CLINICAL REVIEW

OPEN

Expert Panel Consensus on Clinical Assertion Statements Describing Noninvasive Tools for Diagnosing Nonalcoholic Steatohepatitis

Ira M. Jacobson, MD,* Vincent Wai-Sun Wong, MD,†‡
Laurent Castera, MD, PhD,§‖ Quentin M. Anstee, MBBS, PhD,¶#
Mazen Noureddin, MD, MHSc,** Kenneth Cusi, MD,††‡‡
Stephen A. Harrison, MD,§§ Elisabetta Bugianesi, MD, PhD,‖‖
and Zobair M. Younossi, MD, MPH¶¶##

Goals and Background: A panel of 9 experts in nonalcoholic steatohepatitis gathered to assess multiple components of the diagnostic process.

Materials and Methods: The Clinical Assertion Statements covered screening of patients with type 2 diabetes for high-risk nonalcoholic fatty liver disease, which—if any—noninvasive tests could determine whether to delay or defer biopsy, whether primary care providers and endocrinologists should routinely calculate Fibrosis-4 (FIB-4) scores in patients with nonalcoholic fatty liver disease or those at risk for it, optimal noninvasive tests to stage fibrosis, the need to consider fibrosis in patients with normal transaminase levels, periodic monitoring for progressive fibrosis, whether patients should undergo biopsy before pharmacotherapy, and the clinical utility of genetic testing.

Results and Conclusions: Evidence was presented to support or refute each Clinical Assertion Statement; the panel voted on the nature of the evidence, level of support, and level of agreement with each Statement. Panel level of agreement and rationale of each Clinical Assertion Statement are reported here.

Key Words: fatty liver disease, steatohepatitis, fibrosis, noninvasive test

During the past few decades, the prevalence of non-alcoholic fatty liver disease (NAFLD) has increased dramatically and globally.[1–4] It is important to recognize that NAFLD is an umbrella term that encompasses nonalcoholic steatohepatitis (NASH), as well as non-NASH NAFLD subtypes.[2,5] Current evidence suggests that fibrosis stage is the most important predictor of adverse clinical outcomes and is associated with patient-reported negative outcomes.[4,6,7] In fact, patients with NAFLD and stage > 2 fibrosis should be considered as having high-risk NAFLD.[4,6,7]

In the United States, NASH is the second leading indication for liver transplantation overall, and the most common indication among women and persons over 65 years of age.[8–10] In addition, NASH is among the top causes of hepatocellular carcinoma (HCC) and cirrhosis.[11,12] To predict potential outcomes and identify cases that may benefit from intervention, early diagnosis of high-risk NAFLD is paramount.

Currently, liver biopsy is considered the gold standard for identifying steatohepatitis and hepatic fibrosis stage.[13,14] However, liver biopsy is limited by its invasiveness, sampling issues, and interobserver variability.[13] To address these drawbacks and facilitate the early identification of high-risk NAFLD, a number of noninvasive tests (NITs) have been developed. In this context, most NITs for NASH have not been fully validated. In contrast, most of the efforts have focused on NITs for fibrosis, which fall into 3 categories: (1) clinical tools that combine routine laboratory tests and clinical parameters to calculate a risk score, such as the Fibrosis-4 index (FIB-4), NAFLD Fibrosis Score (NFS), and aspartate aminotransferase (AST) to platelet index (APRI); (2) imaging modalities that measure liver stiffness, the most frequently used being transient elastography (TE); and (3) blood-based biomarkers of hepatic fibrosis, such as the Enhanced Liver Fibrosis (ELF) Test.[7,15–20]

Patients' clinical profiles in combination with NITs may also be helpful. Notably, patients with type 2 diabetes (T2D) are at high risk for NAFLD-related disease progression.[21,22] Further, the risk of advanced fibrosis and mortality increases with the number of metabolic syndrome (MetS) components present.[23–25] Using NITs plus the patient's clinical profiles will not only help establish NAFLD-related prognosis but also guide potential treatment and management options.

This paper can be cited using the date of access and the unique DOI number which can be found in the footnotes.

TABLE 1. Grading Criteria for Clinical Assertion Statements

Nature of Evidence	Level of Support	Level of Acceptance
High: Meta-analysis, > 1 well-designed clinical trial, or systematic review	**A**: There is good evidence to support the statement	**A**: Accept recommendation completely
Moderate: ≥ 1 clinical trial or well-designed cohort or case-controlled study	**B**: There is fair evidence to support the statement	**B**: Accept recommendation with some reservations
Low: Case reports, case series, or flawed clinical trial	**C**: There is poor evidence to support the statement, but recommendations may be made on other grounds	**C**: Accept recommendation with major reservations
Very low: Expert opinion, no direct research evidence (such as only descriptive studies or reports of expert committees)	**D**: There is fair evidence to reject the statement	**D**: Reject recommendation with reservations
	E: There is good evidence to reject the statement	**E**: Reject recommendation completely

MATERIALS AND METHODS

In this review, evidence supporting the role of NITs and genetic testing in the diagnosis of NASH and advanced fibrosis was assessed. An international panel of NASH experts, including 8 hepatologists and 1 endocrinologist with many years of clinical experience and numerous publications on this topic, convened to summarize the evidence related to a series of 7 Clinical Assertion Statements developed by the forum chairs (I.M.J., Z.M.Y.). The statements were developed based on everyday diagnostic and management decisions that clinicians must make in their practices. Each expert was tasked with performing a literature search to identify evidence supporting their assigned Statement(s). Individual search methodology was included in each expert's presentation, detailing search terms used on PubMed, number of publications, and publication sorting method. After each statement-specific presentation, panel members deliberated the merits of the evidence, voted on the level of support provided by the peer-reviewed literature, and determined the level of acceptance (Table 1). The final review summarized the panel's consensus on the level of acceptance of each Clinical Assertion Statement.

RESULTS

Statement 1A: All Adults With T2D Should Be Screened for NAFLD

T2D is strongly associated with both the development and severity of NAFLD. In a systematic review and meta-analysis of 80 studies, the pooled prevalence of NAFLD among patients with T2D was 55.5%, with 37.3% and 17.0% having NASH and advanced fibrosis, respectively.[1] In a recent global review, prevalence rates for NAFLD in T2D were as high as 67% to 70% in studies from West Asia, Europe, and the United States.[3] T2D is also a major risk factor for decompensated cirrhosis and HCC in patients with NAFLD.[26]

Several studies have demonstrated the feasibility of nonspecialist screening for NAFLD and liver fibrosis in patients with T2D using serum tests or TE.[22,27,28] Data on periodic liver assessment in patients with T2D are scarce. In one study, however, 611 patients with T2D underwent paired TE examination separated by 3 years.[29] Incident NAFLD (controlled attenuation parameter increasing to ≥ 248 dB/m) and advanced liver disease (liver stiffness ≥ 10 kPa) developed in 52% and 4.3%, respectively.

Any screening program is meaningful only if early detection of the disease can translate into effective treatments and improved clinical outcomes. In this regard, screening for NAFLD with fibrosis—rather than NAFLD itself—would be most impactful on the management of patients with T2D beyond considerations of lifestyle interventions that apply across the spectrum of NAFLD. In fact, one can assume that the vast majority of patients with T2D have NAFLD, and screening for NAFLD will not be meaningful. In contrast, identifying NAFLD with fibrosis through screening can provide important prognostic information. Specifically, moderate to advanced fibrosis is generally considered to warrant consideration of pharmacological therapy targeting the liver disease itself (F2-F4), while advanced fibrosis (F4 and, on an individualized basis, F3) requires screening for HCC. In a cost-utility analysis, screening for fibrotic NAFLD using ultrasonography plus liver enzymes followed by TE and intensive lifestyle intervention in patients with T2D appears to be cost-effective in the United States, compared with no screening.[30]

While the panel agreed that there is substantial value in screening for advanced hepatic fibrosis in patients at risk of NAFLD, a recommendation to screen all patients with T2D for NAFLD was rejected. The rationale was that the majority of patients with T2D have NAFLD, and the comorbid diagnosis on its own does not currently affect treatment decisions. The nature of the evidence was deemed moderate. Half of the panel felt there was fair evidence to support the statement, whereas other members' positions ranged from poor evidence to support the statement to good evidence to reject the statement.

