Review

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Limitations of non-invasive tests for assessment of liver fibrosis

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Summary

The diagnostic assessment of liver injury is an important step in the management of patients with chronic liver disease (CLD). Although liver biopsy is the reference standard for the assessment of necroinflammation and fibrosis, the inherent limitations of an invasive procedure, and need for repeat sampling, have led to the development of several non-invasive tests (NITs) as alternatives to liver biopsy. Such non-invasive approaches mostly include biological (serum biomarker algorithms) or physical (imaging assessment of tissue stiffness) assessments. However, currently available NITs have several limitations, such as variability, inadequate accuracy and risk factors for error, while the development of a newer generation of biomarkers for fibrosis may be limited by the sampling error inherent to the reference standard. Many of the current NITs were initially developed to diagnose significant fibrosis in chronic hepatitis C, subsequently refined for the diagnosis of advanced fibrosis in patients with non-alcoholic fatty liver disease, and further adapted for prognostication in CLD. An important consideration is that despite their increased use in clinical practice, these NITs were not designed to reflect the dynamic process of fibrogenesis, differentiate between adjacent disease stages, diagnose non-alcoholic steatohepatitis, or follow longitudinal changes in fibrosis or disease activity caused by natural history or therapeutic intervention. Understanding the strengths and limitations of these NITs will allow for more judicious interpretation in the clinical context, where NITs should be viewed as complementary to, rather than as a replacement for, liver biopsy.

Keywords: Liver biopsy; Biomarkers; Elastography; Viral hepatitis; Non-alcoholic fatty liver disease

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Introduction

Histologic assessment of liver disease has been a cornerstone of therapeutic decision making and prognostication in chronic liver disease (CLD) for decades. Liver biopsy is still the established standard for assessment of injury, inflammation, and fibrosis stage, although its role in CLD states, such as chronic hepatitis C (CHC), has been significantly diminished in recent years. The advent of non-invasive approaches for assessment of liver fibrosis, combined with the more recent evolution of simplified direct-acting antiviral (DAA) therapeutic regimens, has now essentially eliminated the need for liver biopsy to differentiate mild from significant (≥F2) disease prior to antiviral therapy for CHC. These non-invasive approaches for assessment of liver fibrosis include various biochemical serum markers, or imaging modalities that provide a physical measure of liver stiffness.[1] There is now increased availability and greater acceptance of non-invasive tests (NITs) as an alternative to biopsy for diagnosis of advanced fibrosis and determination of prognosis in CLD. Current NITs certainly overcome the risks and sampling limitations associated with liver biopsy. However, as these tests become increasingly incorporated into routine clinical practice, there are diagnostic limitations that need to be considered when interpreting results.

Many of the existing serum biomarker panels and imaging tools were developed in relation to a cross-sectional, binary assessment of categorical histopathologic scores in CHC, and not to specifically reflect the variable and dynamic nature of liver fibrogenesis, or the underlying aetiology of CLD. As we consider how to best assess fibrosis progression or regression for antifibrotic therapeutic development without repeat tissue sampling, routinely available NITs do not yet provide sufficiently reliable and sensitive assessment of quantitative changes in fibrosis. Many of the available NITs for fibrosis were validated in viral hepatitis. There is emerging data on the use of NITs to diagnose liver fibrosis related to non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease, autoimmune and cholestatic liver diseases. The diagnostic and prognostic role of NITs in other CLDs was reviewed in the EASL-ALEH Clinical Practice Guidelines.[1] This article will highlight some of the diagnostic limitations of current serum and imaging NITs for fibrosis assessment in viral hepatitis, NAFLD and other CLDs and

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provide practical guidance on the main points to consider when applying NITs in clinical practice (Fig. 1).

Key points

- Interpretation of NITs of fibrosis in clinical practice requires consideration of physiologic variability, quality criteria, co-morbid and behavioural risk factors, liver disease aetiology, and applicability in the specific context of use.
- There are no validated NIT thresholds for longitudinal assessment that correspond to histologic changes in fibrosis, for example to assess regression in advanced disease following antiviral therapy for viral hepatitis.
- Current NITs are not able to diagnose NASH or determine the anti-fibrotic efficacy of emerging therapeutic approaches in NAFLD.

Liver biopsy

Hepatic fibrosis consists of the deposition of extracellular matrix components in highly stable and optically visible fibres within the liver parenchyma.[2] Since histological examination allows for direct visualisation of the liver parenchyma, it is still considered as the reference tool for evaluation of fibrosis. Moreover, liver biopsy remains the only available technique to diagnose non-alcoholic steatohepatitis (NASH).[3] Several semi-quantitative scoring systems have been proposed to stage liver fibrosis. The main classification systems in use for viral hepatitis and NAFLD include the METAVIR and the NASH Clinical Research Network systems (Table 1).[4,5] However, we need to acknowledge that the reference standard has inherent limitations that further reduce the accuracy of NITs.[6,7] Historically, liver biopsy was intended as a tool for differential diagnosis across different aetiologies of liver disease, rather than as a staging tool for liver fibrosis. Liver biopsy is also an invasive procedure that is costly, associated with morbidity and mortality, provides only a cross-sectional interpretation of a dynamic process, and is liable to sampling error due to heterogeneity in fibrosis distribution and interpretation.[8,9] Increasing the length of liver biopsy decreases the risk of such sampling error, and although a 25 mm biopsy length is considered an optimal specimen for an accurate evaluation of non-cirrhotic fibrosis stages, 15 mm with an adequate number of portal tracts has been considered sufficient in prior studies.[10–14] Due to heterogeneity in the fibrosis pattern and distribution based on the aetiology, it is possible that the recommended lengths may also vary across CLDs. The diameter of the core should also be considered. A 16-gauge needle with inner diameter (1.2 mm) larger than a liver lobule (0.5-1 mm) is considered adequate.[14] It is also important to consider the level of expertise of the pathologist, as there is less inter-observer variability between experienced pathologists when using semi-quantitative scoring systems to stage liver fibrosis in CLD.[15–17] The misclassification of biopsy, with greater than 25% false negative/positive rates even for delineating broad overlapping fibrosis stages, is further exacerbated for intermediate (mild-moderate) stage disease. Biopsy has a U-shape accuracy for intermediate stages both due to an observer agreement variability that is worse between F2 and F1, and the relatively small difference in area of fibrosis between F2 and F1.[18] To reduce sampling error and improve specimen quality, laparoscopic and multiple sampling approaches may yield a larger specimen size, but are associated with increased risk and cost.[19]

Despite these limitations, the reference standard can provide useful additional information on fibrosis that is not utilised in routine histologic assessment but should be considered to

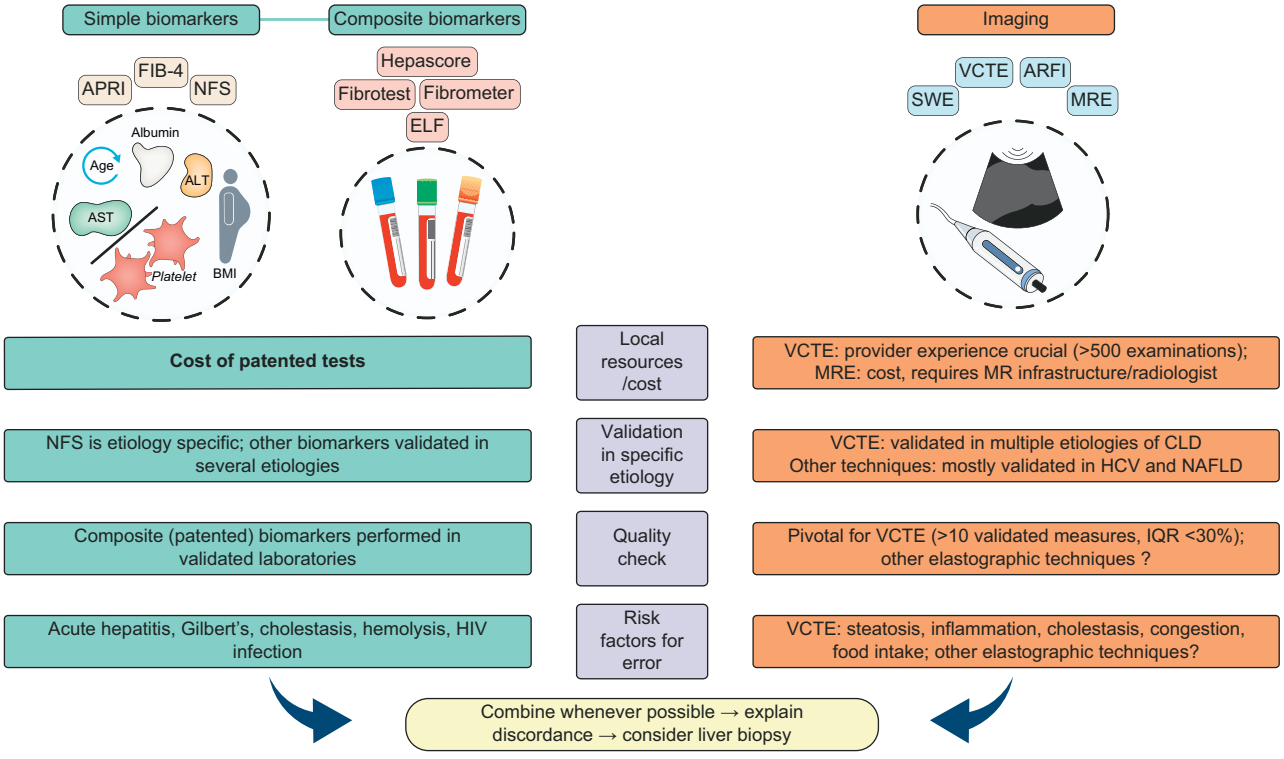

Fig. 1. Guidance and consideration in using NIT for staging liver fibrosis. NIT, non-invasive test.

