1**). VCTE performed as well as liver biopsy, [45] and outperformed FIB-4 in predicting cirrhosis complications and HCC.[46,58] Recent studies have also suggested the added value of dynamic changes in VCTE over time[39,58,63,64]. In the

largest study to date (n=10,920 with serial LSM) in the MASLD population with LSM >15 kPa at baseline, the incidence of LRE was 7.8 per 1000 person-years in patients whose LSM decreased to <10 kPa during follow-up, compared with 38.7 per 1000 person-years when LSM remained >15 kPa.[58] Further prospective studies are needed to confirm that dynamic changes in LSM are useful surrogates for outcomes in patients with CLD. It is particularly pertinent to establish what magnitude of change constitutes a meaningful difference versus normal variation. This is because non-invasive tests can change over weeks and months with fluctuations in weight, alcohol use and after treatment of the underlying disease.

The ability of MRE to predict liver-related outcomes in MASLD has been less extensively reported, based on a few retrospective studies mainly in US expert centers, with a short follow-up[65-68] (**Table 1**). Data on 2D-SWE or pSWE are limited.[69]

Several composite scores, combining VCTE with other parameters, such as Agile 3+, FAST, MEFIB[58] and a sequential algorithm (FIB-4 followed by VCTE) proposed by EASL have shown promising results in predicting LRE in MASLD.[70]

FUTURE DIRECTIONS

Over the past decade, a major paradigm shift in the hepatology field has been the emergence of non-invasive tests to diagnose and risk stratify patients with establishd or suspected chronic liver disease, replacing liver biopsy for that purpose. There is a growing body of evidence demonstrating the ability of NITs to rule in or rule out advanced fibrosis, as well as their predictive value in terms of liver related events. Further efforts are now needed to increase awareness of non-invasive tests and how to screen for advanced fibrosis in people with risk factors for chronic liver disease beyond the hepatology community, as well as to increase the availability of non-invasive tests in primary care settings. The use of artificial intelligence offers

the exciting possibility of improving risk stratification, while reflex testing of at-risk populations can improve linkage to care. For example, the LiverRisk score, which consists of simple clinical and biological parameters, was recently developed in a large European general population cohort [71], and may allow risk stratification for liver outcomes at a population level [72]. Efforts are also focused on the accurate non-invasive staging of significant (rather than advanced) liver fibrosis, as this is now the threshold to initiate treatment in patients with MASLD.[73] Novel biomarkers incorporating omic approaches offer promise to fulfil this unmet need.[74] Finally, the use of non-invasive tests to assess therapeutic response in patients with MASLD [75] is becoming increasingly important given the high prevalence of the disease and the emergence of approved therapies for this population[73].

References

1. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. Journal of hepatology 2023;79:516-37.
2. 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;385:1559-69.
3. Menghini G. One-second needle biopsy of the liver. Gastroenterology 1958;35:190-9.
4. Tapper EB, Lok AS. Use of Liver Imaging and Biopsy in Clinical Practice. N Engl J Med 2017;377:756-68.
5. Gines P, Krag A, Abraldes JG, Sola E, Fabrellas N, Kamath PS. Liver cirrhosis. Lancet 2021;398:1359-76.
6. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. Journal of hepatology 2015;63:237-64.
7. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update. Journal of hepatology 2021;75:659-89.
8. Sterling RK, Duarte-Rojo A, Patel K, et al. AASLD Practice Guideline on imaging-based non-invasive liver disease assessments of hepatic fibrosis and steatosis. Hepatology 2024.
9. Gines P, Castera L, Lammert F, et al. Population screening for liver fibrosis: Toward early diagnosis and intervention for chronic liver diseases. Hepatology 2022;75:219-28.
10. Castera L, Boursier J. Noninvasive Algorithms for the Case Finding of "At-Risk" Patients with NAFLD. Semin Liver Dis 2022;42:313-26.
11. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-26.
12. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.
13. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 2004;127:1704-13.
14. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705-13.
15. Boursier J, Zarski JP, de Ledinghen V, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology 2013;57:1182-91.
16. Castera L, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2019;156:1264-81 e4.
17. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology 2008;47:455-60.
18. Liang JX, Ampuero J, Niu H, et al. An individual patient data meta-analysis to determine cut-offs for and confounders of NAFLD-fibrosis staging with magnetic resonance elastography. Journal of hepatology 2023;79:592-604.
19. Papatheodoridi M, Hiriart JB, Lupsor-Platon M, et al. Refining the Baveno VI elastography criteria for the definition of compensated advanced chronic liver disease. Journal of hepatology 2021;74:1109-16.
20. 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;73:252-62.
21. World Health Organization. Guidelines for the diagnosis, care and treatment of persons with chronic hepatitis B infection,2024.

22. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. Hepatology 2017;66:1486-501.
23. Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology 2023;78:1966-86.
24. EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). Journal of hepatology 2024;81:492-542.
25. Cusi K, Isaacs S, Barb D, et al. American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). Endocr Pract 2022;28:528-62.
26. Kanwal F, Shubrook JH, Adams LA, et al. Clinical Care Pathway for the Risk Stratification and Management of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2021;161:1657-69.
27. 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;77:1797-835.
28. 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;150:626-37.e7.
29. Majumdar A, Campos S, Gurusamy K, Pinzani M, Tsochatzis EA. Defining the Minimum Acceptable Diagnostic Accuracy of Noninvasive Fibrosis Testing in Cirrhosis: A Decision Analytic Modeling Study. Hepatology 2020;71:627-42.
30. Srivastava A, Gailer R, Tanwar S, et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. Journal of hepatology 2019;71:371-8.
31. Kjaergaard M, Lindvig KP, Thorhauge KH, et al. Screening for Fibrosis Promotes Lifestyle Changes: A Prospective Cohort Study in 4796 Individuals. Clin Gastroenterol Hepatol 2024;22:1037-47 e9.
32. Ajmera V, Cepin S, Tesfai K, et al. A prospective study on the prevalence of NAFLD, advanced fibrosis, cirrhosis and hepatocellular carcinoma in people with type 2 diabetes. Journal of hepatology 2023;78:471-8.
33. Castera L, Laouenan C, Vallet-Pichard A, et al. High Prevalence of NASH and Advanced Fibrosis in Type 2 Diabetes: A Prospective Study of 330 Outpatients Undergoing Liver Biopsies for Elevated ALT, Using a Low Threshold. Diabetes Care 2023;46:1354-62.
34. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749-61.
35. de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C. Baveno VII - Renewing consensus in portal hypertension. Journal of hepatology 2022;76:959-74.
36. Abraldes JG, Bureau C, Stefanescu H, et al. Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: The "Anticipate" study. Hepatology 2016;64:2173-84.
37. de Franchis R. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. Journal of hepatology 2015;63:743-52.

