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Non-invasive tests of non-alcoholic fatty liver disease

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Abstract

For the detection of steatosis, quantitative ultrasound imaging techniques have achieved great progress in past years. Magnetic resonance imaging proton density fat fraction is currently the most accurate test to detect hepatic steatosis. Some blood biomarkers correlate with non-alcoholic steatohepatitis, but the accuracy is modest. Regarding liver fibrosis, liver stiffness measurement by transient elastography (TE) has high accuracy and is widely used across the world. Magnetic resonance elastography is marginally better than TE but is limited by its cost and availability. Several blood biomarkers of fibrosis have been used in clinical trials and hold promise for selecting patients for treatment and monitoring treatment response. This article reviews new developments in the non-invasive assessment of non-alcoholic fatty liver disease (NAFLD). Accumulating evidence suggests that various non-invasive tests can be used to diagnose NAFLD, assess its severity, and predict the prognosis. Further studies are needed to determine the role of the tests as monitoring tools. We cannot overemphasize the importance of context in selecting appropriate tests.

Keywords: Fatty liver; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Liver fibrosis; Cirrhosis; Transient elastography; FibroScan; Magnetic resonance imaging

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide and affects around 30% of the adult population in Asia.[1] In the United States, it has already become one of the leading indications for liver transplantation and an important cause of hepatocellular carcinoma (HCC).[2] However, only a small proportion of patients with NAFLD will eventually develop cirrhosis and HCC, and it would not make sense to provide liver-specific treatments and screen for complications in those who would not develop liver-related complications. It is thus important to assess the severity of NAFLD for rational use of medical resources.

Among the features of NAFLD severity, non-alcoholic steatohepatitis (NASH) is the driver of disease progression, whereas liver fibrosis is the link between liver injury and cirrhosis and its complications. Several meta-analyses have clearly demonstrated a strong and dose-response relationship between histologic fibrosis stage and liver-related morbidity and mortality.[3] Besides, a recent prospective study showed that all-cause mortality increased with increasing fibrosis stage in NAFLD patients.[4] While liver biopsy remains the only accepted method to diagnose NASH and the reference standard to evaluate fibrosis, it is an invasive procedure and cannot be widely applied. It is also ill suited as a monitoring test. Liver biopsy is also a suboptimal reference standard with considerable sampling bias and intra-observer and inter-observer variability.[5] Therefore, non-invasive tests for hepatic steatosis, steatohepatitis, and fibrosis have been a hot area of research. In the past few years, many non-invasive tests were developed for clinical use. However, clinicians may face uncertainty in determining which tests to use in various clinical contexts. A consensus scoring system should be developed and validated for clinical application.[6]

Since our group last reviewed this topic,[7] there have been new data on the application of various non-invasive tests in drug trials and clinical care pathways. Various new biomarkers have also been developed. Besides, although biomarkers are most often used in the diagnostic setting, the Food and Drug Administration of the United States has

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Table 1: The United States Food and Drug Administration BEST biomarker categories (Available from: http://fda.gov. [Last accessed on September 26, 2021]).

Categories	Meaning
Susceptibility/risk	Potential for developing a disease or medical condition
Diagnostic	Detect or confirm presence of a disease or condition
Monitoring	Serially for assessing status of a disease or medical condition
Prognostic	Likelihood of a clinical event, disease recurrence or progression
Predictive	Favorable or unfavorable effect from exposure to a medical product or an environmental agent
Pharmacodynamic/ response	Biological response has occurred in an individual who has been exposed to a medical product or an environmental agent
Safety	Likelihood, presence, or extent of toxicity as an adverse effect

BEST: Biomarkers, EndpointS and other Tools.

defined different biomarkers for various clinical settings [Table 1]. In this article, we review the progress in the field focusing on new developments in the past few years.

Methods

This is a narrative review. We searched PubMed and Embase using the following search terms: "nonalcoholic fatty liver disease," "nonalcoholic steatohepatitis," "NAFLD," "NASH," "liver fibrosis," "cirrhosis," "non-invasive tests," "biomarkers," "transient elastography," "liver stiffness measurement," and "imaging." We also extended the literature search by reviewing the reference lists of the articles and meeting abstracts of the Asian Pacific Association for the Study of the Liver, European Association for the Study of the Liver, and American Association for the Study of Liver Diseases. Because of the reference limit, we had to be selective and in general chose meta-analyses over individual studies, and favored larger and more recent studies.

Non-invasive assessment of hepatic steatosis

Hepatic steatosis is the basis to diagnose NAFLD. In patients with concomitant liver diseases such as chronic hepatitis B, the presence of hepatic steatosis is also associated with more severe liver injury and a higher risk of HCC.[8] In addition, improvement in hepatic steatosis may correlate with histologic improvements in NASH and fibrosis during pharmacological treatment. Because hepatic steatosis can change more rapidly than inflammation and fibrosis, it may be an attractive early readout in clinical trials.

Serum biomarkers and scores

According to European guidelines, non-invasive tests can be categorized as blood-based test, methods assessing physical properties of the liver tissue, and imaging methods assessing the anatomy of liver organs.[9] Serum biomarkers are currently used to diagnose or grade steatosis separately, or combined with anthropometric parameters to build models. Serum biomarkers are a group of parameters that are measured and evaluated as indicators of biological processes. In past decades, several biomarker panels were developed to assess hepatic steatosis. SteatoTest including 12 parameters was the first score developed for assessment of the liver steatosis in 2005. A study showed that SteatoTest can accurately estimate liver steatosis determined by liver biopsy in a cohort with 744 patients (area under the receiver operating characteristics curve [AUROC] 0.79 in training cohort, 0.72–0.86 in validation cohort).[10] Fatty liver index (FLI), which includes four items, body mass index (BMI), γ-glutamyltransferase (GGT) level, triglycerides (TGs) level, and waist circumference (WC), was developed in the following year. Bedogni *et al*[11] showed that FLI had high accuracy in diagnosing fatty liver (AUROC 0.84). The components of FLI are simple to obtain, but one of the limitations is that researchers only used ultrasonography instead of biopsy as the reference standard. The hepatic steatosis index (HSI) was developed based on a large cohort with 10,724 health check-up patients in Korea. HSI includes four parameters (serum aspartate aminotransferase [AST] to alanine aminotransferase [ALT] ratio, BMI, presence of diabetes mellitus [DM], and sex). By dual-cutoff values of 30 and 36 HSI score in detecting NAFLD, the HSI can correctly classify 85.6% patients in derivation cohort and 86.3% in validation cohort using ultrasound as reference.[12] In recent years, some new biomarker panels in assessing steatosis were constructed based on large cohort datasets and novel approaches. The Korean-NAFLD (K-NAFLD) score including four components (sex, WC, systolic blood pressure, and TG) was derived and validated in a cohort with >3000 patients. K-NAFLD showed significantly predictive impact on metabolic risk factors, but external validation was needed in a future study.[13] NAFL screening score was developed for detecting NAFLD in a large cohort with 46,493 patients from two centers and then validated in a cohort with 1996 patients. The NAFL screening score was comprised of simple clinical parameters, including classifications of age, BMI, fasting plasma glucose, uric acid, TG, and AST-to-ALT ratio. The NAFL screening score was established separately for men and women and had high accuracy in both training and validation cohorts.[14] The NAFL risk score with five parameters was developed to predict 4-year risk for NAFL from 8226 patients without fatty liver. NAFL risk score had good performance for predicting NAFL, especially in the female cohort.[15] NAFLD ridge score with six laboratory parameters was developed using novel machine learning method based on 922 patients. Researchers used proton magnetic resonance spectroscopy (^{1}H-MRS) as