Table 1. METAVIR and NASH CRN staging systems for liver fibrosis.

METAVIR		NASH CRN	
Degree	**Description**	**Degree**	**Description**
0	None	0	None
1	Periportal fibrosis	1 a	Mild (delicate) zone 3 perisinusoidal fibrosis
		1b	Moderate (dense) zone 3 perisinusoidal fibrosis
		1c	Portal/periportal fibrosis only
2	Periportal fibrosis with few bridges or septa	2	Zone 3 perisinusoidal fibrosis with portal/periportal fibrosis
3	Bridging fibrosis	3	Bridging fibrosis
4	Cirrhosis	4	Cirrhosis

NASH, non-alcoholic steatohepatitis.

assess the efficacy of antifibrotic approaches.[20] In order to get more accuracy and objectivity for the quantification of fibrosis, pathologists have developed approaches based on image analysis, such as computerised or digital morphometry. However, even when using fully automated techniques, morphometry remains time-consuming and is not recommended for routine practice. A major advantage of morphometry is that it provides a finite quantitative scale (collagen proportionate area [CPA]) which is linear and more accurate than those determined by semi-quantitative scoring methods.[12,21] Morphometry has been readily adopted for clinical trials, but these studies have shown the non-linearity relationship between CPA and semi-quantitative stages of fibrosis, a further demonstration that fibrosis stage does not equate to "amount" of fibrosis.[22,23] Indeed, the CPA was developed to sub-classify patients with cirrhosis, rather than to provide a continuous measure of pre-cirrhotic fibrosis.[24] Other refined approaches for fibrosis assessment in the context of antifibrotic efficacy, include spatial organisation of fibrosis using fractal geometry, assessment of *in situ* fibrillar collagen using non-linear optical microscopy and second-harmonic generation, or image-based quantitation of architectural parameters.[25–27] These techniques are not readily available and have a limited role in routine clinical practice.

Non-invasive tools for liver fibrosis staging

Serum biomarkers

Fibrogenesis is a dynamic process involving extracellular matrix synthesis and degradation. Fibrosis is regulated by host genetic factors, and involves complex cellular interactions occurring in a rich pro-fibrogenic microenvironment of inflammatory cytokines and adipokines, as well as angiogenic and neuroendocrine signals.[20,28] Host co-morbid factors such as the metabolic syndrome or alcohol provide further imbalance in the fibrogenic cascade. Serum biomarkers have the potential to reflect these dynamic changes, and thus the ability to assess matrix turnover earlier in the disease process. This could help to identify patients at risk of progressive fibrosis, allowing for earlier intervention or closer surveillance. Despite significant progress in our understanding of the pathobiology of fibrogenesis, none of the routinely available NITs have been validated as monitoring biomarkers, as extensive long-term longitudinal data are lacking. Box 1 summarises the main criteria of an ideal marker of fibrosis. Many of the current serum biomarker algorithms adopted into the clinical setting include a combination of either "direct markers", that are mostly complex proteins derived from myofibroblasts and extracellular matrix remodelling, or "indirect markers" that are relatively simple biochemical tests which estimate disease severity. Of note, in the case of patented serum biomarkers, few studies have been conducted independently from the developers of these tests. Moreover, although an improvement in accuracy is observed with patented compared to simple markers, their widespread application remains limited by their cost and availability. Various other combinations of cytokines, chemokines, genetic polymorphisms, microRNAs, and post-translational modified glycoproteins have also been proposed as candidate biomarkers of fibrosis in CLD but have not yet been validated or made routinely available outside research laboratories.

Imaging elastography

Over the past 15 years, a major advance in liver fibrosis staging has been the introduction of liver stiffness measurement (LSM) using ultrasound (US) or magnetic resonance (MR)-based techniques. Some of these devices have been readily adopted into clinical practice as point-of care tests to complement serum biomarkers of fibrosis and clinical assessment in CLD. Imaging elastography parameters are reported as m/s or kPa and vary depending on device-related technical factors such as shear wave frequency, signal acquisition, and software. At present, reported LSM thresholds cannot be compared across different elastography platforms. However, the Quantitative Imaging Biomarkers Alliance continues to develop and refine protocols and hardware/software standards for imaging elastography.

Vibration-controlled transient elastography

Monodimensional US vibration-controlled transient elastography (VCTE, Echosens, Paris, France), was the first US-based technique to be introduced. VCTE is a rapid, safe, and reproducible procedure for LSM assessment that can be performed at the bedside with immediate results. This represents a true point-of-care LSM assessment and is the most widely used and validated technique for non-invasive imaging assessment of liver fibrosis. Quality measures are established for VCTE, and require at least 10 validated measurements and an interquartile range (IQR, that reflects variations among LSM) <30% of the median value (IQR/LSM ≤30%).[29] Interpretation of the LSM must be in the context of these quality metrics, and prior studies have shown that the highest accuracy for fibrosis staging is obtained with the more stringent IQR/LSM ≤10%. Around 15% of results may be unreliable, and failure to obtain any LSM occurs in ~3% of patients, mostly due to obesity or operator inexperience (<500 examinations).[30,31] VCTE results indicating IQR/LSM >30% in conjunction with LSM ≥7.1 kPa are particularly unreliable.[32] In routine clinical practice, LSM readings can still be obtained in most patients, and so quality measures are often overlooked. This important limitation is seldom appreciated by the requesting provider. VCTE may not

Box 1. Ideal biomarkers of fibrosis in chronic liver disease.

- Easy to perform
- Cost-effective
- Readily available
- Provides early diagnosis
- High diagnostic accuracy
- Correlates with extracellular matrix deposition
- Validated independently of manufacturer across different etiology of liver disease
- Follows longitudinal change in fibrosis progression/regression
- Tissue specific
- Provides prognosis
- Not influenced by physiologic variation (for example, due to age, gender, diet, bodyhabitus, exercise, diurnal variation)
- Reproducible characteristics across diagnostic platforms
- Minimal variation across multiethnic populations
- Avoids further invasive or other complex diagnostic testing

be obtained in patients with a narrow intercostal space or ascites. Normal LSM range varies according to the population, as demonstrated in a healthy South Asian cohort with normal alanine aminotransferase (ALT) and an LSM range of 3.2–8.5 kPa, with higher LSM readings reported in underweight and obese patients.[33]

VCTE XL probes generate lower LSM than M probes, and validated XL thresholds for fibrosis stage in viral hepatitis and NAFLD have not yet been established. VCTE accuracy decreases with body mass index (BMI) >30, and the XL probe is still associated with unreliable LSM rates of 15–25% in non-Asian NAFLD cohorts.[34,35] Using M and XL probes for obese and non-obese patients with NAFLD yields comparable LSM and diagnostic performance.[35] Besides obesity and operator inexperience, other important LSM confounders that are independent of fibrosis, include inflammation, cholestasis, congestion, food intake and portal vein thrombosis.[36,37] Elevation in ALT >120 IU/L, or significant necro-inflammatory activity, confounds results by increasing LSM, and could place patients with F0-2 fibrosis into the "cirrhotic" LSM range.[38] From a practical perspective, VCTE assessments are scheduled throughout the entire day, so a strict requirement for a 2–3 hour fasting period is often not feasible and is often overlooked for initial clinic visits and point-of-care testing. A 600 Kcal meal will increase LSM for 1–2 hours, and could place a patient with CHC and moderate-advanced fibrosis into the cirrhotic range.[39] Alcohol excess, amyloidosis, or other co-morbid conditions that lead to hepatic congestion (right heart failure) or cholestasis will also significantly elevate LSM (Table 2). VCTE should be performed by an experienced operator in fasting patients, considering ALT levels, BMI, alcohol intake, and other co-morbid states. LSM thresholds for significant fibrosis and cirrhosis have been validated for CHC but vary in chronic hepatitis B (CHB), HIV-HCV coinfection, and other CLDs. A recent technical review from the American Gastroenterology Association (AGA) was not able to provide a recommendation on an acceptable LSM threshold for the diagnosis of NAFLD cirrhosis.[40] Simplifying VCTE thresholds, such as the proposed "rule of 5" for ruling-in or -out compensated advanced liver disease and progression to clinically significant portal hypertension, may provide a more practical application for routine clinical use.[41]

Shear wave elastography

Several other US-based elastography techniques based on strain ("static") or shear wave ("dynamic") imaging have been incorporated into US devices. Static imaging elastography appears to have limited utility for diagnosis of liver fibrosis due to i) the need for operator-dependent manual application of stress to induce deformation in liver tissue, and ii) physiologic motion artefact, resulting in significant variability in the reported parameters. Acoustic radiation force impulse refers to the use of ultrasonic compression pulses to generate shear waves; it is incorporated into point shear wave elastography (pSWE) devices such as Virtual Touch™ Tissue Quantification (Siemens Healthcare, Erlangen, Germany) or ElastPQ™, (EPIQ7 ultrasound system, Philips Healthcare, Bothell, WA, USA), amongst others. Several devices incorporating 2-D ShearWave Elastography are now available (Aixplorer®, Supersonic Imagine, Aix-en-Provence, France; LOGIQ E9 ShearWave Elastography, GE Healthcare, WI, USA; ElastQ, Phillips Healthcare, Netherlands; Aplio 500 i-series, Canon Medical Systems, Japan) amongst others.[42] These devices are being increasingly adopted into practice as B-mode visualisation provides an advantage over VCTE. However, there is a lack of uniformity in commercial system design, variability in shear wave frequency, sampling rates, and other technical parameters that limit the comparison of LSM across manufacturer systems. In general, compared to other NITs, there is less data regarding the diagnostic utility of SWE for CLD. Quality criteria for SWE are not as established as VCTE, and many of the confounding factors that elevate VCTE LSM also influence SWE.[1] Additional physiologic factors such as physical exertion, transmitted cardiac impulses, and breathing cycle must be considered (Table 2). Other limitations include the narrow range of values (0.5–4.4 m/s) for pSWE, which limits the ability to define strict thresholds for fibrosis stage and variation in the region of interest chosen by the operator. In addition, compared to VCTE, greater technical and anatomical expertise are required, limiting applicability to dedicated centres.[43]