38. Stafylidou M, Paschos P, Katsoula A, et al. Performance of Baveno VI and Expanded Baveno VI Criteria for Excluding High-Risk Varices in Patients With Chronic Liver Diseases: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2019;17:1744-55.e11.
39. Semmler G, Yang Z, Fritz L, et al. Dynamics in Liver Stiffness Measurements Predict Outcomes in Advanced Chronic Liver Disease. Gastroenterology 2023;165:1041-52.
40. Semmler G, Lens S, Meyer EL, et al. Non-invasive tests for clinically significant portal hypertension after HCV cure. Journal of hepatology 2022;77:1573-85.
41. Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. Hepatology 2013;57:1357-65.
42. Unalp-Arida A, Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. Hepatology 2017;66:84-95.
43. Innes H, Morling JR, Buch S, Hamill V, Stickel F, Guha IN. Performance of routine risk scores for predicting cirrhosis-related morbidity in the community. Journal of hepatology 2022;77:365-76.
44. Vilar-Gomez E, Vuppalanchi R, Gawrieh S, Samala N, Chalasani N. CAP and LSM as determined by VCTE are independent predictors of all-cause mortality in the US adult population. Hepatology 2023;77:1241-52.
45. Mozes FE, Lee JA, Vali Y, et al. Performance of non-invasive tests and histology for the prediction of clinical outcomes in patients with non-alcoholic fatty liver disease: an individual participant data meta-analysis. Lancet Gastroenterol Hepatol 2023;8:704-13.
46. Boursier J, Vergniol J, Guillet A, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. Journal of hepatology 2016;65:570-8.
47. Hagstrom H, Nasr P, Ekstedt M, Stal P, Hultcrantz R, Kechagias S. Accuracy of Noninvasive Scoring Systems in Assessing Risk of Death and Liver-Related Endpoints in Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol 2019;17:1148-56 e4.
48. Lee J, Vali Y, Boursier J, et al. Prognostic accuracy of FIB-4, NAFLD fibrosis score and APRI for NAFLD-related events: A systematic review. Liver Int 2021;41:261-70.
49. Pearson M, Nobes J, Macpherson I, et al. Enhanced liver fibrosis (ELF) score predicts hepatic decompensation and mortality. JHEP Rep 2024;6:101062.
50. Rasmussen DN, Thiele M, Johansen S, et al. Prognostic performance of 7 biomarkers compared to liver biopsy in early alcohol-related liver disease. Journal of hepatology 2021;75:1017-25.
51. Sanyal AJ, Harrison SA, Ratziu V, et al. The Natural History of Advanced Fibrosis Due to Nonalcoholic Steatohepatitis: Data From the Simtuzumab Trials. Hepatology 2019;70:1913-27.
52. Younossi ZM, Anstee QM, Wai-Sun Wong V, et al. The Association of Histologic and Noninvasive Tests With Adverse Clinical and Patient-Reported Outcomes in Patients With Advanced Fibrosis Due to Nonalcoholic Steatohepatitis. Gastroenterology 2021;160:1608-19 e13.
53. Munteanu M, Pais R, Peta V, et al. Long-term prognostic value of the FibroTest in patients with non-alcoholic fatty liver disease, compared to chronic hepatitis C, B, and alcoholic liver disease. Aliment Pharmacol Ther 2018;48:1117-27.
54. Foucher J, Chanteloup E, Vergniol J, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 2006;55:403-8.

55. Corpechot C, Carrat F, Gaouar F, et al. Liver stiffness measurement by vibration-controlled transient elastography improves outcome prediction in primary biliary cholangitis. Journal of hepatology 2022;77:1545-53.
56. Hegmar H, Wester A, Aleman S, et al. Liver stiffness predicts progression to liver-related events in patients with chronic liver disease - A cohort study of 14 414 patients. Liver Int 2024;44:1689-99.
57. Vergniol J, Foucher J, Terrebonne E, et al. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. Gastroenterology 2011;140:1970-9, 9 e1-3.
58. Lin H, Lee HW, Yip TC, et al. Vibration-Controlled Transient Elastography Scores to Predict Liver-Related Events in Steatotic Liver Disease. JAMA 2024;331:1287-97.
59. 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;72:581-9.
60. Pons M, Rivera-Esteban J, Ma MM, et al. Point-of-Care Noninvasive Prediction of Liver-Related Events in Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol 2024;22:1637-45 e9.
61. Ciardullo S, Muraca E, Zerbini F, Perseghin G. Liver stiffness is associated with all-cause mortality in patients with NAFLD: A systematic review and meta-analysis. Liver Int 2023;43:2604-10.
62. Shen Y, Wu SD, Wu L, et al. The prognostic role of liver stiffness in patients with chronic liver disease: a systematic review and dose-response meta-analysis. Hepatol Int 2019;13:560-72.
63. Gawrieh S, Vilar-Gomez E, Wilson LA, et al. Increases and decreases in liver stiffness measurement are independently associated with the risk of liver-related events in NAFLD. Journal of hepatology 2024;81:600-8.
64. Petta S, Sebastiani G, Vigano M, et al. Monitoring Occurrence of Liver-Related Events and Survival by Transient Elastography in Patients With Nonalcoholic Fatty Liver Disease and Compensated Advanced Chronic Liver Disease. Clin Gastroenterol Hepatol 2021;19:806-15 e5.
65. Gidener T, Ahmed OT, Larson JJ, et al. Liver Stiffness by Magnetic Resonance Elastography Predicts Future Cirrhosis, Decompensation, and Death in NAFLD. Clin Gastroenterol Hepatol 2021;19:1915-24 e6.
66. Han MAT, Vipani A, Noureddin N, et al. MR elastography-based liver fibrosis correlates with liver events in nonalcoholic fatty liver patients: A multicenter study. Liver Int 2020;40:2242-51.
67. Higuchi M, Tamaki N, Kurosaki M, et al. Longitudinal association of magnetic resonance elastography-associated liver stiffness with complications and mortality. Aliment Pharmacol Ther 2022;55:292-301.
68. Ajmera V, Kim BK, Yang K, et al. Liver Stiffness on Magnetic Resonance Elastography and the MEFIB Index and Liver-Related Outcomes in Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis of Individual Participants. Gastroenterology 2022;163:1079-89 e5.
69. Trebicka J, Gu W, de Ledinghen V, et al. Two-dimensional shear wave elastography predicts survival in advanced chronic liver disease. Gut 2022;71:402-14.
70. Boursier J, Hagstrom H, Ekstedt M, et al. Non-invasive tests accurately stratify patients with NAFLD based on their risk of liver-related events. Journal of hepatology 2022;76:1013-20.