reference diagnostic standard, which made the results more robust. NAFLD ridge score showed high accuracy both in training and validation cohorts.[16]

Abdominal ultrasonography

Different imaging approaches were developed to assess hepatic steatosis of liver tissue including ultrasound-based techniques, such as abdominal ultrasonography and controlled attenuation parameter (CAP), and magnetic resonance-based techniques such as MRI-estimated proton density fat fraction (MRI-PDFF) [Table 2]. Abdominal ultrasonography is currently the first-line diagnostic approach of hepatic steatosis due to its low cost and wide availability. Because of different scatter and attenuation of ultrasound waves by different tissues, focal of steatosis tissue present brighter than other parenchyma in ultrasound examinations. In a large meta-analysis based on 49 studies and 4720 participants, ultrasonography showed high accuracy in detecting moderate-to-severe steatosis. (AUROC 0.93).[17] The Hamaguchi scoring system was developed based on ultrasonographic findings. Three grading components, bright liver and hepatorenal echo contrast (0–3), deep attenuation (0–2), and vessel blurring (0–1), comprise the Hamaguchi score. The scoring system

Table 2: Non-invasive tests of hepatic steatosis.

Test	Description	Accuracy	Application	Limitations
Blood-based test				
SteatoTest	GGT, total bilirubin, α2m, ApoA1, haptoglobin, ALT, BMI, total cholesterol, TG, and glucose adjusted for age and gender	AUROC 0.72–0.86 for steatosis >5% (Sn 85%–100%, Sp 83%–100%; by dual cut-offs)	SteatoTest value <0.3 can exclude grade 2–4 steatosis; SteatoTest >0.72 is suggestive of grade 2–4 steatosis.	α2m, ApoA1, haptoglobin are not available in routine examination; high cost.
FLI	BMI, GGT, TG, WC	AUROC 0.84 in detecting fatty liver (Sn 87%, Sp 86%; by dual cut-offs)	A simple biomarker panel to detect fatty liver; FLI values <30 rule out fatty liver, and values >60 rule in fatty liver.	Ultrasound instead of biopsy was used as the reference standard
HSI	AST, ALT, BMI, DM presence and sex	AUROC 0.82 in detecting NAFLD (Sn 93.1%, Sp 93.1%; by dual cut-offs)	An easy biomarker panel to detect fatty liver; HSI values <30 rule out fatty liver, and values >36 rule in fatty liver.	Ultrasound instead of biopsy was used as the reference standard
K-NAFLD score	Sex, WC, SBP, TG	AUROC 0.93 in detecting NAFLD (PPV 99%, NPV 72.3%; by dual cut-offs)	An easy scoring system to identify NAFLD; K-NAFLD values <−3.285 rule out NAFLD, and values >0.884 rule in NAFLD.	Use NAFLD liver fat score instead of biopsy as the reference standard
NAFL screening score	Age, BMI, fasting plasma glucose, uric acid, triglyceride, and AST to ALT ratio	AUROC 0.825 for males (Sn 79.9%, Sp 66.3%), 0.861 for females (Sn 89.4%, Sp 69%) in detecting NAFL	A simple score to detect NAFL. Cut-off of NAFL screening score 33 for male 29 for female.	Ultrasound instead of biopsy was used as the reference standard
NAFL risk score	BMI, TG multiplied by GGT, ratio of AST and ALT, LDL-C and HDL-C, uric acid	AUROC 0.739 for males, 0.823 for females in predicting 4-year risk of NAFL	A simple score to predict 4-year risk of NAFL. Low-risk score group for male (0–6.5), for female (0–12.5). High-risk score group for male (7–18), for female (13–18).	Ultrasound instead of biopsy was used as the reference standard
NAFLD ridge score	ALT, HDL-C, TG, HbA1c, WBC, and hypertension presence	AUROC 0.88 in detecting NAFLD (Sn 92%, Sp 90%; by dual cut-offs)	An accurate novel score with machine learning approach to predict NAFLD; NAFLD ridge scores <0.24	Low PPV (69%)

(*continued*)

Table 2
(continued).