Statement 1B: Primary Care Providers (PCPs) and Endocrinologists Should Routinely Calculate FIB-4 in Patients With NAFLD and Those Who Are at Significant Risk for NAFLD (eg, Patients With T2D) and Refer Those With FIB-4 > 1.3 for Specialty Evaluation

FIB-4 is a validated NIT for liver fibrosis that takes into consideration age, platelet count, and AST and alanine aminotransferase (ALT) levels.[31] It can be calculated using online calculators or mobile apps. Despite FIB-4 being inferior to TE when assessing for advanced fibrosis, head-to-head cross-sectional comparisons demonstrated it to be more accurate than other generic serum-based fibrosis scores, such as APRI and NFS.[32,33] In a meta-analysis comparing different NITs, FIB-4 had an area under the

TABLE 2. The Wilson and Jungner Classic Screening Criteria and the Case for NAFLD Screening in Patients With T2D[40]

Factors	Criteria	Does NAFLD Meet the Criteria?
Disease	The condition sought should be an important health problem	**Yes.** NAFLD affects at least 50% of patients with T2D and is an important cause of cirrhosis and HCC
	There should be a recognized latent or early symptomatic stage	**Yes.** it takes years or decades before NASH progresses to cirrhosis, hepatic decompensation, and HCC
	The natural history of the condition, including development from latent to declared disease, should be adequately understood	**Maybe.** Although the natural history of NAFLD is well defined, the progression rate is highly variable, and some patients may have regression of disease. The prediction of disease progression is inaccurate
Setting	Facilities for diagnosis and treatment should be available	**Maybe.** Many hepatology clinics can provide adequate diagnosis and treatment for NAFLD, but the majority of patients are in primary care and nonspecialist settings. The clinical care pathway is ill-defined in most regions
Diagnosis	There should be a suitable test or examination	**Yes.** NITs for hepatic steatosis and advanced fibrosis are available and extensively evaluated
	The test should be acceptable to the population	**Yes.** NITs have been tested in various settings, and acceptability does not appear to be an issue
	Case finding should be a continuing process and not a "once-and-for-all" project	**Maybe.** Few studies have examined the serial use of NITs in a longitudinal manner
Treatment	There should be an accepted treatment for patients with recognized disease	**Yes.** Lifestyle modification is effective in improving NASH and liver fibrosis. Current guidelines support the use of vitamin E and/or pioglitazone in selected patients with NASH
	There should be an agreed policy on whom to treat	**Yes.** Regulators and expert panels agree that patients with NASH and advanced fibrosis should be prioritized for treatment
Cost-effectiveness	The cost of case finding should be economically balanced in relation to possible expenditure on medical care as a whole	**Maybe.** A few studies suggest that case finding is cost-effective, but this is region-specific and context-specific

HCC indicates hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NIT, noninvasive test; T2D, type 2 diabetes.

receiver operating characteristic curve (AUROC) of 0.84 for the diagnosis of advanced fibrosis (F3 and F4).[34]

In the general population, FIB-4 has > 95% negative predictive value (NPV) for excluding future development of cirrhosis, hepatic decompensation, HCC, and liver-related death.[35,36] When measured repeatedly, individuals whose FIB-4 is persistently <1.3 have the lowest risk of liver-related morbidity and mortality.[36] As for all diagnostic tests, the positive predictive value (PPV) of FIB-4 is dependent on the regional prevalence of advanced fibrosis. FIB-4 is more likely to yield false-positive results when applied to patients without fatty liver, those with normal ALT levels, and those above 65 years of age.[37,38] A lower cutoff of 2.0 improves the specificity of FIB-4 in patients above 65 years of age without affecting sensitivity.[38]

Only a few studies have evaluated the use of FIB-4 within a clinical care pathway. In 1 example, PCPs in 2 clinical commissioning groups in the United Kingdom performed initial FIB-4 testing for patients with NAFLD.[39] Patients with FIB-4 <1.3 (71% of 1452 patients) were considered low risk and continued to receive primary care management. Those with FIB-4 > 3.25 (3%) were referred to hepatologists. Patients with FIB-4 between 1.3 and 3.25 (26%) had an ELF panel performed for further characterization. Compared with standard-of-care data, this pathway reduced unnecessary referrals by 81% and increased the detection of advanced fibrosis and cirrhosis by 5- and 3-fold, respectively.

Overall, the panel accepted the statement that PCPs and endocrinologists should routinely calculate FIB-4 in patients with or at risk for NAFLD and refer those with FIB-4 > 1.3 for further evaluation. Some members of the panel, however, voted to accept the statement with reservations. There was fair to good evidence to support the statement, and the panel felt the nature of the evidence was at least moderate. The general acceptance of Statement 1B by the panel is consistent with the emphasis in the discussion of Statement 1A on not screening for NAFLD alone but, rather, on screening for fibrotic NAFLD as an initial step. Here, the panel takes the issue of screening for fibrosis a step further by recommending the widespread use of FIB-4. In light of the absence of any cost, immediate accessibility, and acceptable accuracy, FIB-4 can be readily applied in primary care and other nonspecialist settings to identify patients with or at risk for advanced fibrosis. The emphasis on advocating FIB-4 for widespread adoption in this and the prior statement is complementary to the appropriate use of imaging to identify NAFLD or other hepatobiliary diseases and the use of serologic tests to evaluate patients with elevated liver enzymes. The subsequent decision to refer or arrange additional testing should depend on the specific healthcare setting and the availability of different tests and specialty resources (Table 2).

Statement 2: In Patients With NAFLD and TE Results Predicting F3-F4 Fibrosis, an Alternative Noninvasive Approach Predicting F3-F4 Fibrosis Justifies Deferral of Liver Biopsy

Because the fibrosis stage is the main predictor of long-term outcomes, identifying NAFLD patients with advanced fibrosis is critical as they are at the highest risk of complications and death.[6,41,42] Given the extremely high number of
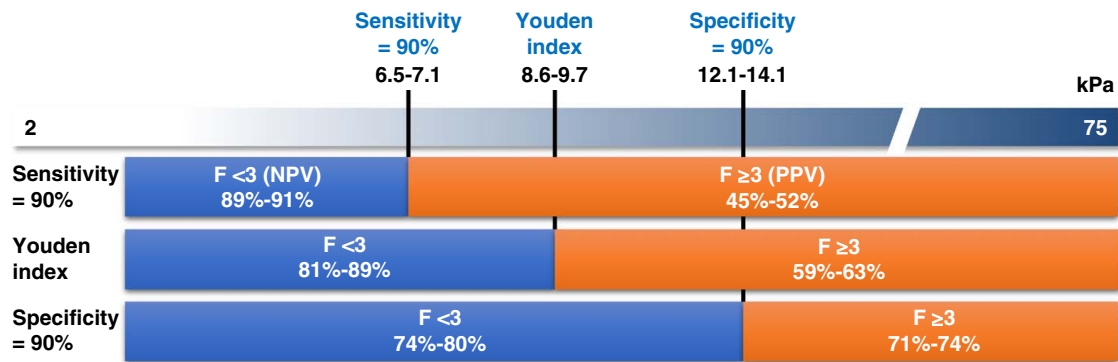
FIGURE 1. The negative predictive value (NPV) is better than the positive predictive value (PPV) for transient elastography (TE).[44,45]

at-risk patients, liver biopsy in all patients with NAFLD is unsuitable. NITs are now widely used to risk-stratify NAFLD patients—namely, ruling out/ruling in advanced fibrosis/cirrhosis.[20,43] TE (eg, FibroScan) is the most widely available imaging-based NIT with the largest amount of data on NAFLD.[43] In a recent meta-analysis, TE had good diagnostic accuracy for advanced fibrosis (AUROC: 0.88 with medium probe and 0.85 with extra-large probe) and for cirrhosis (AUROC: 0.92 with medium probe and 0.94 with extra-large probe).[34] In a recent (individual) patient data meta-analysis (N = 230), TE was outperformed by magnetic resonance elastography (MRE) for detecting advanced fibrosis (AUROC 0.84 vs. 0.93, respectively, $P = 0.001$).[18] However, given its cost and limited availability, MRE is not suitable for routine use in clinical practice.