Magnetic resonance elastography

Magnetic resonance elastography (MRE) incorporates a modified phase-contrast method to image the micron-level displacements associated with mechanical generated shear wave propagation. In contrast to US elastography, quality metrics are established across platforms.[44] Limitations include cost and availability, along with patient-dependent factors such as presence of magnetically susceptible implants, compliance with breath-hold, and claustrophobia. Iron overload, higher BMI, and significant ascites are also associated with technical failure (Table 2).[45] Diagnostic MRE thresholds for fibrosis stage vary between studies, and optimal liver stiffness thresholds derived from meta-analyses of mostly retrospective data, with different histologic scoring systems, require further validation.[46]

Aetiology-specific considerations and limitations of NITs

Chronic hepatitis C

Several of the currently available serum fibrosis marker panels were initially developed in CHC cohorts to guide interferon (IFN)-based therapy.[47–51] Other marker panels included a mixed CLD population but algorithms were also adopted for CHC.[52,53] Improving the diagnostic role of biomarkers of fibrosis has included Standards for Reporting of Diagnostic Accuracy Studies

Table 2. Limitations of current non-invasive serum and imaging tests.

Type of limitation	Serum biomarkers	Transient elastography (VCTE)	Shear wave elastography	MRE
Technical limitations	Not Liver specific	Requires training and experience for validated quality criteria No B-mode image and unable to select liver region of interest	Requires dedicated US training Quality criteria not yet validated Unable to compare reported parameters of shear wave speed (range 0.5–4.4 m/s) or Young's modulus (2–150 kPa) between US devices, VCTE, or MRE	Requires specialised technician or radiologist
Discrimination of adjacent fibrosis stages	No	No	No	No
Performance for intermediate fibrosis stage	Poor	Overlapping LSM range	Limited data	Overlapping LSM range
Cost and availability	Patented marker panels not readily available and costly	Not widely reimbursed Access concerns in resource limited practices	Not readily available outside specialised centres	Costly Not available outside dedicated radiology centres
False positivity	Haemolysis, Gilbert's disease, cholestasis, immune thrombocytopenia, inflammation, age, exercise, non-fasting	Acute hepatitis, inflammation, non-fasting, exercise, hepatic venous congestion, inflammation or infiltration, alcohol excess, cholestasis, steatosis, portal vein thrombosis	Left *vs.* Right hepatic lobe, acute hepatitis, hepatic inflammation or infiltration, non-fasting, exercise, right heart failure, extrahepatic cholestasis, breathing cycle (end-expiration *vs.* end-inspiration)	Inflammation, cholestasis, hepatic venous congestion, postprandial state, and right heart failure
Failure	Indeterminate "grey zone" scores in 30-50% for simple markers (NFS, APRI, FIB-4)	Higher failure rates than serum tests: operator inexperience, narrow intercostal space, body habitus, ascites	Higher failure rates than serum tests: BMI, tissue depth >2–3 cm below skin surface	Higher failure than serum tests: waist circumference/BMI, claustrophobia, iron deposition, massive ascites, higher field strength (3 T *vs.* 1.5 T)
Thresholds	Variable for simple markers across aetiologies	Variable across aetiologies	Not validated across aetiologies	Vary between gradient-recalled echo *vs.* echo planar imaging, 2D *vs.* 3D acquisition, 40 *vs.* 60 Hz, and across aetiologies
Differentiation between simple steatosis and NASH	No	No	No	No
Follow-up of dynamic fibrosis changes	No	No	No	No

APRI, AST-platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CLD, chronic liver disease; FIB-4, fibrosis-4; LSM, liver stiffness measurement; MRE, Magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; US, ultrasound; VCTE, vibration-controlled transient elastography.

(STARD),[54] and recommendations on statistical methods to account for "spectrum bias" between the study and reference population, quality measures to reduce observer and sampling variability, and using combined imaging or sequential algorithms for improved reliability.[55]

The most widely validated serum fibrosis marker in CLD is the FibroTest (FT, BioPredictive, Paris, France; HCV FibroSURE, Labcorp, Burlington, NC).[47] Potential false results for FT and other marker algorithms which utilise bilirubin (HepaScore, PathWest, UWA, Australia) may also be associated with falsely elevated results in the presence of haemolysis, Gilbert's syndrome or cholestasis (Table 2). Other patented and non-patented algorithms incorporating aminotransferases (aspartate aminotransferase [AST] to platelet ratio index [APRI], FibroMeter [Echosens, Paris, France], Forns Index, fibrosis- 4 [FIB-4]) may be falsely positive in acute hepatitis. Hyaluronic acid levels may be influenced by age[56] or postprandial state.[57] HIV coinfection may result in thrombocytopenia, or drug-induced elevations in bilirubin or gamma-glutamyltransferase, which can also affect the diagnostic accuracy of serum marker panels. Chronic or systemic inflammatory states may produce false positive results in marker algorithms that incorporate acute phase reactants, such as hyaluronic acid, α-2 macroglobulin, platelets, N-terminal pro-collagen peptide, gamma globulin (including Enhanced Liver Fibrosis Score [ELF] (Siemens Healthcare, Erlangen, Germany), FT, Hepascore, Fibroindex, FibroSpect II (Prometheus Labs. Inc., San Diego, CA), FibroMeter).

Simple, cheap and readily available algorithms such as APRI or FIB-4 are associated with "indeterminate" range scores in 30–50% of patients, representing a significant limitation and requirement for secondary diagnostic tests. A prior systematic review of 10 different simple and complex biomarker panels concluded that clinically relevant predictive values (positive predictive value ≥90% and negative predictive value ≥95%) for significant fibrosis could be obtained for only 35% of patients with CHC.[58] Even under ideal performance parameters of 0.9 for biopsy sensitivity and specificity, and disease prevalence of 40%, a perfect marker would not exceed an area under the curve (AUC) of 0.9 for stage ≥F2.[6]

In a landmark virtual biopsy study, biopsy performance was noted to be lower for intermediate stages, and length >25 mm did not significantly increase accuracy for METAVIR staging.[8] This represents an inherent limitation of biomarker studies

and, as a result, non-invasive markers often misclassify patients with intermediate stages of fibrosis.[59] In an attempt to overcome the diagnostic limitations of existing serum markers, that were developed to reflect a broad spectrum of injury across F2-F4, a study in >800 patients with CHC evaluated a customised multiplex array platform of 37 candidate serum biomarkers for adjacent stage differentiation. However, their diagnostic performance for differentiating adjacent METAVIR stages was comparable to FT, with only a modest AUC range of 0.51–0.72.[60] In general, the diagnostic performance of serum markers is better for cirrhosis than significant fibrosis in CHC.[1,61] This observation may be of relevance in the era of DAA treatment. DAA therapies with high efficacy are now available for CHC, and accurate fibrosis staging prior to treatment is less clinically relevant than detection of bridging fibrosis or cirrhosis, which can guide continued post-treatment surveillance. However, pre-treatment staging of liver fibrosis remains pivotal for identification of patients with liver cirrhosis, who require screening for hepatocellular carcinoma (HCC) and oesophageal varices. Moreover, given the significant HCV-associated disease burden in many countries such as the United States, there is a continued need for NITs that can monitor fibrosis progression in patients that are still awaiting[62] or have been declined treatment.[63] Many patients with advanced disease that have been treated remain at risk of disease progression from co-morbid, metabolic, and behavioural factors following sustained virologic response (SVR).[64] Although compensated cirrhosis may regress to an earlier fibrosis stage following antiviral therapy,[65] stellate cell activation, portal inflammation, and sinusoidal capillarisation may persist for several years following SVR.[66] Serum biomarker scores may decline following SVR,[67–70] indicating that these indices may be influenced by biochemical responses following antiviral therapy. However, very few studies have assessed both histology and biomarkers following SVR.[71] In the DAA era, biopsies for CHC have become obsolete, and there is a greater dependence on NITs both pre-and post-treatment to determine fibrosis stage. Proposed NIT algorithms for post-SVR monitoring of patients with CHC have not been validated for clinical outcomes.[72] Routine use of NITs after SVR in patients with advanced disease is associated with a high false negative rate, and there is no consensus on the degree of improvement in non-invasive thresholds that would constitute a clinically relevant change in prognosis, or one that correlates with fibrosis regression[1] (Table 3).