Test	Description	Accuracy	Application	Limitations
			rule out NAFLD, and scores >0.44 rule in NAFLD.	
Imaging methods				
Abdominal USG	Different scatter and attenuation of ultrasound wave by different tissues, focal of steatosis tissue present brighter than other parenchyma	AUROC 0.93 for presence of steatosis (Sn 60%–80%, Sp 80%–100%)	Most widely used imaging methods to detect fatty liver; Easy to perform and low cost; Available in most medical centers across the world.	Low sensitivity in obese patients and patients with mild fatty liver.
Hamaguchi score	Based on USG findings including bright liver and hepatorenal echo contrast (0–3), deep attenuation (0–2), vessel blurring (0–1)	AUROC 0.98 for diagnosing NAFLD (Sn 48.1%, Sp 79.7%)	Provide accurate information about fatty liver, visceral obesity and the metabolic syndrome in people without alcohol consumption. Hamaguchi score >2 rule in NAFLD; Hamaguchi score >1 rule in visceral obesity.	Not validated in large cohort.
QUS score	Based on RF raw data in QUS, numbers of ultrasonographic features can be obtained.	AUROC 0.82 in detecting NASH (Sn 90.5%, Sp 94.5% by dual cut-offs)	A novel and reliable score using machine learning approach to detect NASH. QUS score <6.0 rule out NASH, and >7.5 rule in NASH.	Diagnostic performance decline in patients with severe obesity.
CAP	Use the decline in the amplitude of ultrasound waves in the liver parenchyma to estimate the degree of hepatic steatosis.	AUROC 0.8 in detecting any grade hepatic steatosis (Sn 69%, Sp 82%)	Easy and accurate approach with good feasibility to detect steatosis; Low failure rate.	Cannot distinguish reliably of different steatosis grades.
ATT	The degree of attenuation of the US beam is color-coded by different liver tissues.	AUROC 0.94 in diagnosing moderate–serve steatosis (Sn 90%, Sp 100%)	High accuracy for moderate–serve steatosis.	Suboptimal reference standard (computed tomography)
ATI	ATT estimates hepatic steatosis from differences in attenuation of the received RF signals.	AUROC 0.83 for S0–2 *vs*. S3 (Sn 86%, Sp 69%)	Good diagnostic performance in NASH and grade of steatosis.	Not validated in large cohort; not available in most medical centers.
MRI-PDFF	An imaging biomarker to measure the liver fat based on proton MRS.	AUROC 0.99 in detecting any grade of steatosis (Sn 96%, Sp 100%)	Most accurate method to detect and quantify liver steatosis.	High cost and limited availability

α2m: α2-macroglobulin; 2D-SWE: Two-dimensional shear wave elastography; ALT: Alanine aminotransferase; ApoA1: Apolipoprotein A1; AUROC: Area under the receiver-operating characteristics curve; AST: Aspartate aminotransferase; ATT: Attenuation coefficient; ATI: Attenuation imaging; BMI: Body mass index; CAP: Controlled attenuation parameter; DM: Diabetes mellitus; FLI: Fatty liver index; GGT: γ-glutamyltransferase; HbA1c: Hemoglobin A1C; HDL-C: High-density lipoprotein cholesterol; HSI: Hepatic steatosis index; LDL-C: Low-density lipoprotein cholesterol; MRI-PDFF: Magnetic resonance imaging proton density fat fraction; MRS: Magnetic resonance spectroscopy; NA: Not applicable; NAFL: Non-alcoholic fatty liver; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NPV: Negative predictive value; PPV: Positive predictive value; QUS: Quantitative ultrasound; RF: Radiofrequency; SBP: Systolic blood pressure; Sn: Sensitivity; Sp: Specificity; TG: Triglycerides; USG: Ultrasonography; WBC: White blood cell; WC: Waist circumference.

further improved diagnostic performance in NAFLD patients with metabolic risk factors (AUROC 0.98). By standardized reporting and scoring, the score improved the intra-observer and inter-observer agreement for the diagnosis of NAFLD.[18] Recently, a number of new ultrasound techniques have been developed to quantitatively assess hepatic fat. The quantitative ultrasound (QUS) method is based on a transducer that can detect the radiofrequency (RF) of echoes. When ultrasonic waves pass though soft tissue, such as steatotic tissue, energy absorption, reflection, and scattering take place. The wave energy transmitted back to the transducer constitutes RF. In conventional ultrasound examination, most information of RF is lost, but in QUS, RF data can be used by back scattered US signals and attenuation coefficient (ATT) calculator.[19] According to this mechanism, techniques based on calculation of the ATT include CAP, attenuation imaging (ATI), and ATT. Among these methods, CAP has been already widely used in clinical practice (see the next section). Despite the paucity of data, the diagnostic performance of ATT and ATI for different steatosis grades is satisfactory.[20,21] Based on RF raw data in QUS, one can obtain and analyze a number of ultrasonic features. A novel machine learning score, QUS score that includes 18 predictive RF features, was developed from a biopsy-proven NAFLD cohort to detect NASH. The score showed satisfactory diagnostic performance both in all patients and subgroup analysis.[22]

Controlled attenuation parameter

Ultrasound energy is dissipated more rapidly in a steatotic liver. CAP by vibration-controlled transient elastography (VCTE) captures the decline in the amplitude of ultrasound waves in the liver parenchyma to estimate the degree of hepatic steatosis. In two individual patient data meta-analyses, CAP measured by the M or XL probes had an AUROC of around 0.80 in detecting hepatic steatosis.[23,24] There is considerable overlap in CAP values across steatosis grades, thus casting doubt on its reliability in monitoring changes in steatosis over time. The cutoff of CAP is also a matter of debate. In the two meta-analyses, the optimal cutoffs to detect fatty liver were 248 dB/m and 297 dB/m using the M and XL probes, respectively.[23,24] In two recent prospective studies, the suggested cutoffs to detect S1 steatosis were 244 and 295 dB/m separately.[25,26] However, studies from the USA consistently yielded higher optimal cutoffs of around 300 dB/m.[27] It is unclear if the higher BMI in American cohorts was the cause of the discrepancy. One international multi-center study suggests that patients with an interquartile range of CAP of >40 dB/m were more likely to have inaccurate results, but the findings require independent confirmation.[28]

The latest model of VCTE supports the SmartExam and CAP measurement using the continuous method. In the original model, an operator obtains ten CAP measurements and uses the median value to represent the degree of steatosis. The new continuous CAP allows continuous measurements of CAP during the entire examination and captures roughly 200 CAP values during the same examination time. Preliminary data suggest that continuous CAP has a lower measurement variability than the original method.[29]

Magnetic resonance imaging

MRS is an alternative method to measure intrahepatic TG non-invasively. ^{1}H-MRS detects hepatic steatosis by measuring proton signals at different locations. MRS is correlated with histopathologic steatosis and has greater capability for accurate identification of mild grade of steatosis than computed tomography and ultrasound.[30] It has been used to detect hepatic steatosis and quantify the amount of liver fat in many epidemiologic studies.[31,32] However, limited availability, need for expertise in protocol prescription, and spectral analysis limit the use of MRS in routine practice.