TE is better at ruling out than ruling in advanced fibrosis. For instance, at a cutoff value of 8 kPa, TE has NPV/ sensitivities > 90% but suboptimal PPV/specificities (< 70%), resulting in false-positive results in 30% to 40% of cases (Fig. 1).[44,45] The high NPV of TE is particularly important in facilitating the ability of PCPs to identify patients not requiring specialty referral.

A dual cutoff strategy with a lower cutoff maximizing sensitivity and a higher cutoff maximizing specificity has been refined recently for NAFLD.[46] In a large, individual-patient meta-analysis based on real-world data in 5648 patients with chronic liver disease, an 8 kPa cutoff had 93% sensitivity to rule out advanced fibrosis, while a 12 kPa cutoff had 88% specificity for ruling in advanced fibrosis in patients with NAFLD (n = 1073).[47] However, this was at the expense of a significant number of unclassified patients (25% to 30%) falling between the 2 cutoffs in whom a liver biopsy would be necessary.[47] In actual practice, a commonly used inflection point for considering liver biopsy by the panelists is 8.0 to 8.5 kPa, but decisions should be individualized based on such factors as diagnostic uncertainty (eg, the need to exclude alternative liver diseases) or consideration of pharmacological treatment, including potential enrollment in clinical trials.

Strategies combining TE with additional NITs have been proposed to decrease the number of unclassified patients and the need for liver biopsy.[48,49] Petta et al[49] demonstrated in a study of 741 patients with biopsy-proven NAFLD that TE paired with NFS or FIB-4 strongly reduced the likelihood of wrongly classified patients (to as low as 2.7% to 2.6%), but at the price of a high uncertainty area (ranging from 54.1% to 58.2%) and a low overall accuracy (ranging from 43% to 39.1%).

Overall, the panel disagreed with the recommendation to defer liver biopsy in patients with TE results predicting advanced fibrosis (either by rejecting the statement outright or accepting it with reservations), because of the suboptimal PPV with TE. The majority felt the nature of the evidence was moderate and that there was fair evidence to support the statement, or that there was poor evidence, but recommendations could be made on other grounds. Providing a specific cutoff value for TE may improve the precision of the statement, but TE results alone are not sufficient to defer liver biopsy. If a widely available and low-cost NIT, or combination of tests, were to attain a higher positive predictive value than TE, this question could be reconsidered. In addition, the finding in a patient with a TE score of F3-F4 accompanied by overt evidence of cirrhosis, such as cirrhotic morphology on imaging accompanied by signs of portal hypertension by laboratory criteria or imaging, could obviate the need for biopsy. The panel suggested replacing "alternative NITs" with "additional NITs" to improve this diagnostic approach.

Statement 3: The ELF Test Could Provide an Alternative to TE to Estimate Fibrosis Stage in NAFLD

In general, NITs do not accurately discriminate between stepwise fibrosis stages. Most aim to differentiate between mild (F0-F2) versus advanced (F3-F4) fibrosis/cirrhosis. Because the fibrosis stage is predictive of long-term outcomes, NITs that detect advanced fibrosis/cirrhosis (including FIB-4, ELF, and TE) provide both diagnostic and prognostic information.[15,50–52] Blood-based biomarkers of fibrosis may be indirect calculations (eg, FIB-4, NFS) or directly detected circulating by-products of collagen turnover (eg, ELF test).

The ELF test comprises 3 markers of collagen turnover: *procollagen III N-terminal peptide*, *hyaluronic acid*, and *tissue inhibitor of metalloproteinase 1*. In a cohort of 192 NAFLD patients, ELF achieved an AUROC of 0.93 (0.88 to 0.98) compared with 0.89 (0.81 to 0.97) for NFS in the detection of advanced fibrosis (F3-F4).[53] However, ELF exhibited higher NPV than PPV. The most comprehensive analysis of ELF performance is a large-scale meta-analysis that included > 4500 NAFLD cases.[16] This study assessed ELF's sensitivity/specificity and performance characteristics across a range of disease-prevalence scenarios using several previously published ELF thresholds (Table 3). Lower cutoffs exhibited high sensitivity but limited specificity. In contrast, the diagnostic performance of the ELF test at

TABLE 3. Meta-analysis Assessing Performance of ELF Across a Range of Disease Prevalence Scenarios[16]

Cutoff	Sensitivity	Specificity	Prevalence	PPV	NPV
7.70	0.93	0.34	0.05	0.07	**0.99**
			0.10	0.14	**0.98**
			0.20	0.26	**0.95**
			0.30	0.38	**0.92**
			0.40	0.49	**0.88**
			0.50	0.59	**0.83**
9.80	0.65	0.86	0.05	0.20	**0.98**
			0.10	0.34	**0.96**
			0.20	0.54	**0.91**
			0.30	0.66	**0.85**
			0.40	0.75	0.79
			0.50	**0.82**	0.71
10.51	0.51	0.93	0.05	0.26	**0.97**
			0.10	0.43	**0.94**
			0.20	0.63	**0.88**
			0.30	0.75	**0.81**
			0.40	**0.82**	0.74
			0.50	**0.87**	0.65
11.30	0.36	0.96	0.05	0.34	**0.97**
			0.10	0.52	**0.93**
			0.20	0.71	**0.86**
			0.30	**0.81**	0.78
			0.40	**0.87**	0.69
			0.50	**0.91**	0.60

Bold numbers represent predictive values > 0.80.
ELF indicates Enhanced Liver Fibrosis Test; NPV, negative predictive value; PPV, positive predictive value.

higher thresholds was found to be limited in low-prevalence settings. Thus, clinicians should carefully consider the pretest probability in their practice setting, as this will substantially affect the test's utility.[16]

Adopting an "intention to diagnose" approach in the clinical practice setting, the performance of TE and ELF were considered broadly comparable despite ELF exhibiting lower sensitivity but higher specificity.[54] In 186 NAFLD patients, a TE threshold of 9.7 kPa had a sensitivity/specificity of 91%/65%, whereas an ELF threshold of 9.8 had a sensitivity/specificity of 72%/90% for detecting F3-F4 fibrosis.[54] Available evidence suggests that ELF performance is improved if used as a second-line test within a sequential testing strategy to increase pretest probability.[39,48] A direct comparison of FIB-4+TE versus FIB-4+ ELF in the STELLAR trial demonstrated a sensitivity/specificity of 77%/89% versus 69%/92%, respectively for advanced fibrosis, again with ELF exhibiting lower sensitivity but marginally higher specificity.[48] Real-world data from a sequential FIB-4+ELF care pathway demonstrated reduced referral of mild disease.[39]

Overall, the panel's consensus was to accept the statement that ELF could be an alternative to TE, although with clear reservations, as the performances of these tests are similar but not entirely equivalent and real-world head-to-head data were lacking. The panel felt the nature of the evidence was moderate to high, with most feeling the level of support in the peer-reviewed literature was fair to poor, but recommendations could be made on other grounds. A number of other novel diagnostics were considered potentially viable alternatives to TE given sufficient evidence and regulatory approval [eg, NIS4[55] (a panel testing for miR-34a-5p, YKL-40, alpha2-macroglobulin, and hemoglobin A1c combined] or panels incorporating N-terminal propeptide of type III collagen (PRO-C3)[56,57]]. Other important reservations focused on the impact of pretest probability on ELF performance—meaning that ELF's performance, if used as a single test to positively identify cases with advanced fibrosis in a low-prevalence primary care setting—would not exceed 50% and was likely to be <25% (Table 3). While this may be the case for most biomarkers, the panel questioned whether the marginal performance benefits beyond such inexpensive tests such as FIB-4 were sufficient to advocate for wide adoption of ELF and whether the test was as convenient as a point-of-care test (eg, TE) that can give an immediate result. The general consensus was that ELF may have a place as a second-line test following use of FIB-4, but further data are required.