Elastography techniques have mostly been validated in the context of CHC. Several thresholds have been proposed to identify patients with stage >F2 fibrosis and with F4. As for the serum-based marker panels, imaging elastography is also unable to reliably differentiate between adjacent fibrosis stages, and there is considerable overlap in LSM for intermediate stage disease. Differing VCTE devices (FS402 and FS502) may provide discordant results for stage F2-3 in CHC and should be considered for follow-up LSM assessments.[73]

For patients with CHC, an important clinical consideration in the current DAA era is the role of imaging elastography following SVR. Routine use of NITs either alone or in combination is not recommended in non-cirrhotic patients during therapy or after achieving SVR.[1] Prior studies have indicated VCTE may improve following CHC antiviral therapy, and this likely relates to the associated early biochemical response.[68,74,75] Longer duration follow-up at 3 years is required to assess favourable changes in LSM.[76] Based on very limited evidence, the technical review on VCTE by AGA suggested that low-risk patients without metabolic comorbidities, history of alcohol excess, or HBV-HIV coinfection, and with a post-SVR VCTE of ≤9.5 kPa may be considered for discharge from a dedicated liver clinic.[40] Other proposed algorithms for following patients with CHC after DAA therapy have not been validated against clinical outcomes or liver biopsy.[77] However, in patients with advanced fibrosis, post-SVR VCTE thresholds that predict low risk of clinical outcomes or regression of cirrhosis, have not been established. A prior paired-biopsy study in 33 patients with CHC, with cirrhosis treated with IFN-based therapy, indicated that VCTE had a sensitivity of 61% and specificity of 95% at

Table 3. Special considerations when using NITs to diagnosis fibrosis by aetiology of CLD.

	Hepatitis C	Hepatitis B	NAFLD	Alcohol-related liver disease	Cholestatic and autoimmune liver diseases
Validation	VCTE +++ Indirect markers +++ Direct markers +	VCTE ++ Indirect markers ++ Direct markers +	VCTE ++ Indirect markers +++ Direct markers +	VCTE ++ Indirect markers + Direct markers +	VCTE + Indirect markers + Direct markers +
Cut-off	≥F2 F4	≥F2 F4	≥F3 F4	≥F2 F4	≥F2 F4
Applicability	Cautious interpretation of VCTE post-SVR	Risk of false positivity of VCTE with ALT flares	Reduced VCTE reliability at higher BMI	Adjust VCTE cut-off according to AST and bilirubin and cautious interpretation after alcohol withdrawal	Potential risk of VCTE false positivity with cholestasis and significant transaminitis
Clinical relevance	Identification of cirrhosis pre-treatment to start screening for HCC and oesophageal varices	Identification of cirrhosis to start screening for HCC (for patients not already falling in high risk categories independently of fibrosis stage), and oesophageal varices; Identification of significant liver fibrosis, as guidance for antiviral treatment together with ALT and HBV DNA	Identification of cirrhosis to start screening for HCC and oesophageal varices Identification of significant liver fibrosis as guidance for antifibrotic treatment (currently available only in context of clinical trials)	Identification of cirrhosis to start screening for HCC and oesophageal varices	Identification of cirrhosis to start screening for HCC and oesophageal varices

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; SVR, sustained virologic response; VCTE, vibration-controlled transient elastography.

LSM <12 kPa for detecting stage F4 at ~5 years post-SVR.[78] Thus, VCTE appears to have poor clinical utility as a screening tool to assess cirrhosis regression following SVR. Furthermore, there is no guidance on the optimal timing of repeated VCTE assessments post-SVR to assess regression from advanced fibrosis. At present, patients with pre-treatment liver cirrhosis should continue surveillance for HCC indefinitely, even if NITs no longer suggest the presence of cirrhosis. Compared to the IFN-based treatment era, patients with CHC and decompensated disease are now eligible for DAA therapy. Associating post-SVR fibrosis regression with changes in VCTE or other NITs in patients with advanced fibrotic injury becomes more challenging and less clinically relevant than assessing changes in liver-related outcomes.

Regarding surveillance for varices, Baveno VI recommendations state that patients with CHC, with LSM <20 kPa and platelet count>150,000, may safely forego endoscopic screening for oesophageal varices. These patients can be followed by yearly repetition of VCTE and platelet count.[79] However, as is the case for HCC surveillance, a decline in LSM post-SVR should not prevent the clinician from continued surveillance endoscopy based on pre-treatment liver cirrhosis.

Chronic hepatitis B

Serum markers of fibrosis have not been widely adopted for chronic hepatitis B (CHB) infection, as management decisions for CHB consider not only disease severity, but also HBV DNA, liver aminotransferases, and HBV e-antigen status, among other variables.[80] Variable natural history, immune activity, and inflammatory flares will influence the reliability of current NITs.[81] An independent meta-analysis of 16 studies in CHB concluded that FT was suboptimal for identifying significant fibrosis.[82] Simple serum markers require further validation in patients with CHB and significant fibrosis in inactive or immune tolerant states.[83] Antiviral therapy in CHB results in viral suppression and fibrosis regression, including the reversal of cirrhosis.[84,85] Despite the low cost, ease of interpretation, and access advantages in resource limited settings, simple markers such as APRI and FIB-4 have limited diagnostic accuracy for moderate-advanced stage disease in CHB and do not reflect changes in fibrosis. These indices were evaluated in a cohort of 575 patients with CHB enrolled in a clinical registration trial. At baseline, 113/139 (81%) patients with cirrhosis had an APRI score ≤2 and 173/195 (89%) patients with advanced fibrosis had a FIB-4 score ≤3.25. APRI and FIB-4 did not correlate with histologic fibrosis regression observed at 5 years.[86] Other serum markers that incorporate liver aminotransferases or acute phase reactants are also likely to be associated with false negatives following antiviral therapy, and other non-invasive tools such as imaging elastography have been proposed for risk assessment and management in CHB.[1,87] For CHB, VCTE thresholds for significant fibrosis stages (F2-F4) are variable and may be lower than CHC, particularly for the diagnosis of liver cirrhosis.[88] Liver inflammation during a viral flare or reactivation will result in higher VCTE LSM.[89,90] In patients with CHB and cirrhosis, LSM improves with continued antiviral therapy.[91] The prognostic role of VCTE in CHB is established.[92] However, the correlation between changes in VCTE and improved histology after antiviral therapy or following disease progression have not yet been determined, nor has the ability of VCTE to predict liver-related outcomes in patients with inactive CHB. There is limited data on the utility of other elastography methods for the diagnosis and prognostication of CHB-related fibrosis.[1]

Non-alcoholic fatty liver disease

Several patented and non-patented combined serum biomarker and clinical models have been developed to predict advanced fibrosis in NAFLD. Many of these were developed in CHC and thresholds have been modified and adapted for NAFLD-related advanced fibrosis.[93] The NAFLD fibrosis score (NFS) and FIB-4 score are the most widely validated of these non-proprietary tests, and due to the use of simple tests and the availability of free online calculators, they are regarded as clinically useful tools for identifying patients at a higher risk of advanced fibrosis.[94] However, these tests are associated with "indeterminate" range scores in at least 30% of cases.[95,96] NFS and FIB-4 have reduced specificity in older patients, and new thresholds for patients aged ≥65 years have been proposed.[97] These tests were developed in cohorts with a higher prevalence of advanced fibrosis and not as screening tools. Therefore, they require the use of sequential diagnostic tests with higher specificity for detecting advanced fibrosis in non-tertiary centre populations. Several algorithms for NAFLD risk stratification have been proposed based on blood markers alone, or in combination with imaging elastography, but they require further validation.[93,96] Patented serum markers for NAFLD (FibroMax, BioPredictive, Paris, France; ELF, FibroMeter) are not as easily available or as cost-effective as NFS or FIB-4, and their diagnostic utility in identifying patients with advanced fibrosis who have discordant scores from the first-line indirect serum (or imaging elastography) tests requires validation. There may also be variable diagnostic performance for proprietary markers between diabetic and non-diabetic cohorts,[98] and potential ethnic differences that influence test accuracy need to be resolved.[99] While the incorporation of newer biomarkers such as N-terminal type III collagen propeptide (Pro-C3) may improve the accuracy of biomarker panels,[100] cost and availability issues will limit their routine clinical use for now.

Emerging functional genomic technologies have been applied to develop more accurate blood-based biomarkers for NASH.[96] However, validating these complex and expensive methodologies for a heterogenous disease state such as NAFLD has been difficult, limiting their clinical application to date. For example, protein-profiling technologies have identified glycoproteins and other post-translational peptides as markers of NASH,[101,102] and metabolomic-profiling technologies have identified additional lipid metabolites as potential biomarkers of NASH.[103–105] Recent algorithms have also incorporated the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) I148 M, rs738409 polymorphism into predictive models for NASH.[106] This single nucleotide polymorphism is strongly associated with more hepatic fat deposition and fibrosis, with recent data linking it with a higher risk of liver-related events and death in patients with NAFLD.[107] Other approaches using plasma DNA methylation,[108] modified single-nucleotide aptamer-based assays,[109] circulating microRNA,[110] and gut microbiome metagenomic profiling, are examples of the wealth of promising data that is generated by technological advances,[111] but also highlight the need for further studies to validate these candidate NASH biomarkers. There will be issues regarding cost, reproducibility, and high-throughput capability. Importantly, NASH encompasses a spectrum of histologic injury

that includes steatosis, lobular inflammation, hepatocyte clarification/ballooning and fibrotic injury.[112] None of these technologies have yet identified a marker that consistently provides an accurate diagnosis to reflect these histologic features of NASH.