MRI-PDFF is an imaging biomarker to measure the liver fat over the entire liver and is the most accurate method to detect and quantify liver steatosis. MRI-PDFF has almost perfect correlation with ^{1}H-MRS and is more sensitive than histology in quantifying longitudinal changes of liver fat content.[33] It is also more accurate than CAP in identifying all grades of hepatic steatosis in NAFLD patients with a high AUROC of 0.99.[34]

MRI-PDFF response is a potential surrogate for histologic improvement. Several studies have shown a correlation between a reduction in MRI-PDFF (usually taken as a ≥30% relative reduction) and a 2-point improvement in the NAFLD activity score, resolution of NASH, and fibrosis improvement.[35,36] This test has been used as an end point to examine efficacy of various drugs to assess treatment response in several early phase trials in NASH. However, the MRI-PDFF response is probably drug-specific, and some studies have failed to demonstrate an association with histologic response.[37] Moreover, MRI-PDFF can be underestimated in patients with advanced fibrosis. The high cost and limited availability limit the routine clinic use of this test.

Non-invasive assessment of NASH

NASH is defined as the presence of hepatic steatosis, lobular inflammation, and hepatocyte ballooning. Patients with NASH have faster fibrosis progression and are at an increased risk of cirrhosis and HCC.[38] Currently, the US Food and Drug Administration and the European Medicines Agency would consider conditional approval of a drug if it can lead to resolution of NASH with no worsening of fibrosis, or improvement in fibrosis without worsening of NASH.[39] The main problem of using resolution of NASH as a histologic endpoint stems from the intra-observer and inter-observer variability of its diagnosis.[5] The suboptimal reliability of histologic diagnosis of NASH also hampers the development of non-invasive tests for this condition.

Serum cytokeratin-18 (CK-18) fragment

CK-18 fragment is the most widely studied biomarker of NASH so far.[40] CK-18 fragment is derived from hepatocyte apoptosis and can be detected in serum by immunoassay. In NASH (but not NAFL) patients and NASH animal models, increased hepatocyte death caused by apoptosis is usually present. Apoptosis leads to activation of effector caspase (mainly caspase-3), which cleaves many different substrates in cells, including CK-18, which is the main intermediate protein in the liver, leading to the characteristic morphological changes of apoptosis.

M30 enzyme-linked immunosorbent assay detects caspase cleaved K18 fragment and cell apoptosis, while M65 enzyme-linked immunosorbent assay detects total cell death. In two meta-analyses, the pooled AUROC of CK-18 was 0.82 with a sensitivity of 66% to 78% and specificity of 82% to 87%.[41,42] To increase CK-18 sensitivity, several studies tried to combine it with other biological parameters, such as soluble Fas (sFas) levels, uric acid, serum Golgi protein 73, adiponectin and resistin, or ALT and presence of metabolic syndrome.[43-46]

Other serum NASH biomarkers under investigation

The ALT level was one of the simple biomarkers of NASH. Previous studies have found that the frequency of NASH in individuals with normal ALT was 11% whereas the frequency was 29% in those with elevated ALT. At two times the upper limit of normal, ALT has a specificity of 60% for NASH.[47]

Several studies have found a significant relationship between the number of metabolic syndrome components and the probability of NASH in patients with NAFLD.[48-50] Recently, several approaches using genetic biomarkers have been proposed, including single nucleotide polymorphisms located in *PNPLA3*, such as the NASH Score (*PNPLA3* genotype, AST, and fasting insulin), the NASH ClinLipMet Score (glutamate, isoleucine, glycine, lysophosphatidylcholine 16:0, phosphoethanolamine 40:6, AST, fasting insulin, and *PNPLA3* genotype), individualized polygenic risk score (sex, presence of metabolic syndrome, homeostatic model assessment for insulin resistance, AST, *PNPLA3,* and *HSD17B13* genotypes), and the NASH prothrombin time (PT) scoring system (*PNPLA3* and *TM6SF2* genotypes, diabetes status, insulin resistance, AST and high-sensitivity C-reactive protein), expression of non-coding RNAs, specifically microRNAs, such as the miR-122.[7,51,52]

Several predictive models for the diagnosis of NASH combine clinical and laboratory parameters. The predictive models include the hypertension, increased ALT, and insulin resistance, Palekar score (age, sex, AST, BMI, AST/ALT ratio, and hyaluronic acid [HA]), Gholam score (AST and DM), oxNASH (13-hydroxyl-octadecadienoic acid/linoleic acid ratio, age, BMI, and AST), NAFIC score (ferritin, insulin, and type IV collagen 7s), acNASH index (AST-to-creatinine ratio), and NashTest (Biopredictive, Paris, France), a proprietary formula including 12 variables (age, sex, height, weight, serum levels of TG, cholesterol, a2-macroglobulin, apolipoprotein A1, haptoglobin, GGT, aminotransferases ALT, AST, and total bilirubin).[53,54]

Magnetic resonance imaging

LiverMultiScan is a non-invasive MRI-based biomarker to evaluate levels of liver fat, liver iron content, fibrosis, and inflammation. The technology is comprised of corrected T1 (cT1), T2, and liver fat assessment by advanced MRI. LiverMultiScan measures the amount of iron in the liver to correct for its effect on T1 – cT1 as excess iron in the liver reduces T1 relaxation time and leads to underestimation of liver disease. cT1 also correlates with necroinflammation and fibrosis and may serve as a non-invasive method in NASH.[55] Phosphorus MRS is a method to measure and interpretate some spectral signals. It shows different biochemical changes in NAFLD patients with NASH and non-NASH and is accurate for NASH diagnosis.[56]

Magnetic resonance elastography (MRE) is a technology to assess the mechanical properties of biologic tissues and estimate the tissue stiffness by MRI imaging combined with low-frequency vibrations. It examines the entire liver and has the advantages of less sample error, low failure rate, and high repeatability compared with biopsy procedures. Imaging parameters including multi-frequency 3D-MRE (mf3D-MRE) depiction of damping ratio (DR), shear stiffness (SS), and MRI-PDFF depiction of fat fraction (FF) showed significant differences between NASH and non-NASH. DR at 40 Hz and SS at 60 Hz showed best correlation with NASH. The non-invasive method by Allen *et al*,[57] which combines these parameters of mf3D-MRE and MRI-PDFF, provides good ability to diagnose NASH and stratifies the severity of NASH. This technical approach provides a promising alternative to liver biopsy for the estimation of liver histology and disease activity.

Recently, radiomics have been widely applied in MRI imaging studies. This technique can extract different imaging features for quantitative analysis. With the help of machine learning, the entirety of the strength of radiomics can be harnessed. In the past decades, some studies showed that T2-weight imaging may correlate with hepatic inflammation.[58,59] Recently, Chen *et al*[60] established a radiomics signature based on T2-weight imaging, which can accurately predict hepatic inflammation (AUROC 0.8 in training cohort, 0.77 and 0.75 in validation cohort). Radiomics techniques provide more opportunities for quantitative analysis in the NAFLD field.