Statement 4: NASH Should Be Confirmed by Liver Biopsy Before Initiating Pharmacological Therapy in NAFLD

In response to the difficulties in using liver biopsy to confirm NASH, new data have shifted the focus toward NASH patients with stage 2 and higher fibrosis. These are stages that correlate with disease morbidity and mortality and potential candidacy for targeted therapies.[42,58,59] The 2018 AASLD Guidance recommended that pharmacological therapy should be predicated upon the finding of F2 fibrosis or greater on liver biopsy.[13]

Recent research has focused on identifying patients with NASH+NAFLD Activity Score (NAS) ≥ 4+ ≥ F2, primarily to replace biopsy, which is currently required for clinical trials.[60] One promising NIT is the FibroScan-AST (FAST) score, which combines the controlled attenuation parameter score and stiffness assessed by TE with AST.[61] In the derivative/validation cohorts study, the test performed well, with AUROC of ~0.80 and 2 author-determined cutoffs: the first (≤ 0.35) to rule out NASH+NAS ≥ 4+ ≥ F2 and the other (≥ 0.67) to rule in NASH+NAS ≥ 4+ ≥ F2.[62] Greater than 30% of patients fell into the indeterminate zone.[62] Nevertheless, the test is a promising alternative to liver biopsy for determining which NASH patients should be started on therapy.

Similarly, use of NIS4 was tested in a phase 2 NASH clinical trial cohort and validated in external cohorts.[55] NIS4 achieved an AUROC of ~0.80 and, similarly, had 2 cutoffs to rule in or rule out NASH+NAS ≥ 4+ ≥ F2. In addition, some patients' NIS4 scores fell in the indeterminate zone (~30%).

It is plausible that one of these tests could be used to identify patients who are candidates for therapy, or that tests such as TE or ELF could be used, especially in a combination or sequential approach.[48] The panel felt that it is reasonable to initiate NASH therapies based on these approaches, especially in individuals at high risk of NASH-associated fibrosis. It should be noted, however, that many biomarkers have shown to correlate with histologic improvement (eg, ALT decline), steatosis/steatohepatitis [eg, magnetic resonance imaging (MRI)-estimated proton-density fat fraction changes], and fibrosis (eg, 25% reduction of stiffness on TE). Further, multiparametric (cT1) MRI is useful to assess steatohepatitis changes.[63] Given the
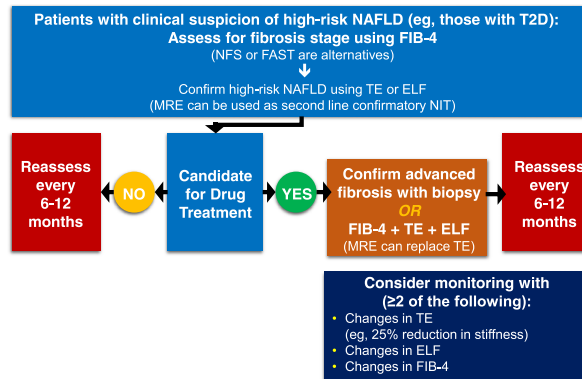
FIGURE 2. An algorithm for fibrosis assessment and monitoring based on discussion of the Clinical Assertion Statements. ELF indicates Enhanced Liver Fibrosis Test; FAST, FibroScan-AST; FIB-4, Fibrosis-4; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NIT, noninvasive test; NFS, NAFLD Fibrosis Score; T2D, type 2 diabetes TE, transient elastography.

correlation of histology with clinical liver events, there is growing evidence that NITs can also predict clinical liver events, thus strengthening the rationale for use of NITs to determine the initiation of NASH therapies.[50,64–66]

Overall, panel members voted to reject the statement that NASH should be confirmed via liver biopsy before initiating pharmacological therapy in NAFLD, but some thought the statement could be accepted with major reservations. The panel felt that the level of support in the peer-reviewed literature was poor, but there may be other grounds for making a recommendation. The panel agreed that the nature of evidence for the statement was moderate. The panel took into account the present armamentarium of off-label agents being used for NASH and felt that biopsy is not necessary in every case. The panel also recognized that targeted agents currently in clinical trials may require liver biopsy following regulatory approval, given that their ongoing clinical trials are based on histologic inclusion criteria and endpoints. There was consensus that use of NITs to determine therapy initiation was reasonable in high-risk patients, such as those with T2D, especially if a "best practice approach" is used (Fig. 2).

Statement 5: In Patients With NAFLD and Metabolic Risks, Presence of Hepatic Fibrosis Should Be Considered Regardless of Level of Transaminase Elevation

This statement targets PCPs, who often do not consider the risk of hepatic fibrosis in patients with NAFLD and metabolic risk factors, particularly in those with normal transaminases.[67] "Consideration of hepatic fibrosis" may include diagnostic efforts and interventions or eventual referral to a specialist. The statement may challenge deep-rooted PCP-referral patterns that rely heavily on transaminase elevation. Emerging literature suggests that an approach based on transaminase elevation likely will miss many patients with NASH and clinically significant fibrosis (≥F2)—stages that correlate with adverse liver outcomes.[42]

While organizations' guidelines agree that patients with NAFLD, cardiometabolic risk factors, and elevated transaminases should be evaluated for hepatic fibrosis, disagreement exists on how to proceed if transaminases are "normal" in the presence of the other 2 variables. Various guidelines recommend for[68,69] and against[13,70] routine

screening for NAFLD in high-risk groups. The American Association for the Study of Liver Diseases (AASLD) recommends a "high index of suspicion for NAFLD and NASH in patients with T2D," while other guidelines recommend that clinicians "consider" screening high-risk individuals (age above 50 y, T2D, or MetS).[68,71] The American Diabetes Association suggests that patients with prediabetes or T2D and elevated ALT or NAFLD per ultrasound "should be evaluated" for steatohepatitis and liver fibrosis.[72]

New evidence may have the potential to unify guidelines on screening patients with NAFLD and cardiometabolic risks for clinically significant fibrosis (≥F2), regardless of transaminase levels. In recent studies, advanced fibrosis in high-risk populations has ranged from 3% to 10% using FIB-4 or NFS[28,73,74] and from 6% to 18% by liver stiffness measure using TE[27,75,76] or MRE.[77] Mean ALT and AST levels in those with advanced fibrosis were between 20 and 30 IU/L, with only a minority of individuals having elevated transaminases (≤20%). As most earlier studies were performed in Southeast Asia,[27,73,75,76] it has been unclear until recently whether the absence of transaminase elevation represented racial/ethnic differences or less-severe metabolic disease compared with Western populations. However, similar results have emerged in recent studies from Europe[78,79] and the United States.[22,74,80,81] Portillo-Sanchez et al[80] reported hepatic fibrosis prevalence of 56% among 103 patients with T2D who underwent liver biopsy (mean ALT and AST levels were 28 and 23 IU/L, respectively). In another recent study that screened for advanced fibrosis using TE in 825 adults with T2D, the mean plasma aminotransferases were not elevated in those with either F3 (ALT 29 IU/L, AST 26 IU/L) or F4 (ALT 35 IU/L, AST 33 IU/L).[81] Finally, Lomonaco et al[22] employed TE to screen 561 patients with T2D while attending their routine general medicine, family medicine, or endocrinology clinic appointments. About 1 in 6 patients had clinically significant fibrosis (≥F2), and 9% had F3 to F4 advanced fibrosis, but only 28% of those with fibrosis had elevated plasma AST or ALT. A confirmatory liver biopsy was done in about one third of the patients.