Biomarkers have an increasingly important role as endpoints to support drug approval in clinical trials targeting advanced fibrosis and NASH.[113] These potential "surrogate" biomarkers will have to reflect changes in the risk of fibrosis progression and in clinically meaningful endpoints. Based on a meta-analysis of paired biopsy data in a relatively small number of patients, on average it took 14.3 years and 7.1 years for fibrosis to progress by 1-stage in patients with simple steatosis and NASH, respectively.[114] Fibrosis stage may improve in around 20% of patients over this period and worsen more rapidly in a similar proportion. Recent longitudinal data from the NASH Clinical Research Network indicated that simple markers had modest AUCs for the prediction of fibrosis progression (0.66 [for NFS], 0.70 [APRI], and 0.73 [FIB-4]) in 292 patients with paired biopsies over a median period of 2.6 years. Model scores adjusted for baseline fibrosis stage were associated with progression of fibrosis, but not regression.[115] The prevalence of significant fibrosis (F2-F4) was ~50% in this study. It remains to be determined whether these simple NITs, or combinations with imaging elastography, can be used to monitor fibrosis progression in lower prevalence settings. Phase II and III NAFLD clinical trials have incorporated several biomarkers such as ELF, Pro-C3, FT amongst other discovery initiatives. A recent phase IIb study of simtuzumab for stage 3 and 4 NASH was stopped at 96 weeks because of primary non-efficacy in histologic endpoints, compared to placebo. There was improvement in histologic fibrosis by 1-stage in ~20% of stage 3 patients and ~10% with baseline cirrhosis. Progression to cirrhosis was observed in ~20% at 96 weeks across the treatment groups. However, despite these histologic changes, no significant differences were observed between the simtuzumab and placebo groups through week 96 in liver biochemistry, ELF score, FT, or NFS.[116] In a recent 12-week clinical trial of NGM282 (a fibroblast growth factor 19 analogue) in 43 patients with NAFLD, significant reductions in pro-C3 and ELF, but not ALT, were observed in patients with histologic response compared to non-responders.[117] Fibrosis biomarker panels that incorporate liver aminotransferases and acute phase reactants will need to be interpreted with caution, as therapies may improve necroinflammation but not fibrosis over a relatively short study duration.[118] Further data from ongoing NASH clinical trials, with repeat tissue sampling and incorporation of NITs of function (HepQuant, Greenwood Village, CO),[119] will provide more information on whether these biomarkers accurately reflect changes in histology or clinical endpoints following therapeutic interventions.

Recently, imaging elastography and other MR-based quantitative assessments of steatosis, including controlled attenuation parameter (CAP) and MRI-estimated proton density fat fraction (MRI-PDFF), were reviewed in patients with NASH.[93] CAP thresholds are associated with overlap between grade 2 and 3 steatosis, influenced by metabolic factors such as type II diabetes mellitus and BMI, and potential differences between M and XL probe values need to be resolved. Although change in steatosis is not a clinical endpoint in phase III studies, CAP is unlikely to be as useful in following change in steatosis as MRI-PDFF or ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS), although there may be uncertainty in the diagnostic accuracy of these MR-based techniques for detection of steatosis in advanced fibrosis.[120–122] Of note, the measure of CAP can be useful in correcting the VCTE LSM thresholds in order to reduce the risk of overestimation of fibrosis stage induced by hepatic steatosis.[123]

None of the available imaging methods can reliably differentiate the transition from simple steatosis to NASH. For advanced fibrosis (F3-F4), optimal VCTE LSM thresholds for M or XL probes have not been defined, as NAFLD studies independent of clinical trials have been conducted in small heterogeneous cohorts with variable prevalence of advanced fibrosis, and using differing histologic scoring systems.[124] The AGA technical review on elastography was not able to provide a pooled estimate for VCTE LSM that could accurately diagnose cirrhosis.[40] Subsequently, a recent multicentre study from the United Kingdom evaluating VCTE using M or XL probes in 373 patients with NAFLD noted optimal thresholds for advanced fibrosis and cirrhosis of 9.7 kPa and 13.6 kPa, respectively.[125] However, baseline data from a recent phase III clinical trial for NAFLD F3-4 with available VCTE in >1,700 patients, reported a median LSM of 16.5 kPa in their cohort, with an optimal upper LSM threshold of 11.4 kPa to rule-in F3-F4. Although this was a high F3-F4 prevalence cohort by selection, there was no single threshold for their serum and imaging NITs that could balance optimal sensitivity and specificity.[126] VCTE thresholds for advanced fibrosis will need to be validated in other NAFLD clinical trials performed in diabetic and multi-ethnic cohorts. Similar studies are also required to determine the diagnostic utility of SWE in advanced NAFLD.

There is increasing data regarding the validity of MRE compared to VCTE and US elastography for the diagnosis of advanced fibrosis in patients with NAFLD.[93] Similar to VCTE limitations, MRE thresholds also vary across study cohorts and optimal values derived from pooled data require further validation in non-tertiary centres and multi-ethnic cohorts.[127] Phase II clinical trials in NAFLD have readily adopted MRE and MRI-PDFF outcomes such as ≥30% reductions in PDFF as surrogates of favourable changes in NASH histology.[128] In a phase II study of NAFLD stage F2-F3, a 1-stage improvement in fibrosis at 24 weeks was observed in 21/62 patients that received selonsertib (a selective inhibitor of apoptosis signal-regulating kinase 1).[129] However, there were no significant corresponding changes in VCTE or MRE, and the optimal threshold to predict improvement in NASH was a relative reduction in MRI-PDFF of 25% with a modest AUC of 0.70.[130] Until further data from larger clinical cohorts are available, there are no established absolute or relative changes in MRI-PDFF, US elastography, or MRE that correspond to clinical outcomes, improvement or worsening of NASH or fibrosis stage on biopsy. Defining changes in NITs in the context of therapeutic development remains a challenge.[118]

Alcohol-related liver disease

Several studies have investigated the performance of NITs in patients with alcohol-related liver disease. The most validated NITs in alcohol-related liver disease include FT, APRI, FIB-4, ELF and VCTE. Reported cut-offs are similar to those used in CHC, with specificity ranging from 72% to 98% for the detection of F2-F4 fibrosis and from 71% to 94% for the detection of cirrhosis. VCTE has been shown to be superior to serum markers. However, the cut-off value for liver cirrhosis varies significantly across studies, ranging from 11.5 to 25.8 kPa.[131] A recent meta-analysis of VCTE based on individual patient data included 10

studies comprising 1,026 patients.[132] AST and bilirubin concentrations had a significant effect on LSM, with higher concentrations associated with higher stiffness values. As such, the meta-analysis concluded that in alcohol-related liver disease, VCTE assessments of liver fibrosis should consider AST and bilirubin concentrations by using specifically adjusted liver stiffness cut-offs. As LSM encompasses the sum of all pathological features of alcohol-related liver disease, including inflammation, ballooning and fibrosis, this parameter also improves soon after alcohol withdrawal.[131] LSM is influenced by changes in biochemical activity following alcohol withdrawal, as evidenced by improving LSM with declining AST.[133,134] Potential differences in LSM based on ambulatory clinic *vs.* inpatient cohorts need to be further defined,[135] and LSM should be interpreted carefully in patients with liver cirrhosis to account for these variables (Table 3).

Cholestatic and autoimmune liver diseases

A few studies have assessed NITs in cholestatic liver disease, particularly primary biliary cholangitis. To date, VCTE is the best NIT for performance, data robustness, validation status, and prognostic relevance, followed by APRI, ELF, and hyaluronic acid, without marked differences among the latter 3 markers. In primary biliary cholangitis, as with other fibrosis indices, LSM by VCTE exhibits a non-linear relationship with fibrosis stage, explaining better performance for extreme ends compared to intermediate stages of the fibrosis spectrum.[136] There are fewer studies on NITs in primary sclerosing cholangitis. Thus far, VCTE is the most widely validated assessment, even in terms of correlation with clinical outcomes. In primary sclerosing cholangitis, special attention should be paid to patients with jaundice to exclude biliary obstruction by dominant strictures of major bile ducts before performing VCTE. Indeed, obstructive cholestasis is known to significantly impact LSM, irrespective of liver fibrosis.[136,137] In autoimmune hepatitis (AIH), a recent meta-analysis including 16 studies with 861 patients demonstrated that VCTE may perform better than simple serum markers, including APRI and FIB-4.[138] A retrospective study of 108 patients with AIH suggested that, in contrast to CHB, neither ALT levels nor hepatic inflammation affect the accuracy of LSM in the detection of fibrosis.[139] However, it is likely that significant AIH-associated inflammation would influence LSM, as complete biochemical remission is associated with a decline in LSM.[140] Thus, as with alcohol-related liver disease, liver aminotransferases should also be considered during interpretation of LSM in patients with AIH.

Combination algorithms

Given the limitations and variability of NITs, several studies have proposed combining them in diagnostic algorithms. Sequential and synchronous combinations of tests, including FT, APRI, FibroMeter and HepaScore have been proposed, with resulting diagnostic accuracy over 90% for both significant liver fibrosis (F2-F4) and cirrhosis.[141,142] Recent guidelines suggest that the combination of 2 unrelated NITs may provide better accuracy and may overcome some of the limitations of a single test. The EASL-ALEH Clinical Practice Guidelines propose combining VCTE with serum biomarkers in CHC as this approach is the most widely validated.[1] When VCTE and serum biomarker results are in concordance, the diagnostic accuracy for significant fibrosis, but not for cirrhosis, is increased. In cases of unexplained discordance, a liver biopsy should be performed if the results would change patient management. In CHB, VCTE is better at predicting advanced liver fibrosis and cirrhosis than serum markers.[1] The diagnostic benefit of combining TE with a serum biomarker for fibrosis evaluation has not been established. In recent years, the sequential use of markers and risk stratification tools have been tested to improve referral pathways in NAFLD. In a prospective cohort study, Srivastava and colleagues evaluated a pathway for the management of patients with NAFLD, aimed at improving the detection of cases of advanced fibrosis and cirrhosis and avoiding unnecessary referrals to secondary care. Over 3,000 patients entered the pathway, based on the sequential use of FIB-4 and ELF, which reduced unnecessary referrals from primary care to secondary care by 81%.[143] Along the same lines, Davyduke and colleagues proposed a "FIB-4 first" strategy on a VCTE-based pathway for 597 patients with suspected NAFLD referred from primary care. This staged risk-stratification model using FIB-4 and VCTE could obviate the need for up to 87% of further assessments.[144] Combining at-risk clinical characteristics (hazardous alcohol intake, elevated ALT, metabolic risk factors) with serum markers and VCTE in primary care programmes yielded many more diagnoses of advanced liver disease in patients with alcohol-related liver disease or NAFLD.[145,146] These combined approaches are important, but require validation based on disease aetiology, ethnicity and prevalence, as well as consideration of regional socioeconomic limitations due to differing healthcare payor systems, access to US elastography, and specialist care.