71. Graupera I, Thiele M, Ma AT, et al. LiverScreen project: study protocol for screening for liver fibrosis in the general population in European countries. BMC Public Health 2022;22:1385.
72. Serra-Burriel M, Juanola A, Serra-Burriel F, et al. Development, validation, and prognostic evaluation of a risk score for long-term liver-related outcomes in the general population: a multicohort study. Lancet 2023;402:988-96.
73. Harrison SA, Bedossa P, Guy CD, et al. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. N Engl J Med 2024;390:497-509.
74. Ghosh S, Zhao X, Alim M, Brudno M, Bhat M. Artificial intelligence applied to 'omics data in liver disease: towards a personalised approach for diagnosis, prognosis and treatment. Gut 2025;74:295-311.
75. Rinella ME, Dufour JF, Anstee QM, et al. Non-invasive evaluation of response to obeticholic acid in patients with NASH: Results from the REGENERATE study. Journal of hepatology 2022;76:536-48.

Figures Legend

Figure 1. Diagnosing liver fibrosis stages and progression, using clinical signs, liver biopsy, or non-invasive tests.

Liver disease is often asymptomatic until complications occur, therefore a clinical diagnosis is typically made at the stage of decompensated cirrhosis. Liver biopsy is usually reserved to aid the diagnosis of liver disease of unclear aetiology, when the degree of inflammation needs assessment or when there is significant discrepancy in non-invasive fibrosis assessment. The use of non-invasive fibrosis tests allows testing for liver fibrosis in asymptomatic individuals who might have risk factors but not an established diagnosis of chronic liver disease. This has become particularly relevant with the increasing prevalence of steatotic liver disease. The figure demonstrates how different histological stages of fibrosis can be diagnosed using clinical parameters and the four most used non-invasive fibrosis tests, with their corresponding cut-offs across the fibrosis spectrum.

Abbreviations: FIB-4, fibrosis-4 score; ELF, Enhanced liver fibrosis score, VCTE, vibration controlled transient elastography; MRE, magnetic resonance elastography.

Figure 2. Non-invasive tests for liver fibrosis assessment.