Clinically, these studies imply that if about 10% of adults with T2D have ≥F3 fibrosis, universal screening of the estimated 30 million adults with T2D in the United States would identify about 3 million cases of advanced

fibrosis or cirrhosis, many of whom are without elevated aminotransferases. Screening of adults with T2D would also be supported by a recent study that suggests screening followed by 1 year of lifestyle modification or pioglitazone treatment was cost-effective in preventing future end-stage liver disease.[30] There is greater uncertainty for patients with NAFLD and obesity without T2D, where the prevalence of hepatic steatosis and fibrosis is lower.[1,26,42,82–84] Recently, Barb et al[74] examined 3841 adults from the National Health and Nutrition Examination Survey (NHANES) 2015-2016 database and concluded that steatosis was primarily driven by obesity, while diabetes doubled the risk of advanced fibrosis. Screening by ALT and AST levels (ie, rather than by FIB-4) would have failed to identify most of those at risk of clinically significant fibrosis.

Overall, there was a split in the panel's level of acceptance of the statement that in patients with NAFLD and metabolic risks the presence of hepatic fibrosis should be considered regardless of level of transaminase elevation. Some accepted the recommendation completely or with some reservations, while some rejected it with reservations. Two third of the panel members considered the nature of evidence to support the statement of moderate quality.

Considerations that weighed in the discussion were: (1) a need to refine the best screening approach; (2) adoption of evidence-based lower transaminase cutoffs to improve case finding and minimize workload and costs; and (3) establishment of a cost-effective treatment strategy. Overall, there was a strong(er) rationale to screen for hepatic fibrosis regardless of transaminase levels in patients with T2D and NAFLD than in patients with obesity in the absence of diabetes.

Statement 6: All Patients With NAFLD Should Undergo NIT Every 2 Years to Assess for Progressive Fibrosis

Disease progression is heterogenous, nonlinear, and dependent on disease activity and fibrosis severity.[6,85] Using a Markov model to forecast the incidence of NAFLD progression between 2015 and 2030, it has been suggested that the incidence of decompensated cirrhosis and liver deaths will increase by 168% and 178%, respectively, over this 15-year period.[86] Thus, it is reasonable to determine a testing strategy to identify those patients at greatest risk of disease progression.

A meta-analysis of patients with NAFLD (nonalcoholic fatty liver or NASH) demonstrated that the timeframe for a 1-stage progression in fibrosis was roughly 14.3 years. This was compared with 7.1 years for those with biopsy-confirmed NASH.[87] In both analyses, ~20% of patients were found to be rapid progressors as defined by fibrosis progression from stage 0 to stage 3 or 4 over the period of the study. The most important clinical predictors of progression were hypertension and the AST/ALT ratio. Other studies have shown that T2D is an independent predictor of advanced liver disease, and fibrosis progression may occur in as short as 18 months.[88,89] Among a large German NAFLD population, cardiovascular disease, T2D, hypertension, obesity, and renal impairment were all independently predictive of mortality.[90]

Fibrosis progression over time can be assessed noninvasively. Data from a retrospective analysis of 292 patients with paired liver biopsies demonstrated that APRI, FIB-4, and NFS were significantly associated with changes in collagen deposition.[91] More recently, data from > 2000 patients with biopsy-proven stage 3 or 4 NASH showed that

increases in NIT values (eg, FIB-4, ELF, NFS) are associated with increased risks of both histologic and clinical disease progression, with some of these increases in NIT values occurring within 12 months.[52] Elastography (TE, MRE) and MRI-based imaging modalities (including multiparametric MRI) also are predictive of long-term patient outcomes.[64,92] In the case of multiparametric MRI, changes may be seen as early as 20 months.[92]

Sequential testing combining a serologic test with an imaging test is likely to minimize indeterminate cases and improve diagnostic accuracy. A recent study at 2 primary care clinics in Northeast England evaluated sequential testing with FIB-4 followed by TE in > 400 patients (more than 35 y of age) with T2D.[93] This 2-tier NIT strategy led to a nearly 7-fold increase in detection of advanced fibrosis. Additional support comes from an individual-patient data meta-analysis of 37 studies comprising > 5700 patients. Sequential testing using FIB-4 and TE improved sensitivity and specificity to rule in or rule out advanced fibrosis.[94]

Overall, the panel accepted the statement that patients with NAFLD should undergo NITs for progressive fibrosis every 1 to 2 years. The majority accepted the statement with some reservations, though some panel members rejected the recommendation with reservations. The nature of evidence to support the assertion was moderate and the level of support in the peer-reviewed literature was graded as either fair or poor evidence, but recommendations could be made on other grounds. There was concern that screening all patients with NAFLD every 1 to 2 years using NITs may not be helpful, as most patients would not progress within this time frame. However, there was general agreement that noninvasive monitoring is helpful and should be longitudinally documented. Patients with T2D or MetS, including hypertension or an elevated AST/ALT ratio, may benefit from more-frequent screening using a sequential testing strategy, which appears to be cost-effective.[30]

Statement 7: Genetic Testing for *PNPLA3* and Other Genetic Markers Could Be Useful in the Management of NAFLD or NASH

A large fraction of hepatic fat variability is determined by inherited factors.[95] Genetic variants, such as *PNPLA3* I148M, *TM6SF2* E167K, *MBOAT7* rs641738, *GCKR* P446L, *HSD17B13* rs72613567, and other rare variants, have been strongly linked to risk for liver disease severity in NAFLD.[95,96] Overall, *PNPLA3* and *TM6SF2* variants show the highest genome-wide significance for increased risk of advanced liver damage, HCC, and (for *PNPLA3* only) mortality, independent of other risk factors including fibrosis.[95] Conversely, *HSD17B13* loss-of-function variants have been linked to robust protection against liver inflammation, cirrhosis, and HCC.[95]

In a seminal study, Liu et al[97] validated a strong genetic association between the common *PNPLA3* variant p.I148M and the risk of developing HCC. The study demonstrated a 5-fold increased risk of HCC in NAFLD [GG vs. CC genotype: odds ratio (OR), 5.05; 95% CI: 1.47-17.29; $P = 0.01$] and up to 12-fold increased risk of HCC in the UK general population (GG vs. CC genotype: OR, 12.19; 95% CI: 6.89-21.58; $P < 0.0001$). The variant had a high specificity for NAFLD-related HCC at the population level, but it did not allow the identification of a specific at-risk population.[97] In a re-analysis of the data, the population-attributable risk of *PNPLA3* rs738409 for HCC was 55%, with an AUROC of 0.68 attributable to this variant.[98] The

NPV was substantially greater than the PPV, suggesting that the *PNPLA3* rs738409 genotype could be useful to select individuals who are least likely to develop HCC.