Conclusion

Liver fibrosis staging is a vital part of the clinical management of CLD of any aetiology. Various NITs and their use in combination may help guide clinical decision making, reduce the number of specialistic referrals from primary care, and obviate the need for a significant number of invasive biopsy procedures. However, current NITs have several limitations, such as a lack of discrimination of NASH *vs.* simple steatosis, a lack of validation for longitudinal assessment and for fibrosis regression following therapeutic interventions (antiviral therapy in viral hepatitis). Knowledge of pitfalls intrinsic to NITs, particularly risk factors for false results, is of paramount importance for appropriate interpretation in clinical practice.

Abbreviations

AGA, American Gastroenterology Association; ALT, alanine aminotransferase; APRI, AST-platelet ratio index; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; CAP, controlled attenuation parameter; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CLD, chronic liver disease; CPA, collagen proportionate area; DAA, direct-acting antiviral; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; FLIP, fatty liver inhibition of progression; HCC, hepatocellular carcinoma; IFN, interferon; LSM, liver stiffness measure; MR, magnetic resonance; MRE, magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; NITs, non-invasive tests; SVR, sustained virologic response; US, ultrasound; VCTE, vibration-controlled transient elastography.

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Conflicts of interest
The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions
The authors contributed equally to the production of this manuscript.

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References
[1] European Association for Study of Liver, Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH clinical practice guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63:237–264.
[2] Bedossa P, Paradis V. Liver extracellular matrix in health and disease. J Pathol 2003;200:504–515.
[3] European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388–1402.
[4] Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994;20:15–20.
[5] Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, Network NCR. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810–820.
[6] Mehta SH, Lau B, Afdhal NH, Thomas DL. Exceeding the limits of liver histology markers. J Hepatol 2009;50:36–41.
[7] Poynard T, Halfon P, Castera L, Charlotte F, Le Bail B, Munteanu M, et al. Variability of the area under the receiver operating characteristic curves in the diagnostic evaluation of liver fibrosis markers: impact of biopsy length and fragmentation. Aliment Pharmacol Ther 2007;25:733–739.
[8] Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449–1457.
[9] Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. Hepatology 2009;49:1017–1044.
[10] Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001;344:495–500.
[11] Cholongitas E, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, et al. A systematic review of the quality of liver biopsy specimens. Am J Clin Pathol 2006;125:710–721.
[12] Standish RA, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. Gut 2006;55:569–578.
[13] Schiano TD, Azeem S, Bodian CA, Bodenheimer Jr HC, Merati S, Thung SN, et al. Importance of specimen size in accurate needle liver biopsy evaluation of patients with chronic hepatitis C. Clin Gastroenterol Hepatol 2005;3:930–935.
[14] Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. J Hepatol 2003;39:239–244.
[15] Bedossa P. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565–575.
[16] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
[17] Westin J, Lagging LM, Wejstal R, Norkrans G, Dhillon AP. Interobserver study of liver histopathology using the Ishak score in patients with chronic hepatitis C virus infection. Liver 1999;19:183–187.
[18] Rousselet MC, Michalak S, Dupre F, Croue A, Bedossa P, Saint-Andre JP, et al. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology 2005;41:257–264.
[19] Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614–2618.
[20] Karsdal MA, Manon-Jensen T, Genovese F, Kristensen JH, Nielsen MJ, Sand JM, et al. Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2015;308:G807–G830.
[21] Goodman ZD, Becker Jr RL, Pockros PJ, Afdhal NH. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. Hepatology 2007;45:886–894.
[22] Goodman ZD, Stoddard AM, Bonkovsky HL, Fontana RJ, Ghany MG, Morgan TR, et al. Fibrosis progression in chronic hepatitis C: morphometric image analysis in the HALT-C trial. Hepatology 2009;50:1738–1749.
[23] McHutchison J, Goodman Z, Patel K, Makhlouf H, Rodriguez-Torres M, Shiffman M, et al. Farglitazar lacks antifibrotic activity in patients with chronic hepatitis C infection. Gastroenterology 2010;138:1365–1373. 1373.e1-2.
[24] Tsochatzis E, Bruno S, Isgro G, Hall A, Theocharidou E, Manousou P, et al. Collagen proportionate area is superior to other histological methods for sub-classifying cirrhosis and determining prognosis. J Hepatol 2014;60:948–954.
[25] Sandrini J, Boursier J, Chaigneau J, Sturm N, Zarski JP, Le Bail B, et al. Quantification of portal-bridging fibrosis area more accurately reflects fibrosis stage and liver stiffness than whole fibrosis or perisinusoidal fibrosis areas in chronic hepatitis C. Mod Pathol 2014;27:1035–1045.
[26] Xu S, Wang Y, Tai DC, Wang S, Cheng CL, Peng Q, et al. qFibrosis: a fully-quantitative innovative method incorporating histological features to facilitate accurate fibrosis scoring in animal model and chronic hepatitis B patients. J Hepatol 2014;61:260–269.
[27] Gailhouste L, Le Grand Y, Odin C, Guyader D, Turlin B, Ezan F, et al. Fibrillar collagen scoring by second harmonic microscopy: a new tool in the assessment of liver fibrosis. J Hepatol 2010;52:398–406.
[28] Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: concept to treatment. J Hepatol 2015;62:S15–S24.
[29] Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835–847.
[30] Castera L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828–835.
[31] Wong GL, Wong VW, Chim AM, Yiu KK, Chu SH, Li MK, et al. Factors associated with unreliable liver stiffness measurement and its failure with transient elastography in the Chinese population. J Gastroenterol Hepatol 2011;26:300–305.
[32] Boursier J, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebail B, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology 2013;57:1182–1191.
[33] Das K, Sarkar R, Ahmed SM, Mridha AR, Mukherjee PS, Dhali GK, et al. "Normal" liver stiffness measure (LSM) values are higher in both lean and obese individuals: a population-based study from a developing country. Hepatology 2012;55:584–593.
[34] Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, et al. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 2012;55:199–208.
[35] Wong VW, Irles M, Wong GL, Shili S, Chan AW, Merrouche W, et al. Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease. Gut 2019;68:2057–2064.
[36] Tapper EB, Castera L, Afdhal NH. FibroScan (vibration-controlled transient elastography): where does it stand in the United States practice. Clin Gastroenterol Hepatol 2015;13:27–36.
[37] Huang R, Gao ZH, Tang A, Sebastiani G, Deschenes M. Transient elastography is an unreliable marker of liver fibrosis in patients with portal vein thrombosis. Hepatology 2018;68:783–785.
[38] Tapper EB, Cohen EB, Patel K, Bacon B, Gordon S, Lawitz E, et al. Levels of alanine aminotransferase confound use of transient elastography to diagnose fibrosis in patients with chronic hepatitis C virus infection. Clin Gastroenterol Hepatol 2012;10(8):932–937.e1.
[39] Arena U, Lupsor Platon M, Stasi C, Moscarella S, Assarat A, Bedogni G, et al. Liver stiffness is influenced by a standardized meal in patients with chronic hepatitis C virus at different stages of fibrotic evolution. Hepatology 2013;58:65–72.