Non-invasive assessment of fibrosis

Fibrosis is the natural response to tissue injury. When there is ongoing liver injury, fibrosis would become counterproductive. Accumulating fibrosis would eventually lead to thick fibrous septae and distorted liver architecture, that is, the development of cirrhosis. As fibrosis is the common path of different chronic liver diseases towards cirrhosis, it comes as no surprise that the severity of fibrosis has strong correlation with HCC and cirrhotic complications.[3] Apart from resolution of NASH, improvement in fibrosis with no worsening of NASH is another accepted histologic endpoint for conditional approval of NASH drugs.[39]

Simple fibrosis scores

Simple fibrosis scores incorporate "indirect" markers of liver fibrosis along with some clinical parameters to improve the accuracy [Table 3].[61] The AST/ ALT ratio and AST/platelet ratio (APRI) were initially derived from chronic hepatitis C (CHC) cohorts.[62,63] They can be calculated easily but have relatively low accuracy in diagnosing advanced fibrosis in patients with NAFLD (AUROC 0.66–0.74 and AUROC 0.74, respectively).[64]

Table 3: Non-invasive tests of hepatic fibrosis.

Test	Description	Accuracy	Application	Limitations
Simple fibrosis scores				
AST/ALT ratio	AST and ALT	AUROC 0.66–0.74 for F3 fibrosis (Sn 40%, Sp 80%)	Reasonable NPV to exclude advanced liver fibrosis and cirrhosis	Modest accuracy
APRI	AST and platelet count	AUROC 0.74 for F3 fibrosis (Sn 66.5%, Sp 71.7%)	High NPV to exclude advanced liver fibrosis and cirrhosis Low values associated with low risk of liver-related events	Modest accuracy
FIB-4 index	Age, AST, ALT, and platelet count	AUROC 0.84 for F3-4 fibrosis (Sn 82%, Sp 93%; by dual cut-offs)	High NPV to exclude advanced liver fibrosis and cirrhosis Low values associated with low risk of liver-related events	Poor performance in patients <35 years of age; less specific in patients >65 years of age
NFS	Age, BMI, impaired fasting glucose or DM, AST/ALT ratio, platelet count, and serum albumin levels	AUROC 0.82 for F3-4 fibrosis (Sn 73%–82%, Sp 96%–98%; by dual cut-offs)	High NPV to exclude advanced liver fibrosis and cirrhosis Low values associated with low risk of liver-related events	Poor performance in patients <35 years of age; less specific in patients >65 years of age; the inclusion of irreversible parameters (age and diabetes) limits its use as a monitoring tool
BARD score	BMI, AST/ALT ratio and DM presence	AUROC 0.70–0.83 for F3-4 fibrosis (Sn 87%, Sp 73%; by dual cut-offs)	A widely used score to predict advanced fibrosis; A BARD score of 2–4 points was associated with advanced fibrosis.	Modest accuracy; interpretation of BMI might vary across different ethnicity
HFS	Sex, age, homeostatic model assessment score, presence of diabetes, AST, and albumin, and platelet counts	AUROC 0.85 for F3-4 fibrosis (Sn 74%, Sp 97% by dual cut-offs)	A simple score to predict advanced fibrosis; HFS <0.12 rule out advanced fibrosis, >0.47 rule in advanced fibrosis	Modest accuracy in French patients; need be validated in DM-free cohort.
Specific fibrosis biomarkers				
HA	Main structural role in the formation of ECM	AUROC 0.89 for F3-4 fibrosis (Sn 85.0%, Sp 79.7%)	Component of a few blood panels	Not suggested to use alone
PIIINP	A direct measure of type III collagen formation in tissues.	AUROC 0.78 for ≥F2 fibrosis in CHB (Sn 48.1%, Sp 79.7%)	Component of a few blood panels	Not suggested to use alone
Pro-C3	Reflect true synthesis of type III collagen	AUROC 0.73 for ≥F3 fibrosis (Sn 60%, Sp 74%)	Correlated well with steatohepatitis and fibrosis stage	Less well studied outside NAFLD
TIMP1	Regulate matrix metalloproteinases and inhibit ECM degradation	AUROC 0.97 for NASH (Sn 96.7%, Sp 100%)	High accuracy for NASH and included in a few blood panels	Not suggested to use alone
Specific fibrosis panels				
ELF	PIIINP, HA, and TIMP1	AUROC 0.83 for F3-4 fibrosis (Sn 65%, Sp 86%) by cut-off 9.8	Predict clinical outcomes and disease progression in patients with chronic liver diseases	Not sensitive to early stages of fibrosis; costly; limited availability outside the UK

(*continued*)

Table 3
(continued).

Test	Description	Accuracy	Application	Limitations
MLA	BMI, Pro-C3, type-IV collagen, AST to GGT ratio.	AUROC 0.9 in training cohort, and 0.89 in validation cohort in detecting significant fibrosis ($F \geq 2$), 0.997 and 0.989 in detecting advanced fibrosis ($F \geq 3$) and cirrhosis respectively.	Distinguished diagnostic performance in detecting significant fibrosis, advanced fibrosis, and cirrhosis	Costly, not available in large cohort
Individual risk nomogram	Waist-to-height ratio, HA, Pro-C3, chitinase-3-like protein 1, and CK-18 neoepitope M65.	AUROC 0.83 in detecting significant fibrosis ($F \geq 2$) (Sn 69%, Sp 82%)	A novel nomogram in detecting significant fibrosis	Costly, need further external validation
FibroTest	GGT, total bilirubin, α2m, apolipoprotein AI and haptoglobin	AUROC 0.88 for F3-4 fibrosis (Sn 95%, Sp 71%) by cut-off 0.30	Good diagnostic performance for advanced fibrosis	Not sensitive to early stages of fibrosis; costly; more extensively studied in viral hepatitis
FibroMeter NAFLD	Body weight, prothrombin index, and serum levels of ALT, AST, ferritin, and fasting glucose	AUROC 0.94 for ≥F2 fibrosis (Sn 78.5%, Sp 95.9%)	Accurate marker of liver fibrosis specifically in patients with chronic liver disease due to NAFLD	High cost and not routinely available
FibroMeter VCTE	FibroMeter NAFLD and LSM	AUROC 0.94 for F3-4 fibrosis (Sn 70%, Sp 93%)	Improve the diagnosis of advanced fibrosis than LSM alone	High cost; not routinely available
Hepascore	Age, sex and serum levels of bilirubin, GGT, HA and α2m	AUROC 0.81 for F3-4 fibrosis (Sn 75.5%, Sp 84.1%)	A widely used algorithm to assess liver fibrosis severity and predict clinical outcome in chronic liver diseases	Influenced by age and sex; Not routinely available
Imaging biomarkers				
VCTE	Measure the velocity of an elastic shear wave propagating through the liver; M and XL probes	AUROC 0.93–0.95 for F3-4 fibrosis with M probe (Sn 91%–92%, Sp 75%–88%); AUROC 0.84–0.95 for F3-4 fibrosis with XL probe (Sn 75%–88%, Sp 74%–82%)	High NPV to exclude advanced fibrosis, modest PPV to rule in advanced fibrosis or cirrhosis; Quick and painless; Reduce the need for biopsy	High cost and not widely available in clinic settings
p-SWE	Short-duration acoustic pulses that propagate shear waves and generate localized, micrometer-scale displacements in tissue	AUROC 0.91–0.95 for F3-4 fibrosis (Sn 73%–96%, Sp 82%–92%)	Easily implemented on modified commercial ultrasonography machines; More accurate than TE in obese patients	Quality criteria not well defined
2D-SWE	The interrogation of the tissue by ARFIs induced into the tissues by focused ultrasonic beams and captures the propagation of resulting shear waves in real time	AUROC 0.80–0.98 for F3-4 fibrosis (Sn 71.4%, Sp 94.4%)	Easily implemented on a commercially available ultrasonography machine; A quantitative estimation of liver stiffness can be performed in a certain region of interest	Experienced operators needed; Quality criteria not well defined