Overall, no single genetic variant provides adequate risk stratification in NAFLD. The contribution of *PNPLA3* I148M alone to NAFLD heritability may explain as much as 5% to 10% of the total variation in liver fat. Although remarkable, this proportion is modest for a relevant clinical predictor.[99] However, combining numerous variants in polygenic risk scores (PRSs; calculated as a weighted sum of disease-risk alleles carried by an individual) is an attractive approach, but there are currently very few data on their clinical usefulness.[95]

Variants in *PNPLA3*, *TM6SF2*, *GCKR*, and *MBOAT7* were recently combined in a PRS-hepatic fat content (HFC), then adjusted for *HSD17B13* (PRS-5).[100] In a NAFLD cohort, PRSs were associated with a ~12-fold increased OR for severe fibrosis ($P < 10^{-27}$ for both PRS-HFC and PRS-5) and a ~9-fold increased OR for HCC (OR, 9.2; 95% CI: 5.2-16.3; $P = 2.7 \times 10^{-14}$ for PRS-HFC; and OR, 9.1; 95% CI: 5.2-16.0; $P = 1.6 \times 10^{-14}$ for PRS-5). A PRS-HFC value ≥ 0.532 yielded 43% sensitivity and 80% specificity in a NAFLD cohort, compared with 27% and 90%, respectively, in the general population. In another study, a comprehensive polygenic genetic risk score including 181 rare pathogenic variants was superior to evaluating *PNPLA3* I148M and *TM6SF2* E167K alone, but adding genetic risk score to a score based on clinical risk factors did not improve the clinical score's performance.[101]

Overall, the panel's consensus was to either reject the statement completely or reject with reservations. Most of the panel members rated the nature of evidence as moderate, whereas some deemed it low or very low. Similarly, the majority of the panel deemed the level of support as fair evidence to reject, though a few felt there was poor evidence to support the statement or good evidence to reject the statement. Current evidence suggests that *PNPLA3* I148M variants are risk factors for the full spectrum of liver damage in NAFLD, but the prognostic value of genotyping is not sufficient for prediction at an individual level, particularly in view of the very low PPV. In complex traits in diseases like NAFLD, ORs for the effect of these traits on disease progression and severity are relatively modest, whereas ORs of about 50 are required to ensure that diagnostic tests do not suffer from too many false positives. The panel generally agreed that studies of larger cohorts with similar levels of baseline fibrosis and longer duration of longitudinal follow-up are needed to assess genetic risk systematically.

DISCUSSION

Deliberations about the nature of the published evidence, the strength of the evidence supporting the statements, and the degree of agreement or disagreement by the international panel of NAFLD experts underscored a lack of sufficient evidence in the peer-reviewed literature to achieve a clear consensus on several important issues regarding the use of NITs. The process used to develop this document anticipated the possibility of discordance between the level of evidence for a statement and the recommendation to accept or reject it. For example, for Statement 1A, the majority of the panel felt there was fair published evidence to support the recommendation to screen all patients with T2D for NAFLD, but as a clinical recommendation, it was rejected with some or no

reservations by all the panelists. As the majority of patients with T2D can be presumed to have NAFLD, the panelists felt that greater value should be ascribed to PCPs and endocrinologists in determining whether such patients have evidence of fibrosis. Highlighting FIB-4 as the recommended NIT for this purpose reflects its simplicity, low-cost, immediate availability, and competitive performance with other NITs in comparative studies.

The panel's disagreement with Statement 2, which asserts that a TE score predicting F3-F4 fibrosis could obviate the need for liver biopsy, reflects the evidence that the PPV of TE for ruling in advanced fibrosis or cirrhosis is exceeded by its NPV for ruling out these histologic stages. The panel agreed, however, that additional NITs, whether blood-based biomarkers or MRE, may be sufficient. In some instances with compelling evidence of cirrhosis and portal hypertension on routine laboratory assessment or imaging, a correspondingly high TE score might also be sufficient.

The utility of the ELF test as a potential alternative to TE, when combined with FIB-4, was accepted in Statement 3, albeit with reservations. This recommendation was based upon the lack of availability of TE in many practice settings, the demonstration of predictive value for clinical outcomes [the basis on which it was approved by the Food and Drug Administration (FDA) in August 2021], and studies in which ELF combined with FIB-4 has improved PPV and NPV for fibrosis and reduced the number of indeterminate results.

In contrast to the recommendation in the 2018 AASLD Guidance Document on NAFLD,[13] the panelists rejected Statement 4 that a liver biopsy demonstrating steatohepatitis and fibrosis is necessary before initiation of pharmacological therapy. The panelists emphasized fibrosis rather than steatohepatitis as the major prognostic determinant. Also cited were 2 newer NITs (the FAST score and NIS4) that can potentially predict NASH+NAS $\geq 4+$ and $\geq F2$, which are the criteria used to determine eligibility for many trials investigating new drugs for NASH.[61]

The majority of the panelists accepted the recommendation in Statement 5. The presence of hepatic fibrosis should be considered regardless of transaminase levels in patients with NAFLD and metabolic risks. However, some members of the panel rejected the statement, and there was general agreement that there is poor to fair evidence in the literature supporting the statement as a cost-effective clinical approach. All panelists agreed, however, that normal transaminases do not preclude the possibility of fibrosis, especially in patients with T2D.

The recommendation in Statement 6 that all patients with NAFLD should undergo NITs for fibrosis every 1 to 2 years was generally accepted. This may identify patients in whom pharmacological therapy would be warranted or, in more advanced cases, patients who need HCC screening. The panelists emphasized, however, that other screening intervals may be appropriate, depending upon the clinical variables, including metabolic factors, laboratory or imaging results, and prior NIT results.

The panel rejected Statement 7, which asserts that genetic testing for *PNPLA3* and other genetic markers would be useful at present in the clinical management of NAFLD. Despite the association between genetic variants at the *PNPLA3*, *TM6SF2*, and other loci and the development or progression of NAFLD, an evidence base for incorporating genetic profiling into treatment pathways is

currently lacking. The panelists advocated for further research aimed at subphenotyping patients with NASH and correlating genetic variants with response to novel therapies.

In conclusion, NITs for hepatic fibrosis play a critical role in the evaluation of patients with NAFLD. In addition to liver specialists, PCPs, endocrinologists and other clinicians should be familiar with the use of blood-based NITs to assess for hepatic fibrosis in patients with NAFLD. In general, a diagnosis of advanced fibrosis or cirrhosis should not be based upon a single NIT in the absence of compelling standard laboratory and radiologic data indicating cirrhosis. The added utility of combining blood-based NITs and elastography to enhance fibrosis assessment should be better understood by all clinicians, reserving liver biopsy primarily for fibrosis assessment when NITs yield equivocal results or there is discordance between NITs. The panel's consensus was that histologic evidence of steatohepatitis is not a prerequisite for initiation of pharmacological management in patients with strong NIT-based evidence of moderate or advanced fibrosis. Further, the panelists agreed that periodic elastography-based monitoring at individualized intervals should be employed to assess for progression of fibrosis. Assessment of genetic variability in *PNPLA3* and other loci does not play a standard role in current practice, but it is an important priority for future research.

ACKNOWLEDGMENTS

REFERENCES

1. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. *J Hepatol.* 2019;71:793–801.
2. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease: meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* 2016;64:73–84.
3. Stefan N, Cusi K. A global view of the interplay between nonalcoholic fatty liver disease and diabetes. *Lancet Diabetes Endocrinol.* 2022;10:284–296.
4. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* 2018;15:11–20.
5. Fazel Y, Koenig AB, Sayiner M, et al. Epidemiology and natural history of non-alcoholic fatty liver disease. *Metabolism.* 2016;65:1017–1025.
6. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology.* 2020;158:1611.e12–1625.e12.
7. Younossi ZM, Loomba R, Anstee QM, et al. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. *Hepatology.* 2018;68: 349–360.
8. Noureddin M, Vipani A, Bresee C, et al. NASH leading cause of liver transplant in women: updated analysis of indications for liver transplant and ethnic and gender variances. *Am J Gastroenterol.* 2018;113:1649–1659.
9. Younossi ZM, Stepanova M, Ong J, et al. Nonalcoholic steatohepatitis is the most rapidly increasing indication for liver transplantation in the United States. *Clin Gastroenterol Hepatol.* 2021;19:580.e5–589.e5.
10. Younossi ZM, Marchesini G, Pinto-Cortez H, et al. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: implications for liver transplantation. *Transplantation.* 2019;103:22–27.
11. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology.* 2015;62:1723–1730.