[40] Singh S, Muir AJ, Dieterich DT, Falck-Ytter YT. American Gastroenterological Association Institute technical review on the role of elastography in chronic liver diseases. Gastroenterology 2017; 152:1544–1577.
[41] de Franchis R. Expanding consensus in portal hypertension: report of the Baveno VI Consensus Workshop: stratifying risk and individualizing care for portal hypertension. J Hepatol 2015;63:743–752.
[42] Kennedy P, Wagner M, Castera L, Hong CW, Johnson CL, Sirlin CB, et al. Quantitative elastography methods in liver disease: current evidence and future directions. Radiology 2018;286:738–763.
[43] Tang A, Cloutier G, Szeverenyi NM, Sirlin CB. Ultrasound elastography and MR elastography for assessing liver fibrosis: part 1, principles and techniques. AJR Am J Roentgenol 2015;205:22–32.
[44] Serai SD, Yin M, Wang H, Ehman RL, Podberesky DJ. Cross-vendor validation of liver magnetic resonance elastography. Abdom Imaging 2015;40:789–794.
[45] Wagner M, Corcuera-Solano I, Lo G, Esses S, Liao J, Besa C, et al. Technical failure of MR elastography examinations of the liver: experience from a large single-center study. Radiology 2017;284:401–412.
[46] Singh S, Venkatesh SK, Wang Z, Miller FH, Motosugi U, Low RN, et al. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. Clin Gastroenterol Hepatol 2015;13:440–451.e6.
[47] Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 2001; 357:1069–1075.
[48] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–526.
[49] Patel K, Gordon SC, Jacobson I, Hezode C, Oh E, Smith KM, et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. J Hepatol 2004;41:935–942.
[50] Adams LA, Bulsara M, Rossi E, DeBoer B, Speers D, George J, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem 2005;51:1867–1873.
[51] Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002; 36:986–992.
[52] Cales P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konate A, et al. A novel panel of blood markers to assess the degree of liver fibrosis. Hepatology 2005;42:1373–1381.
[53] Rosenberg WM, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 2004;127:1704–1713.
[54] Boursier J, de Ledinghen V, Poynard T, Guechot J, Carrat F, Leroy V, et al. An extension of STARD statements for reporting diagnostic accuracy studies on liver fibrosis tests: the Liver-FibroSTARD standards. J Hepatol 2015;62:807–815.
[55] Guha IN, Myers RP, Patel K, Talwalkar JA. Biomarkers of liver fibrosis: what lies beneath the receiver operating characteristic curve? Hepatology 2011;54:1454–1462.
[56] Lindqvist U, Laurent TC. Serum hyaluronan and aminoterminal propeptide of type III procollagen: variation with age. Scand J Clin Lab Invest 1992;52:613–621.
[57] Fraser JR, Gibson PR. Mechanisms by which food intake elevates circulating levels of hyaluronan in humans. J Intern Med 2005; 258:460–466.
[58] Parkes J, Guha IN, Roderick P, Rosenberg W. Performance of serum marker panels for liver fibrosis in chronic hepatitis C. J Hepatol 2006;44:462–474.
[59] Cales P, de Ledinghen V, Halfon P, Bacq Y, Leroy V, Boursier J, et al. Evaluating the accuracy and increasing the reliable diagnosis rate of blood tests for liver fibrosis in chronic hepatitis C. Liver Int 2008;28:1352–1362.
[60] Patel K, Remlinger KS, Walker TG, Leitner P, Lucas JE, Gardner SD, et al. Multiplex protein analysis to determine fibrosis stage and progression in patients with chronic hepatitis C. Clin Gastroenterol Hepatol 2014;12(12):2113–2120.e1-3.
[61] Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. Ann Intern Med 2013;159:372.
[62] Chhatwal J, Wang X, Ayer T, Kabiri M, Chung RT, Hur C, et al. Hepatitis C disease burden in the United States in the era of oral direct-acting antivirals. Hepatology 2016;64:1442–1450.
[63] Gowda C, Lott S, Grigorian M, Carbonari DM, Saine ME, Trooskin S, et al. Absolute insurer denial of direct-acting antiviral therapy for hepatitis C: a National Specialty Pharmacy Cohort Study. Open Forum Infect Dis 2018;5:ofy076.
[64] Lee YA, Friedman SL. Reversal, maintenance or progression: what happens to the liver after a virologic cure of hepatitis C? Antiviral Res 2014;107:23–30.
[65] Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002;122:1303–1313.
[66] D'Ambrosio R, Aghemo A, Rumi MG, Ronchi G, Donato MF, Paradis V, et al. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. Hepatology 2012;56:532–543.
[67] Fontana RJ, Bonkovsky HL, Naishadham D, Dienstag JL, Sterling RK, Lok AS, et al. Serum fibrosis marker levels decrease after successful antiviral treatment in chronic hepatitis C patients with advanced fibrosis. Clin Gastroenterol Hepatol 2009;7:219–226.
[68] Vergniol J, Foucher J, Castera L, Bernard PH, Tournan R, Terrebonne E, et al. Changes of non-invasive markers and FibroScan values during HCV treatment. J Viral Hepat 2009;16:132–140.
[69] Patel K, Friedrich-Rust M, Lurie Y, Grigorescu M, Stanciu C, Lee CM, et al. FibroSURE and FibroScan in relation to treatment response in chronic hepatitis C virus. World J Gastroenterol 2011;17:4581–4589.
[70] Patel K, Benhamou Y, Yoshida EM, Kaita KD, Zeuzem S, Torbenson M, et al. An independent and prospective comparison of two commercial fibrosis marker panels (HCV FibroSURE and FIBROSpect II) during albinterferon alfa-2b combination therapy for chronic hepatitis C. J Viral Hepat 2009;16:178–186.
[71] Poynard T, Ngo Y, Munteanu M, Thabut D, Massard J, Moussalli J, et al. Biomarkers of liver injury for hepatitis clinical trials: a meta-analysis of longitudinal studies. Antivir Ther 2010;15:617–631.
[72] Trivedi HD, Patwardhan VR, Malik R. Chronic hepatitis C infection - noninvasive assessment of liver fibrosis in the era of direct acting antivirals. Dig Liver Dis 2019;51:183–189.
[73] Parra-Ruiz J, Sanjuan C, Munoz-Medina L, Vinuesa D, Martinez-Perez MA, Hernandez-Quero J. Letter: accuracy of liver stiffness measurement - a comparison of two different FibroScan devices. Aliment Pharmacol Ther 2014;39:1434–1435.
[74] Ogawa E, Furusyo N, Toyoda K, Takeoka H, Maeda S, Hayashi J. The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin. Antiviral Res 2009;83:127–134.
[75] Hezode C, Castera L, Roudot-Thoraval F, Bouvier-Alias M, Rosa I, Roulot D, et al. Liver stiffness diminishes with antiviral response in chronic hepatitis C. Aliment Pharmacol Ther 2011;34:656–663.
[76] Stasi C, Arena U, Zignego AL, Corti G, Monti M, Triboli E, et al. Longitudinal assessment of liver stiffness in patients undergoing antiviral treatment for hepatitis C. Dig Liver Dis 2013;45:840–843.
[77] Trivedi HD, Lin SC, T Y Lau D. Noninvasive assessment of fibrosis regression in hepatitis C virus sustained virologic responders. Gastroenterol Hepatol (N Y) 2017;13:587–595.
[78] D'Ambrosio R, Aghemo A, Fraquelli M, Rumi MG, Donato MF, Paradis V, et al. The diagnostic accuracy of Fibroscan for cirrhosis is influenced by liver morphometry in HCV patients with a sustained virological response. J Hepatol 2013;59:251–256.
[79] de Franchis R, Baveno VIF. Expanding consensus in portal hypertension: report of the Baveno VI Consensus Workshop: stratifying risk and individualizing care for portal hypertension. J Hepatol 2015;63:743–752.
[80] Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016;63:261–283.
[81] Castera L. Hepatitis B: are non-invasive markers of liver fibrosis reliable? Liver Int 2014;34(Suppl 1):91–96.
[82] Salkic NN, Jovanovic P, Hauser G, Brcic M. FibroTest/Fibrosure for significant liver fibrosis and cirrhosis in chronic hepatitis B: a meta-analysis. Am J Gastroenterol 2014;109:796–809.
[83] Kumar M, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, et al. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology 2008;134:1376–1384.