(*continued*)

Table 3
(continued).

Test	Description	Accuracy	Application	Limitations
MRE	A modified phase-contrast method to image the propagation of the shear wave in the liver parenchyma.	AUROC 0.89–0.96 for F3-4 fibrosis (Sn 85.7%, Sp 90.8%)	Implemented on a regular MRI machine; assess the entire liver; standardize mechanical parameters for SS	High cost; not widely available; time-consuming; limit to patients with recognized MRI contraindications
MEFIB index	MRE combined with FIB-4	AUROC 0.85–0.95 for ≥stage 2 fibrosis	High PPV ruling in patients with ≥stage 2 fibrosis	Influenced by age; high cost; not widely available; time-consuming; limit to patients with recognized MRI contraindications

α2m: α2-macroglobulin; 2D-SWE: Two-dimensional shear wave elastography; ARFI: Acoustic radiation force impulse; ALT: Alanine aminotransferase; AUROC: Area under the receiver-operating characteristics curve; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase to platelet ratio index; BMI: Body mass index; BARD score: Calculated from BMI, AST:ALT ratio and DM presence; CK-18: Cytokeratin-18; DM: Diabetes mellitus; ELF: Enhanced liver fibrosis; ECM: Extracellular matrix; FIB-4 index: Fibrosis-4 index; GGT: γ-Glutamyltransferase; HFS: Hepamet fibrosis score; HA: Hyaluronic acid; LSM: Liver stiffness measurement; MLA: Machine learning algorithm; MRE: Magnetic resonance elastograph; MRI: Magnetic resonance imaging; MEFIB index: MRE combined with FIB-4 index; NA: Not applicable; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NFS: NAFLD fibrosis score; NPV: Negative predictive value; PIIINP: Procollagen III amino-terminal peptide; Pro-C3: Neo-epitope-specific competitive enzyme-linked immunosorbent assay for PIIINP; p-SWE: Point shear wave elastography; PPV: Positive predictive value; SS: Shear stiffness; Sn: Sensitivity; Sp: Specificity; TIMP1: Tissue inhibitor of metalloproteinases 1; TE: Transient elastography; VCTE: Vibration controlled transient elastography.

Fibrosis-4 (FIB-4) index and NAFLD fibrosis score (NFS) are both cost-effective and sensitive panels to rule out patients with advanced fibrosis.[40] FIB-4 consists of age, AST, ALT, and platelet count and was developed in CHC/human immunodeficiency virus (HIV) coinfected patients.[65] A recent meta-analysis (based on 64 studies in 13,046 NAFLD patients) reported that FIB-4 had AUROC of 0.84 for diagnosing advanced fibrosis.[66] The NFS comprises age, BMI, presence of impaired fasting glucose or DM, AST/ALT ratio, platelet count, and serum albumin levels.[67] It has an AUROC of 0.82 in detecting advanced fibrosis, which is comparable to that of the FIB-4 index.[64] Some confounding factors, such as age, platelet counts, diabetes, and prevalence of fibrosis, may influence their diagnostic performance.[40,68] The NFS and FIB-4 index have lower diagnostic accuracy for advanced fibrosis in patients aged >35 years and are less specific in older patients (≥65 years), resulting in a high false positive rate for advanced fibrosis.[69] To rectify this, new age-adjusted cut-offs (FIB-4 = 2.0 and NFS = 0.12) have been proposed to improve the accuracy of the NFS and FIB-4 score in patients aged ≥65 years. These scores have high negative predictive value (NPV) in excluding advanced fibrosis, but the positive predictive value (PPV) is very low, especially in primary care settings with a low prevalence of advanced disease.[70] Recently, Hepamet fibrosis score (HFS) was developed to detect advanced fibrosis and comprised of sex, age, homeostatic model assessment score, presence of diabetes, AST and albumin, and platelet counts. Compared to NFS and FIB-4, the HFS has higher PPV and AUROC, and did not require adjustment for age.[71] These simple fibrosis scores also showed consistently good ability to predict liver-related morbidity and mortality among adults with NAFLD.[72]

Specific fibrosis biomarkers

Specific fibrosis biomarkers incorporate direct markers of fibrogenesis and/or fibrinolysis, and are more accurate than simple fibrosis scores in predicting advanced fibrosis and cirrhosis.[73] The enhanced liver fibrosis (ELF) test is a panel of markers that consists of three components: type III procollagen amino terminal propeptide (PIIINP), HA, and tissue inhibitor of metalloproteinase-1. The ELF panel had good diagnostic accuracy in identifying patients with advanced fibrosis, with an AUROC of 0.83,[74] sensitivity 65%, and specificity 86%, using a high threshold of 9.8 (recommended by Siemens). However, its performance can be influenced by age and gender in patients with CHC, although this would require further validation in patients with NAFLD.[75] When used in primary care settings, a two-step pathway FIB-4 followed by the ELF panel in case of indeterminate results could increase the identification of advanced liver disease while reducing unnecessary referrals to secondary care.[76] The ELF test can also predict liver-related morbidity and mortality in patients with chronic liver disease and may be a useful prognostic tool in clinical practice.[77]