This paper can be cited using the date of access and the unique DOI number which can be found in the footnotes.

12. Younossi Z, Stepanova M, Ong JP, et al. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol.* 2019;17:748.e3–755.e3.

13. Chalasani N, Younossi Z, Lavine JE, 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.

14. Brunt EM, Kleiner DE, Carpenter DH, et al. NAFLD: reporting histologic findings in clinical practice. *Hepatology.* 2021;73:2028–2038.

15. Day J, Patel P, Parkes J, et al. Derivation and performance of standardized Enhanced Liver Fibrosis (ELF) test thresholds for the detection and prognosis of liver fibrosis. *J Appl Lab Med.* 2019;3:815–826.

16. Vali Y, Lee J, Boursier J, et al. Enhanced Liver Fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: a systematic review and meta-analysis. *J Hepatol.* 2020;73:252–262.

17. Thiele M, Madsen BS, Hansen JF, et al. Accuracy of the Enhanced Liver Fibrosis test vs FibroTest, elastography, and indirect markers in detection of advanced fibrosis in patients with alcoholic liver disease. *Gastroenterology.* 2018;154:1369–1379.

18. Hsu C, Caussy C, Imajo 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.e8–637.e8.

19. Younossi ZM, Noureddin M, Bernstein D, et al. Role of noninvasive tests in clinical gastroenterology practices to identify patients with nonalcoholic steatohepatitis at high risk of adverse outcomes: expert panel recommendations. *Am J Gastroenterol.* 2021;116:254–262.

20. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. *J Hepatol.* 2021;75:659–689.

21. Younossi ZM, Tampi RP, Racila A, et al. Economic and clinical burden of nonalcoholic steatohepatitis in patients with type 2 diabetes in the U.S. *Diabetes Care.* 2020;43:283–289.

22. Lomonaco R, Godinez Leiva E, Bril F, et al. Advanced liver fibrosis is common in patients with type 2 diabetes followed in the outpatient setting: the need for systematic screening. *Diabetes Care.* 2021;44:399–406.

23. Golabi P, Paik JM, Arshad T, et al. Mortality of NAFLD according to the body composition and presence of metabolic abnormalities. *Hepatol Commun.* 2020;4:1136–1148.

24. Paik JM, Deshpande R, Golabi P, et al. The impact of modifiable risk factors on the long-term outcomes of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2020;51:291–304.

25. Golabi P, Otgonsuren M, de Avila L, et al. Components of metabolic syndrome increase the risk of mortality in non-alcoholic fatty liver disease (NAFLD). *Medicine (Baltimore).* 2018;97:e0214.

26. Kanwal F, Kramer JR, Li L, et al. Effect of metabolic traits on the risk of cirrhosis and hepatocellular cancer in non-alcoholic fatty liver disease. *Hepatology.* 2020;71:808–819.

27. Kwok R, Choi KC, Wong GL, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut.* 2016;65:1359–1368.

28. Morling JR, Fallowfield JA, Guha IN, et al. Using non-invasive biomarkers to identify hepatic fibrosis in people with type 2 diabetes mellitus: the Edinburgh type 2 diabetes study. *J Hepatol.* 2014;60:384–391.

29. Lee HW, Wong GL, Kwok R, et al. Serial transient elastography examinations to monitor patients with type 2 diabetes: a prospective cohort study. *Hepatology.* 2020;72:1230–1241.

30. Noureddin M, Jones C, Alkhouri N, et al. Screening for nonalcoholic fatty liver disease in persons with type 2 diabetes in the United States is cost-effective: a comprehensive cost-utility analysis. *Gastroenterology.* 2020;159:1985.e4–1987.e4.

31. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology.* 2006;43:1317–1325.

32. Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology.* 2010;51:454–462.

33. Boursier J, Guillaume M, Leroy V, et al. New sequential combinations of non-invasive fibrosis tests provide an accurate diagnosis of advanced fibrosis in NAFLD. *J Hepatol.* 2019;71:389–396.

34. Xiao G, Zhu S, Xiao X, et al. 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.

35. Unalp-Arida A, Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. *Hepatology.* 2017;66:84–95.

36. Hagstrom H, Talback M, Andreasson A, et al. Ability of noninvasive scoring systems to identify Individuals in the population at risk for severe liver disease. *Gastroenterology.* 2020;158:200–214.

37. Wai-Sun Wong V, Lai-Hung Wong G, Woo J, et al. Impact of the new definition of metabolic associated fatty liver disease on the epidemiology of the disease. *Clin Gastroenterol Hepatol.* 2021;19:2161–2171.

38. McPherson S, Hardy T, Dufour JF, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. *Am J Gastroenterol.* 2017;112:740–751.

39. Srivastava A, Gailer R, Tanwar S, et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol.* 2019;71:371–378.

40. Wilson JMG, Jungner G. *Principles and Practice of Screening for Disease.* World Health Organization;; 1968.

41. Simon TG, Roelstraete B, Khalili H, et al. Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. *Gut.* 2021;70:1375–1382.

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

43. Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology.* 2019;156:1264.e4–1281.e4.

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

45. Siddiqui MS, Vuppalanchi R, Van Natta ML, et al. Vibration-controlled transient elastography to assess fibrosis and steatosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* 2019;17:156.e2–163.e2.

46. 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.

47. Papatheodoridi M, Hiriart JB, Lupsor-Platon M, et al. Refining the Baveno VI elastography criteria for the definition of compensated advanced chronic liver disease. *J Hepatol.* 2021;74:1109–1116.

48. Anstee QM, Lawitz EJ, Alkhouri N, et al. Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR Trials. *Hepatology.* 2019;70:1521–1530.

49. Petta S, Wong VW, Camma C, et al. Serial combination of non-invasive tools improves the diagnostic accuracy of severe

liver fibrosis in patients with NAFLD. *Aliment Pharmacol Ther*. 2017;46:617–627.

50. Boursier J, Vergniol J, Guillet A, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. *J Hepatol*. 2016;65:570–578.

51. Angulo P, Bugianesi E, Bjornsson ES, et al. Simple non-invasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology*. 2013;145:782.e4–789.e4.

52. Younossi ZM, Anstee QM, Wai-Sun Wong V, et al. The association of histologic and noninvasive tests with adverse clinical and patient-reported outcomes in patients with advanced fibrosis due to nonalcoholic steatohepatitis. *Gastroenterology*. 2021;160:1608.e13–1619.e13.

53. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology*. 2008;47:455–460.

54. Staufer K, Halilbasic E, Spindelboeck W, et al. Evaluation and comparison of six noninvasive tests for prediction of significant or advanced fibrosis in nonalcoholic fatty liver disease. *United European Gastroenterol J*. 2019;7:1113–1123.

55. Harrison SA, Ratziu V, Boursier J, et al. A blood-based biomarker panel (NIS4) for non-invasive diagnosis of non-alcoholic steatohepatitis and liver fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol*. 2020;5:970–985.

56. Boyle M, Tiniakos D, Schattenberg JM, et al. Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease. *JHEP Rep*. 2019;1:188–198.