Panel A shows blood-based tests and elastography techniques used to assess liver fibrosis, including their context of use, advantages, disadvantages, and confounding factors. The ratio of aspartate aminotransferase (AST) to platelet index (APRI) is calculated as follows: (AST ÷ upper limit of the normal range) ÷ platelet count. The Fibrosis-4 (FIB-4) score is calculated as (age × AST) ÷ (platelet count × √ALT). The NAFLD Fibrosis Score (NFS) is calculated as follows:

−1.675 + 0.037 × age + 0.094 × BMI + 1.13 × IR or diabetes (yes = 1, no = 0) + 0.99 × AST:ALT ratio − 0.013 × platelet count − 0.66 × albumin. The ELF test, FibroTest and FibroMeter are proprietary formula.

The context of use further defines the population in which the NIT will be used and the setting in which it will be used (e.g., in primary care or liver clinics).

Panel B shows the advantages and disadvantages of FIB-4 vs. ELF and of VCTE vs. MRE, in terms of accuracy, evidence (based on hundreds or thousands of patients), availability, and cost.

Confounding factors with the risk of false positive results for FIB-4, ELF and FibroTest and elastography. These should be carefully excluded to avoid mischaracterization of fibrosis burden.

Abbreviations: ALT, alanine aminotransferase; BMI body-mass index, INR, international normalized ratio, IR, insulin resistance. VCTE, Vibration-controlled elastography; p-SWE, point-shear wave elastography; 2D-SWE, two-dimensional shear wave elastography, and MRE, magnetic resonance elastography.

Figure 3. Impact of the prevalence of advanced fibrosis (F3/F4) on performance of non-invasive tests.

Conditional probability plot of FIB-4, ELF, and VCTE for the diagnosis of advanced fibrosis. Sensitivity and specificity for these tests were derived from meta-analyses in MASLD and are: 78% and 81% for FIB4 (cut-off 1.3), [22] 65% and 86% for ELF (cut-off 9.8),[20] and 80% and 90% for VCTE (cut-off 9.6-11.4 KPa) [22]. The pretest probability (prevalence) of advanced fibrosis is shown horizontally on the black line from nil on the left, to 50% on the right. The prevalence varies according to the setting (primary care <10%, diabetology clinic 10-20%, liver clinic 20-50%). The impact that a positive test result with each of the three non-invasive tests (FIB-4,

ELF, or VCTE) will have on that probability of advanced fibrosis (positive predictive value) is plotted for each individual test against the baseline prevalence in the upper panel. Similarly, the impact of a negative test result with each of these tests on the probability of advanced fibrosis (negative predictive value) is plotted against the baseline prevalence in the lower panel.

Example: In a population with a 30% prevalence (dash-red line) of advanced fibrosis (a priori probability of fibrosis), a positive FIB-4, ELF or VCTE test will result in a positive predictive value of 54%, 67% and 76%, respectively. In case of a negative FIB-4, ELF or VCTE test, the negative predictive value is 91%, 88%, and 85%, respectively.

Abbreviations: FIB-4, fibrosis-4 score; ELF, Enhanced liver fibrosis score, VCTE, vibration controlled transient elastography; MRE, magnetic resonance elastography.

Figure 4. Non-invasive sequential algorithm to screen for advanced fibrosis in patients with suspicion for chronic liver disease.

Several factors, such as the presence of cardiometabolic risk factors, hepatic steatosis, alcohol consumption, viral hepatitis or chronic elevation in aminotransferases, should prompt assessment for the presence of advanced liver disease. The goal in this regard, in the primary care setting is to exclude the presence of advanced disease (defined as stage 3 (pre-cirrhosis) or stage 4 (cirrhosis)). Adverse liver related outcomes are demonstrable in this subset of patients and targeted intervention could prevent the development of such outcomes. Further, identification of specific disease states, e.g. viral hepatitis, autoimmune hepatitis, hemochromatosis, will also lead the appropriate disease specific intervention. Initial laboratory evaluation should include a liver chemistry panel, international normalized ratio, and complete blood count with platelets. Patients with FIB-4< 1.3 (or <2.0 in those >65) can

remain in primary care and reassessed depending on their risk factors. Those with FIB-4 $\geq$ 1.3, should undergo secondary testing using locally available modalities. Preferably, VCTE should be done. If not available, other methods of measuring liver stiffness, such as other ultrasound-based elastography or MRE, or ELF testing should be used to further risk stratify the patient. Cutoff values provided consider the prevalence of advanced fibrosis in the primary care setting. If advanced fibrosis is suspected or likely, the patient should be referred to specialty care. In the setting of discordant non-invasive testing, persistent elevation in liver chemistries or concern for an additional or alternate etiology, a liver biopsy may be needed.