A neo-epitope-specific competitive enzyme-linked immunosorbent assay for PIIINP (Pro-C3) reflects true synthesis of type III collagen. A recent study in 570 biopsy-proven patients with NAFLD reported that Pro-C3 was correlated with fibrosis stage, yielding an AUROC of 0.73 for advanced fibrosis.[78] A Pro-C3-based fibrosis algorithm that included age, presence of diabetes, Pro-C3, and platelet count (ADAPT) was developed. ADAPT was superior to the existing fibrosis scores (APRI, FIB-4, NFS, and BARD) at identifying advanced fibrosis with AUROC

of 0.83 and 0.88 in derivation and validation hospital-based NAFLD cohorts.[79] Recently, Feng *et al*[80] developed a novel machine learning algorithm (MLA) based on Pro-C3. MLA was developed by random forest and comprised of BMI, Pro-C3, type-IV collagen, and AST to GGT ratio. MLA showed distinguished performance in detecting significant fibrosis ($F \geq 2$) (AUROC 0.9 in training cohort, and 0.89 in validation cohort). Some novel graphical prediction models, such as nomogram method based on specific fibrosis makers, have also been developed to identify significant fibrosis and fibrotic NASH.[81,82]

FibroTest and FibroMeter are proprietary biomarker panels and have shown good diagnostic performance for advanced fibrosis in some but not all studies.[83-85] Although the specific fibrosis biomarkers are promising, their cost and availability have limited their wider application in routine practice.

Ultrasound elastography

The commonly adopted ultrasound-based elastography techniques include VCTE and acoustic radiation force impulse (ARFI), which can be further divided into point-shear wave elastography (p-SWE) and two-dimension shear wave elastography.[19] These techniques differ in the physical properties used and can be grouped into two major types: TE uses a mechanical external push; ARFI uses an acoustic internal push. Both TE and ARFI are shear wave-based techniques, which measure the speed of shear waves, generated by an external mechanical push in TE or by the push pulse of a focused ultrasound beam in ARFI techniques. The shear wave velocity is calculated and related with liver stiffness measurement (LSM), presented as kilopascal.[19]

TE is a one-dimensional technique performed with the FibroScan machine (Echosens, Paris, France); TE assesses LSM by transmitting a shear wave followed by an ultrasound wave through a probe put on the skin overlying the liver parenchyma.[86] The velocity of the shear wave passing through the liver parenchyma is calculated by Doppler technique. The higher the velocity, the stiffer the liver parenchyma is. Reliable TE results require at least 10 successful attempts and the ratio of interquartile range to median of LSM results to be <0.3.[87] It was once suggested to use different LSM cut-off values for different probes: the cut-off values for M probe, with a 90% sensitivity and specificity to rule-in or rule-out advanced fibrosis, were 7.9 and 9.6 kPa, respectively;[88] those for XL probe were 5.7 and 9.3 kPa, respectively.[89] As obese patients tend to have over-estimated LSM results,[90] it was proposed to unify the LSM cut-off values when M and XL probes are used according to the appropriate BMI.[91] While significantly elevated ALT is a well-known confounding factor of LSM,[92] it is lesser a problem in NAFLD patients. With the more recent FibroScan models, LSM and CAP (discussed above) results are available at the same TE examination; this makes TE a desirable tool to assess liver fibrosis and fat at the same examination.[93] LSM is prognostically more important than CAP, as the risk of adverse clinical outcome increases with LSM but not CAP.[94]

ARFI is implemented in the current ultrasound scanner, without the need of additional hardware or cost. The conventional ultrasound probe automatically produces an acoustic "push" pulse for generating a shear-wave, which passes through the tissue.[86] ARFI appears to be more accurate than TE in obese patients.[95] However, the ARFI techniques have been less extensively evaluated than TE. The correlation between p-SWE and liver biopsy is moderate ($r = 0.71$).[96] It is important to note that different systems and ultrasound frequencies may yield different estimates of shear wave speed, thus affecting the interpretation of results.[19]

Magnetic resonance elastography

MRE adopts a phase contrast imaging method, which depends on mechanical wave propagation to assess the degree of liver stiffness; it is less operator-dependent, less affected by obesity and ascites, and less likely to have technical failure than TE.[97] MRE is useful for diagnosis and staging of liver fibrosis, even if the fibrosis is very mild. MRE has higher diagnostic accuracy than TE (accuracy of TE *vs.* MRE for fibrosis: stage ≥1, 0.82 *vs.* 0.87; stage ≥2, 0.87 *vs.* 0.92; stage ≥3, 0.84 *vs.* 0.93; and stage 4, 0.84 *vs.* 0.94); MRI-based technologies are also preferred for obese patients as TE may fail in some of these subjects.[98] However, MRE is expensive and time-consuming and may be affected by variable respiratory efforts and iron overload.[86] Furthermore, it is important to note that MRE reports the shear modulus in kilopascals, which is three times smaller than the Young modulus used to report the results of the ultrasound techniques.[99]

A novel macromolecular proton fraction (MPF) mapping based on spin-lock (SL) magnetic resonance imaging (MPF-SL MRI) has been evaluated in different stages of liver fibrosis.[100] MPF-SL is designed to measure a relaxation rate that is specific to the magnetization transfer effect by removing the R1ρ relaxation due to the mobile water and chemical exchange pools.[100] Liver MRI examination was performed with a regular 3.0T MRI scanner with two sequences in transverse plane: the MPF-SL protocol and a multi-slice two-dimensional GRE imaging protocol. Liver iron concentration and FF were obtained from GRE imaging data using a freely available MRQuantif software (https://imagemed.univ-rennes1.fr/en/mrquantif/download.php). The accuracy of MPF to distinguish between F0 and F1-2 fibrosis was as high as 0.85.[100]

Conclusions

In this article, we summarized new development in the non-invasive assessment of hepatic steatosis, steatohepatitis, and fibrosis. We cannot overemphasize the importance of context in selecting appropriate tests. Ultrasound scan remains the most commonly used method to diagnose fatty liver because it is widely available and relatively inexpensive. Simple fibrosis scores such as the FIB-4 index and NFS can be easily performed and are reasonable options in primary care and non-hepatology settings. These scores have high enough NPVs to exclude advanced fibrosis and future liver-related events. The best test in case