57. Daniels SJ, Leeming DJ, Eslam M, et al. ADAPT: an algorithm incorporating PRO-C3 accurately identifies patients with NAFLD and advanced fibrosis. *Hepatology*. 2019;69:1075–1086.

58. Younossi ZM, Stepanova M, Rafiq N, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology*. 2011;53:1874–1882.

59. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *J Hepatol*. 2017;67:1265–1273.

60. Anania FA, Dimick-Santos L, Mehta R, et al. Nonalcoholic steatohepatitis: current thinking from the Division of Hepatology and Nutrition at the Food and Drug Administration. *Hepatology*. 2021;73:2023–2027.

61. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol*. 2020;5:362–373.

62. Noureddin N, Alkhouri N, Brown KA, et al. Driving nonalcoholic steatohepatitis forward using the FibroScan aspartate aminotransferase score, but obey the traffic lights. *Hepatology*. 2020;72:2228–2230.

63. Harrison SA, Rossi SJ, Paredes AH, et al. NGM282 improves liver fibrosis and histology in 12 weeks in patients with nonalcoholic steatohepatitis. *Hepatology*. 2020;71:1198–1212.

64. Gidener T, Ahmed OT, Larson JJ, et al. Liver stiffness by magnetic resonance elastography predicts future cirrhosis, decompensation, and death in NAFLD. *Clin Gastroenterol Hepatol*. 2021;19:1915.e6–1924.e6.

65. Are VS, Vuppalanchi R, Vilar-Gomez E, et al. Enhanced Liver Fibrosis Score can be used to predict liver-related events in patients with nonalcoholic steatohepatitis and compensated cirrhosis. *Clin Gastroenterol Hepatol*. 2021;19:1292.e3–1293.e3.

66. Han MAT, Vipani A, Noureddin N, et al. MR elastography-based liver fibrosis correlates with liver events in nonalcoholic fatty liver patients: a multicenter study. *Liver Int*. 2020;40:2242–2251.

67. Gawrieh S, Wilson LA, Cummings OW, et al. Histologic findings of advanced fibrosis and cirrhosis in patients with nonalcoholic fatty liver disease who have normal amino-transferase levels. *Am J Gastroenterol*. 2019;114:1626–1635.

68. 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.

69. Arab JP, Dirchwolf M, Álvares-da-Silva MR, et al. Latin American Association for the study of the liver (ALEH) practice guidance for the diagnosis and treatment of non-alcoholic fatty liver disease. *Ann Hepatol*. 2020;19:674–690.

70. Glen J, Floros L, Day C, et al. Non-alcoholic fatty liver disease (NAFLD): summary of NICE guidance. *BMJ*. 2016;354:i4428.

71. Cusi K. Time to include nonalcoholic steatohepatitis in the management of patients with type 2 diabetes. *Diabetes Care*. 2020;43:275–279.

72. American Diabetes Association. American Diabetes Association standard of care in diabetes. *Diabetes Care*. 2021;44(suppl 1):S1–S244.

73. Mahady SE, Macaskill P, Craig JC, et al. Diagnostic accuracy of noninvasive fibrosis scores in a population of individuals with a low prevalence of fibrosis. *Clin Gastroenterol Hepatol*. 2017;15:1453.e1–1460.e1.

74. Barb D, Repetto E, Stokes M, et al. Type 2 diabetes mellitus increases the risk of hepatic fibrosis in individuals with obesity and nonalcoholic fatty liver disease. *Obesity*. 2021;29:1950–1960.

75. Lai LL, Wan Yusoff WNI, Vethakkan SR, et al. Screening for non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus using transient elastography. *J Gastroenterol Hepatol*. 2019;34:1396–1403.

76. Chen K, Sng WK, Quah JH, et al. Clinical spectrum of non-alcoholic fatty liver disease in patients with diabetes mellitus. *PLoS One*. 2020;15:e0236977.

77. Doycheva I, Cui J, Nguyen P, et al. Non-invasive screening of diabetics in primary care for NAFLD and advanced fibrosis by MRI and MRE. *Aliment Pharmacol Ther*. 2016;43:83–95.

78. Koehler EM, Plompen EP, Schouten JN, et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: the Rotterdam study. *Hepatology*. 2016;63:138–147.

79. Sporea I, Mare R, Popescu A, et al. Screening for liver fibrosis and steatosis in a large cohort of patients with type 2 diabetes using vibration controlled transient elastography and controlled attenuation parameter in a single-center real-life experience. *J Clin Med*. 2020;9:1032.

80. Portillo-Sanchez P, Bril F, Maximos M, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab*. 2015;100:2231–2238.

81. Ciardullo S, Monti T, Perseghin G. High prevalence of advanced liver fibrosis assessed by transient elastography among U.S. adults with type 2 diabetes. *Diabetes Care*. 2021;44:519–525.

82. Stefan N, Häring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol*. 2019;7:313–324.

83. Maximos M, Bril F, Portillo Sanchez P, et al. The role of liver fat and insulin resistance as determinants of plasma amino-transferase elevation in nonalcoholic fatty liver disease. *Hepatology*. 2015;61:153–160.

84. Bril F, Barb D, Portillo-Sanchez P, et al. Metabolic and histological implications of intrahepatic triglyceride content in nonalcoholic fatty liver disease. *Hepatology*. 2017;65:1132–1144.

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

86. Estes C, Razavi H, Loomba R, et al. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018;67:123–133.

87. Singh S, Allen AM, Wang Z, et al. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol*. 2015;13:643.e1–654.e9; quiz e639–e640.

88. Alexander M, Loomis AK, van der Lei J, et al. Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnoses in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts. *BMC Med*. 2019;17:95.

89. Bril F, Kalavalapalli S, Clark VC, et al. Response to pioglitazone in patients with nonalcoholic steatohepatitis with vs without type 2 diabetes. *Clin Gastroenterol Hepatol*. 2018;16:558.e2–566.e2.

90. Canbay A, Kachru N, Haas JS, et al. Patterns and predictors of mortality and disease progression among patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2020;52:1185–1194.

91. Siddiqui MS, Yamada G, Vuppalanchi R, et al. Diagnostic accuracy of noninvasive fibrosis models to detect change in fibrosis stage. *Clin Gastroenterol Hepatol*. 2019;17:1877.e5–1885.e5.

92. Jayaswal ANA, Levick C, Selvaraj EA, et al. Prognostic value of multiparametric magnetic resonance imaging, transient elastography and blood-based fibrosis markers in patients with chronic liver disease. *Liver Int*. 2020;40:3071–3082.

93. Mansour D, Grapes A, Herscovitz M, et al. Embedding assessment of liver fibrosis into routine diabetic review in primary care. *JHEP Rep*. 2021;3:100293.

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

95. Trépo E, Valenti L. Update on NAFLD genetics: from new variants to the clinic. *J Hepatol*. 2020;72:1196–1209.

96. Anstee QM, Darlay R, Cockell S, et al. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort. *J Hepatol*. 2020;73:505–515.

97. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C > G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol*. 2014;61:75–81.

98. Anstee QM, Liu YL, Day CP, et al. Reply to: HCC and liver disease risk in homozygous PNPLA3 p.I148M carriers approach monogenic inheritance. *J Hepatol*. 2015;62:982–983.

99. Visscher PM, Hill WG, Wray NR. Heritability in the genomics era—concepts and misconceptions. *Nat Rev Genet*. 2008;9:255–266.

100. Bianco C, Jamialahmadi O, Pelusi S, et al. Non-invasive stratification of hepatocellular carcinoma risk in non-alcoholic fatty liver using polygenic risk scores. *J Hepatol*. 2021;74:775–782.

101. Pelusi S, Baselli G, Pietrelli A, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. *Sci Rep*. 2019;9:3682.

This paper can be cited using the date of access and the unique DOI number which can be found in the footnotes.