[84] Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381(9865):468–475.
[85] Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52:886–893.
[86] Kim WR, Berg T, Asselah T, Flisiak R, Fung S, Gordon SC, et al. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. J Hepatol 2016;64:773–780.
[87] Branchi F, Conti CB, Baccarin A, Lampertico P, Conte D, Fraquelli M. Non-invasive assessment of liver fibrosis in chronic hepatitis B. World J Gastroenterol 2014;20:14568–14580.
[88] Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol 2011;54:650–659.
[89] Verveer C, Zondervan PE, ten Kate FJ, Hansen BE, Janssen HL, de Knegt RJ. Evaluation of transient elastography for fibrosis assessment compared with large biopsies in chronic hepatitis B and C. Liver Int 2012;32:622–628.
[90] Fraquelli M, Rigamonti C, Casazza G, Donato MF, Ronchi G, Conte D, et al. Etiology-related determinants of liver stiffness values in chronic viral hepatitis B or C. J Hepatol 2011;54:621–628.
[91] Andersen ES, Weiland O, Leutscher P, Krarup H, Westin J, Moessner B, et al. Low liver stiffness among cirrhotic patients with hepatitis B after prolonged treatment with nucleoside analogs. Scand J Gastroenterol 2011;46:760–766.
[92] de Ledinghen V, Vergniol J, Barthe C, Foucher J, Chermak F, Le Bail B, et al. Non-invasive tests for fibrosis and liver stiffness predict 5-year survival of patients chronically infected with hepatitis B virus. Aliment Pharmacol Ther 2013;37:979–988.
[93] Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156:1264–1281.e4.
[94] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–357.
[95] Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med 2011;43:617–649.
[96] Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: clinical prediction rules and blood-based biomarkers. J Hepatol 2018;68:305–315.
[97] McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol 2017;112:740–751.
[98] Bril F, McPhaul MJ, Caulfield MP, Castille JM, Poynard T, Soldevila-Pico C, et al. Performance of the SteatoTest, ActiTest, NashTest and FibroTest in a multiethnic cohort of patients with type 2 diabetes mellitus. J Investig Med 2019;67:303–311.
[99] Xia MF, Yki-Jarvinen H, Bian H, Lin HD, Yan HM, Chang XX, et al. Influence of ethnicity on the accuracy of non-invasive scores predicting non-alcoholic fatty liver disease. PLoS One 2016;11:e0160526.
[100] Daniels SJ, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A, et al. ADAPT: an algorithm incorporating PRO-C3 accurately identifies patients with NAFLD and advanced fibrosis. Hepatology 2019;69:1075–1086.
[101] Miller MH, Walsh SV, Atrih A, Huang JT, Ferguson MA, Dillon JF. Serum proteome of nonalcoholic fatty liver disease: a multimodal approach to discovery of biomarkers of nonalcoholic steatohepatitis. J Gastroenterol Hepatol 2014;29:1839–1847.
[102] Kamada Y, Ono M, Hyogo H, Fujii H, Sumida Y, Mori K, et al. A novel noninvasive diagnostic method for nonalcoholic steatohepatitis using two glycobiomarkers. Hepatology 2015;62:1433–1443.
[103] Loomba R, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. J Lipid Res 2015;56:185–192.
[104] Puri P, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. Hepatology 2009;50:1827–1838.
[105] Barr J, Caballeria J, Martinez-Arranz I, Dominguez-Diez A, Alonso C, Muntane J, et al. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. J Proteome Res 2012;11:2521–2532.
[106] Zhou Y, Oresic M, Leivonen M, Gopalacharyulu P, Hyysalo J, Arola J, et al. Noninvasive detection of nonalcoholic steatohepatitis using clinical markers and circulating levels of lipids and metabolites. Clin Gastroenterol Hepatol 2016;14:1463–1472.e6.
[107] Grimaudo S, Pipitone RM, Pennisi G, Celsa C, Camma C, Di Marco V, et al. Association between PNPLA3 rs738409 C>G variant and liver-related outcomes in patients with non-alcoholic fatty liver disease. Clin Gastroenterol Hepatol 2019. https://doi.org/10.1016/j.cgh.2019.08.011 [Epub ahead of print].
[108] Hardy T, Zeybel M, Day CP, Dipper C, Masson S, McPherson S, et al. Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. Gut 2017;66:1321–1328.
[109] Wood GC, Chu X, Argyropoulos G, Benotti P, Rolston D, Mirshahi T, et al. A multi-component classifier for nonalcoholic fatty liver disease (NAFLD) based on genomic, proteomic, and phenomic data domains. Sci Rep 2017;7:43238.
[110] Szabo G, Bala S. MicroRNAs in liver disease. Nat Rev Gastroenterol Hepatol 2013;10:542–552.
[111] Wooden B, Goossens N, Hoshida Y, Friedman SL. Using big data to discover diagnostics and therapeutics for gastrointestinal and liver diseases. Gastroenterology 2017;152:53–67.e3.
[112] Bedossa P, Patel K. Biopsy and noninvasive methods to assess progression of nonalcoholic fatty liver disease. Gastroenterology 2016;150:1811–1822.e4.
[113] Sanyal AJ, Friedman SL, McCullough AJ, Dimick-Santos L. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392–1405.
[114] Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643–654.e1-9; quiz e39-40.
[115] Siddiqui MS, Yamada G, Vuppalanchi R, Van Natta M, Loomba R, Guy C, et al. Diagnostic accuracy of noninvasive fibrosis models to detect change in fibrosis stage. Clin Gastroenterol Hepatol 2019;17:1877–1885.e5.
[116] Harrison SA, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, et al. Simtuzumab is ineffective for patients with bridging fibrosis or compensated cirrhosis caused by nonalcoholic steatohepatitis. Gastroenterology 2018;155:1140–1153.
[117] Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174–1185.
[118] Cheung A, Neuschwander-Tetri BA, Kleiner DE, Schabel E, Rinella M, Harrison S, et al. Defining improvement in nonalcoholic steatohepatitis for treatment trial endpoints: recommendations from the liver forum. Hepatology 2019;70(5):1841–1855.
[119] Helmke S, Colmenero J, Everson GT. Noninvasive assessment of liver function. Curr Opin Gastroenterol 2015;31:199–208.
[120] Noureddin M, Lam J, Peterson MR, Middleton M, Hamilton G, Le TA, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. Hepatology 2013;58:1930–1940.
[121] Caussy C, Reeder SB, Sirlin CB, Loomba R. Noninvasive, Quantitative Assessment of liver fat by MRI-PDFF as an endpoint in NASH Trials. Hepatology 2018;68:763–772.
[122] Permutt Z, Le TA, Peterson MR, Seki E, Brenner DA, Sirlin C, et al. Correlation between liver histology and novel magnetic resonance imaging in adult patients with non-alcoholic fatty liver disease - MRI accurately quantifies hepatic steatosis in NAFLD. Aliment Pharmacol Ther 2012;36:22–29.
[123] Petta S, Wong VW, Camma C, Hiriart JB, Wong GL, Marra F, et al. Improved noninvasive prediction of liver fibrosis by liver stiffness measurement in patients with nonalcoholic fatty liver disease accounting for controlled attenuation parameter values. Hepatology 2017;65:1145–1155.
[124] 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;66:1486–1501.
[125] Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, 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.
[126] Anstee QM, Lawitz EJ, Alkhouri N, Wong VW, Romero-Gomez M, Okanoue T, et al. Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR trials. Hepatology 2019;70(5):1521–1530.
[127] Hsu C, Caussy C, Imajo K, Chen J, Singh S, Kaulback K, et al. Magnetic resonance vs transient elastography analysis of patients with nonalcoholic fatty liver disease: a systematic review and pooled analysis of individual participants. Clin Gastroenterol Hepatol 2019;17:630–637.e8.
[128] Patel J, Bettencourt R, Cui J, Salotti J, Hooker J, Bhatt A, et al. Association of noninvasive quantitative decline in liver fat content on MRI with histologic response in nonalcoholic steatohepatitis. Therap Adv Gastroenterol 2016;9:692–701.
[129] Loomba R, Lawitz E, Mantry PS, Jayakumar S, Caldwell SH, Arnold H, et al. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial. Hepatology 2018;67:549–559.
[130] Jayakumar S, Middleton MS, Lawitz EJ, Mantry PS, Caldwell SH, Arnold H, et al. Longitudinal correlations between MRE, MRI-PDFF, and liver histology in patients with non-alcoholic steatohepatitis: analysis of data from a phase II trial of selonsertib. J Hepatol 2019;70:133–141.
[131] Moreno C, Mueller S, Szabo G. Non-invasive diagnosis and biomarkers in alcohol-related liver disease. J Hepatol 2019;70:273–283.
[132] Nguyen-Khac E, Thiele M, Voican C, Nahon P, Moreno C, Boursier J, et al. Non-invasive diagnosis of liver fibrosis in patients with alcohol-related liver disease by transient elastography: an individual patient data meta-analysis. Lancet Gastroenterol Hepatol 2018;3:614–625.
[133] Gelsi E, Dainese R, Truchi R, Marine-Barjoan E, Anty R, Autuori M, et al. Effect of detoxification on liver stiffness assessed by Fibroscan(R) in alcoholic patients. Alcohol Clin Exp Res 2011;35:566–570.
[134] Mueller S, Nahon P, Rausch V, Peccerella T, Silva I, Yagmur E, et al. Caspase-cleaved keratin-18 fragments increase during alcohol withdrawal and predict liver-related death in patients with alcoholic liver disease. Hepatology 2017;66:96–107.
[135] Thiele M, Detlefsen S, Sevelsted Moller L, Madsen BS, Fuglsang Hansen J, Fialla AD, et al. Transient and 2-dimensional shear-wave elastography provide comparable assessment of alcoholic liver fibrosis and cirrhosis. Gastroenterology 2016;150:123–133.
[136] Corpechot C. Utility of noninvasive markers of fibrosis in cholestatic liver diseases. Clin Liver Dis 2016;20:143–158.
[137] Millonig G, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Buchler MW, et al. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. Hepatology 2008;48:1718–1723.
[138] Wu S, Yang Z, Zhou J, Zeng N, He Z, Zhan S, et al. Systematic review: diagnostic accuracy of non-invasive tests for staging liver fibrosis in autoimmune hepatitis. Hepatol Int 2019;13:91–101.
[139] Guo L, Zheng L, Hu L, Zhou H, Yu L, Liang W. Transient elastography (FibroScan) performs better than non-invasive markers in assessing liver fibrosis and cirrhosis in autoimmune hepatitis patients. Med Sci Monit 2017;23:5106–5112.
[140] Hartl J, Ehlken H, Sebode M, Peiseler M, Krech T, Zenouzi R, et al. Usefulness of biochemical remission and transient elastography in monitoring disease course in autoimmune hepatitis. J Hepatol 2018;68:754–763.
[141] Boursier J, de Ledinghen V, Zarski JP, Fouchard-Hubert I, Gallois Y, Oberti F, et al. Comparison of eight diagnostic algorithms for liver fibrosis in hepatitis C: new algorithms are more precise and entirely noninvasive. Hepatology 2012;55:58–67.
[142] Sebastiani G, Halfon P, Castera L, Pol S, Thomas DL, Mangia A, et al. SAFE biopsy: a validated method for large-scale staging of liver fibrosis in chronic hepatitis C. Hepatology 2009;49:1821–1827.
[143] Srivastava A, Gailer R, Tanwar S, Trembling P, Parkes J, Rodger A, et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. J Hepatol 2019;71:371–378.
[144] Davyduke T, Tandon P, Al-Karaghouli M, Abraldes JG, Ma MM. Impact of Implementing a "FIB-4 First" strategy on a pathway for patients with NAFLD referred from primary care. Hepatol Commun 2019;3:1322–1333.
[145] El-Gohary M, Moore M, Roderick P, Watkins E, Dash J, Reinson T, et al. Local care and treatment of liver disease (LOCATE) - a cluster-randomized feasibility study to discover, assess and manage early liver disease in primary care. PLoS One 2018;13:e0208798.
[146] Harman DJ, Ryder SD, James MW, Jelpke M, Ottey DS, Wilkes EA, et al. Direct targeting of risk factors significantly increases the detection of liver cirrhosis in primary care: a cross-sectional diagnostic study utilising transient elastography. BMJ Open 2015;5:e007516.