Abbreviations: AST, aspartate aminotransferase; ALT alanine aminotransferase; FIB-4, fibrosis-4 score; ELF, Enhanced liver fibrosis score, VCTE, vibration controlled transient elastography; MRE, magnetic resonance elastography.

Table 1. Staging of liver fibrosis and prognostication of chronic liver disease using non-invasive tests (NITs) of fibrosis

Staging of fibrosis								
NIT	**Etiology**	**Design/patients**	**Cut-offs**	**Sensitivity**	**Specificity**	**LR+**	**LR-**	**Reference**
Significant fibrosis (≥F2)								
APRI	CHB	Meta-analysis (264 studies)	0.5 1.5	72% 29%	64% 92%	2.0 3.7	0.4 0.8	WHO 2024 [21]
ELF	MASLD	Retrospective study (192 participants)	9.0	90%	50%	2	0.18	Guha et al. [17]
VCTE	CHB MASLD	Meta-analysis (264 studies) Meta-analysis (64 studies, 13,046 participants)	6-8 kPa 6.7-7.0 kPa	75% 74%	79% 68%	3.6 2.3	0.3 0.4	WHO 2024 [21] Xiao et al. [22]
MRE	MASLD	IPDM (8 studies, 798 participants)	3.14 kPa	79%	89%	7.3	0.22	Liang et al.[18]
Advanced fibrosis (≥F3)								
FIB-4	CHB MASLD	Meta-analysis (264 studies) Meta-analysis (64 studies, 13,046 participants)	1.45 3.25 1.3 2.67	73% 31% 78% 32%	67% 95% 71% 96%	2.2 6.3 2.7 8	0.4 0.7 0.3 0.7	WHO 2024 [21] Xiao et al. [22]
ELF	MASLD	Meta-analysis (11 studies, 4,452 participants)	9.8	65	86	4.6	0.4	Vali et al.[20]
VCTE	CHB SLD	Meta-analysis (264 studies) Retrospective Real-world multicentre study (1,915 participants)	8-11 kPa 8 kPa 12 kPa	80% 93% 79%	85% 64% 89%	5.4 2.6 6.2	0.2 0.1 0.3	WHO 2024 [21] Papatheodoridi et al.[19]
MRE	MASLD	IPDM	3.53 kPa	87%	88%	7.8	0.14	Liang et al.[18]

		(8 studies, 798 participants)						
Cirrhosis (F4)								
APRI	CHB	Meta-analysis (264 studies)	1 2	57% 29%	73% 89%	2.2 2.6	0.6 0.8	WHO 2024 [21]
ELF								
VCTE	CHB MASLD	Meta-analysis (264 studies) Meta-analysis (64 studies, 13,046 particpants)	11-14 kPa 11.5-12 kPa	83% 78%	89% 89%	7.5 7.1	0.2 0.2	WHO 2024 [21] Xiao et al. [22]
MRE	MASLD	IPDM (8 studies, 798 participants)	4.45 kPa	88%	89%	7.8	0.13	Liang et al.[18]