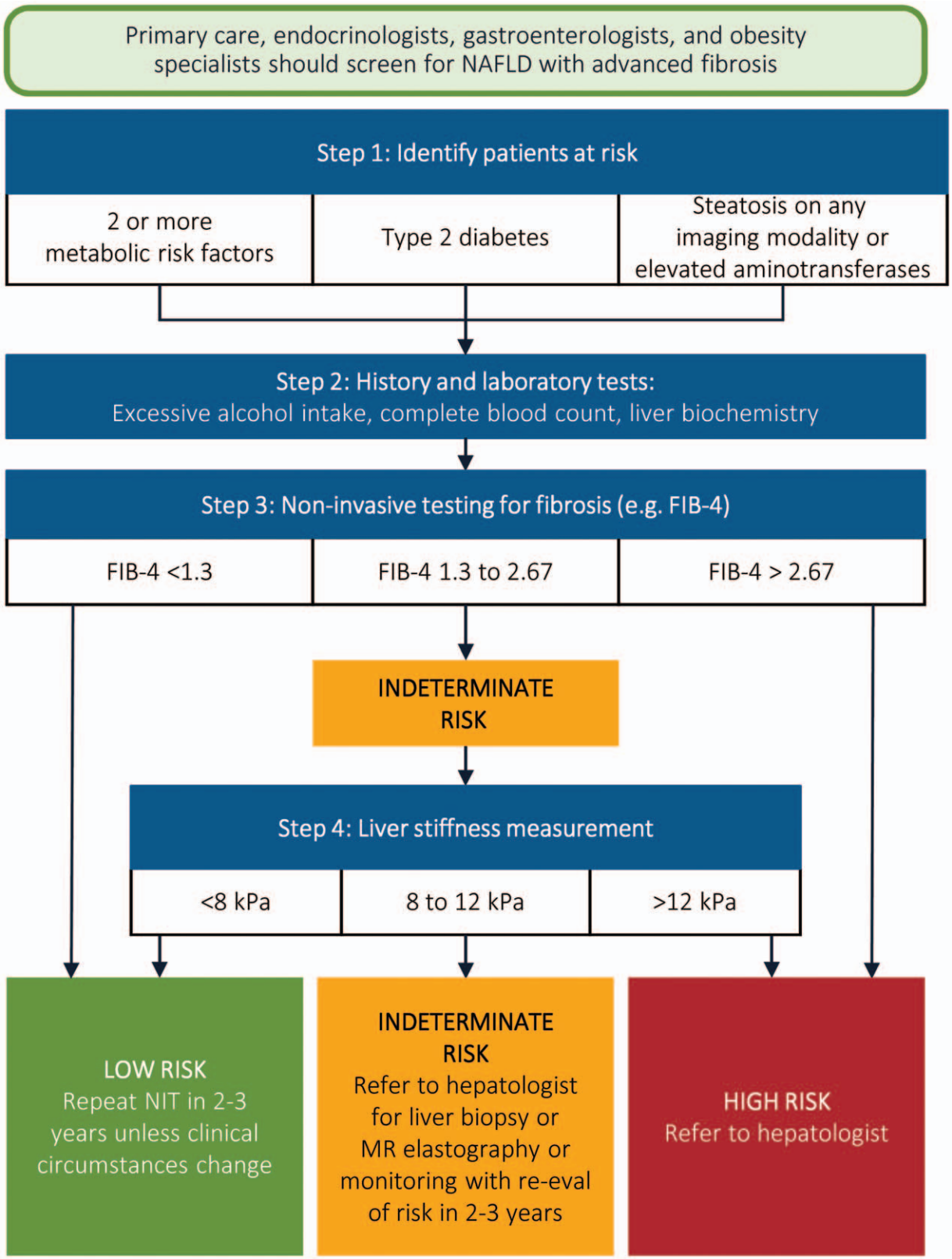

Figure 1: American Gastroenterological Association clinical care pathway for patients with NAFLD (reproduced with permission from Kanwal *et al* and *Gastroenterology*[102]). FIB-4: Fibrosis-4 index; LSM: Liver stiffness measurement; NAFLD: Non-alcoholic fatty liver disease; NIT: Non-invasive tests.

of abnormal simple fibrosis scores would depend on availability, cost, and local expertise. Specific blood biomarkers such as ELF and Pro-C3 or imaging biomarkers including ultrasound elastography and MRE are possible choices. These follow-up tests may be performed by hepatologists or other healthcare providers depending on the local setting.

Surveys have indicated that few countries have established a clinical care pathway for NAFLD.[101] Although it is logical to expect a surge of referrals when more clinicians start evaluating for NAFLD, studies with well-defined clinical care pathways suggest otherwise. In a multi-center study at primary care clinics in the UK, the use of FIB-4 followed by the ELF test in case of indeterminate results in NAFLD patients increased the identification of advanced fibrosis while reducing the number of inappropriate referrals.[76] Recently, the American Gastroenterological Association, in collaboration with members from other professional societies, proposed a clinical care pathway for NAFLD [Figure 1].[102] Compared with similar algorithms, this pathway adopted opinions from primary care physicians and recommends FIB-4 instead of abdominal ultrasonography as the initial assessment, as blood tests are often more accessible than imaging in primary care settings.

Looking ahead, when new drugs for NASH become available, there will be an urgent need to apply non-invasive tests to identify patients needing treatment and monitor treatment response. Data on the performance of non-invasive tests in the current phase 3 clinical trials will be pivotal in shaping clinical care in the years to come.

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Conflicts of interest

Grace Wong has served as an advisory committee member for Gilead Sciences and Janssen, as a speaker for Abbott, Abbvie, Ascletis, Bristol-Myers Squibb, Echosens, Furui, Gilead Sciences, Janssen and Roche, and received research grants from Gilead Sciences. Vincent Wong has served as an advisory board member or consultant for 3V-BIO, AbbVie, Allergan, Boehringer Ingelheim, Echosens, Gilead Sciences, Inventiva, Merck, Novartis, Novo Nordisk, Pfizer, ProSciento, Sagimet Biosciences, TARGET PharmaSolutions, and Terns; and a speaker for Abbott, AbbVie, Echosens, Gilead Sciences, and Novo Nordisk. He has received a research grant from Gilead Sciences to support fatty liver research. He is a co-founder of Illuminatio Medical Technology Limited. The other authors report no conflict of interest.

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