Prognosis								
NIT	**Etiology**	**Design/patients**	**Follow-up median**	**Cut-offs**	**Liver-related events**	**Liver Mortality**	**All-cause Mortality**	**Reference**
General population (GP)								
FIB-4	NA	Retrospective GP-based study NHANES 1988-94 (14,841participants)	19.3 years	Predefined 1.3 2.67	NA	HR : 3.15 (1.33-7.44) HR :25.14 (8.38-75.40)	NA HR : 1.38 (1.21–1.58)	Unalp-Arida et al.[42]
APRI	NA	Retrospective GP-based study NHANES 1988-94 (14,841participants)	19.3 years	Predefined 0.5	NA	HR : 9.44 (5.02-17.73)	HR : 1.46 (1.20–1.78)	Unalp-Arida et al.[42]
LRS	NA	Retrospective GP-based study UK biobank (46,200 participants)	12 years	high-risk 15	NA	HR : 471 (347–641)	NA	Serra-Burriel et al.[72]
VCTE	NA	Retrospective GP-based study NHANES 2017-18	24.4 months	Predefined 10 kPa 20 kPa	NA	NA	HR : 3.4 (1.0–13.8) HR : 5.2 (1.2–22.3)	Vilar-Gomez et al.[44]

		(4192 participants)						
Liver clinics								
FIB-4	MASLD	IPDM (25 studies; 2,518 participants)	57 months	Predefined 1.3 2.67	Composite* 1.3 - 2.67 aHR: 4.29 (2·30–8·00) >2.67 aHR: 18·76 (10.17–34·60)			Mozes et al.[45]
VCTE	MASLD	IPDM (25 studies; 2,518 participants)	57 months	Predefined 10 kPa 20 kPa	Composite* 10 < < 20 kPa aHR: 3.12 (1.94–5.02) >20 kPa aHR: 10.65 (6.53–17.35)			Mozes et al.[45]
	MASLD	Prospective NASH-CRN cohort (1,403 patients)	4.4 years	10 kPa 15 kPa	aHR : 4.0 (2.6-6.4) aHR 5.5 (3.6- 8.5)	NA	NA	Gawrieh et al.[63]
	MASLD	Retrospective international multicenter cohort (16,603 patients)	51.7 months	10 kPa 15 kPa	6 per 1000 Person yr 32.8 per 1000 Person yr	NA	NA	Lin et al.[58]
	MASLD	Meta-analysis (6 studies; 18771 participants)	3.6 years	NA	NA	NA	RR**: 1.03 (1.01-1.05)	Ciardullo et al.[61]
	All liver diseases	Meta-analysis (62 studies; 43,817 participants)	NA	NA	RR**: 1.07 (1.04-1.09)	RR**: 1.11 (1.05- 1.17)	RR**: 1.08 (1.06–1.11)	Shen et al.[62]
MRE	MASLD	IPDM (6 studies; 1,707 participants)	3 years	Predefined 5 kPa 8 kPa	5 < < 8 kPa HR: 11.0 (7.03-17.1) > 8 kPa HR: 15.9 (9.32-27.2)	NA	NA	Ajmera et al.[68]

APRI, Aspartate to platelet ratio index; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 score ; VCTE, vibration-controlled transient elastography ; MRE, magnetic resonance elastography; LRS, LiverRisk score; CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease; LSM, liver stiffness measurement; kPa, kilo Pascal; IPDM, individual patients' data meta-analysis; HR, hazard ratio ; aHR adjusted hazard ratio; WHO, World Health Organization

* Composite endpoint including HCC, ascites, variceal hemorrhage, hepatic encephalopathy, progression to a MELD score of 15 or higher, liver transplantation, and mortality from any cause. ** For one kPa increment in baseline LSM

Key points

- The prognosis and management of chronic liver disease is highly dependent on the extent and progression of liver fibrosis.
- Non-invasive fibrosis tests have revolutionized the practice of hepatology by allowing the identification of patients with advanced fibrosis or cirrhosis before they develop liver complications.
- Non-invasive tests are useful for staging fibrosis in chronic liver disease, diagnosing and risk stratifying cirrhosis, and predicting complications of cirrhosis.
- Cost, availability, and context of use are important considerations when using non-invasive tests in clinical practice.
- The Fibrosis-4 Index, the Enhanced Liver Fibrosis Score and liver stiffness measurement by vibration-controlled transient elastography or magnetic resonance elastography are the most validated and used non-invasive tests.
- Further efforts are now needed to increase awareness of non-invasive tests among healthcare professionals to screen for advanced fibrosis in people with risk factors for chronic liver disease beyond the hepatology community.