Hepatology Publish Ahead of Print
DOI:10.1097/HEP.0000000000000845

AASLD Practice Guideline on blood-based non-invasive liver disease assessments of hepatic fibrosis and steatosis

Richard K. Sterling[1], Keyur Patel[2] Andres Duarte-Rojo[3], Sumeet K. Asrani[4], Mouaz Alsawas[5], Jonathan A. Dranoff[6], Maria Isabel Fiel[7], M. Hassan Murad[5], Daniel H. Leung[8], Deborah Levine[9], Tamar H. Taddei[10], Bachir Taouli[11], and Don C. Rockey[12]

[1] Section of Hepatology, Virginia Commonwealth University, Richmond, Virginia, USA

[2] Division of Gastroenterology and Hepatology, University Health Network, University of Toronto, Toronto, Ontario, Canada

[3] Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[4] Baylor University Medical Center, Dallas, Texas, USA

[5] Mayo Clinic Evidence-based Practice Center, Mayo Clinic, Rochester, Minnesota, USA

[6] Section of Digestive Diseases, Yale School of Medicine, New Haven, Connecticut, USA, and VA Connecticut Healthcare System, West Haven, Connecticut, USA

[7] Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA

[8] Department of Pediatrics, Baylor College of Medicine and Division of Gastroenterology, Hepatology and Nutrition, Texas Children's Hospital, Houston, Texas, USA

[9] Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

[10] Section of Digestive Diseases, Yale School of Medicine, New Haven, Connecticut, USA and VA Connecticut Healthcare System, West Haven, Connecticut, USA

[11] Department of Diagnostic, Molecular and Interventional Radiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

[12] Digestive Disease Research Center, Medical University of South Carolina, Charleston, South Carolina, USA

Running title: Blood-based noninvasive liver disease assessment

Key words: blood-based; hepatic fibrosis; hepatic steatosis; liver biopsy; histology; cirrhosis; stiffness; biomarker; fatty liver; NASH; NAFLD, FIB-4, prognosis, MASLD, NILDA, NIT

Word count: 11,042

Tables/Figures: 11 tables/1 figure, 1 suppl table

Corresponding Author:

Richard K. Sterling

Section of Hepatology

Virginia Commonwealth University

1200 E Broad Street, West Hospital, Rm 1478

Richmond, VA 23298-0341

Phone: 804-828-9034

Fax: 804-828-5348

Email: Richard.sterling@vcuhealth.org

Abbreviations

AASLD: American Association for the Study of Liver Diseases

α1AT: alpha-1-antitrypsin

ALD: alcohol-associated liver disease

ALT: alanine aminotransferase

APRI: AST-to-platelet ratio index

AST: aspartate aminotransferase

AT: ActiTest

AUROC: area under receiver operator curve

BA: biliary atresia

BARD: body mass index, AST/ALT ratio, and presence of type 2 diabetes mellitus

BMI: body mass index

CAP: Controlled attenuation parameter

CF: cystic fibrosis

CFLD: cystic fibrosis liver disease

CLD: chronic liver disease

CRN: clinical research network

CSPH: clinically significant portal hypertension

DAA: direct acting antiviral

DM: diabetes mellitus

DOR: diagnostic odds ratio

ELF: enhanced liver fibrosis

F: fibrosis (used in staging fibrosis with stages F1 to F4)

FIB-4: Fibrosis-4 index

FLI: fatty liver index

GGT: gamma glutamyl transferase

GRADE: Grading of Recommendation Assessment, Development and Evaluation

HCC: hepatocellular carcinoma

HCV: hepatitis C virus

HBV: hepatitis B virus

HBeAg: hepatitis B envelope or “early” antigen

HIV: human immunodeficiency virus

HSI: hepatic steatosis index

HVPG: hepatic vein pressure gradient

IFN: interferon

LAP: lipid accumulation product

LSM: liver stiffness measurement

LR: likelihood ratio

MASLD: metabolic dysfunction-associated steatotic liver disease

METAVIR: meta-analysis of histological data in viral hepatitis

MRI: magnetic resonance imaging

MRE: magnetic resonance elastography

MRI-PDFF: magnetic resonance imaging-proton density fat fraction

NAFLD: nonalcoholic fatty liver disease

NFS: nonalcoholic fatty liver disease fibrosis score

NAS: nonalcoholic fatty liver disease activity score

NASH: nonalcoholic steatohepatitis

NPV: negative predictive value

NILDA: noninvasive liver disease assessments

PICO: patient, intervention, comparison and outcome

PSC: primary sclerosing cholangitis

PBC: primary biliary cholangitis

PPV: positive predictive value

PRO-C3: N-terminal propeptide of type III collagen

PT: prothrombin time

ROC: receiver operating characteristic curve

S: steatosis (used in staging steatosis with stages of 0–3)

SLD: steatotic liver disease

SVR: sustained virologic response

SWE: Shear-wave elastography

T2DM: type 2 diabetes mellitus

TE: transient elastography

TG: triglycerides

US: ultrasound

WC: waist circumference

Disclosures of Conflicts of Interest

Richard K. Sterling: Research Grant to institution: Gilead, AbbVie, Abbott, Roche, and Zydus

Keyur Patel: none (in relation to this manuscript); Consultant: Gilead Sciences; Advisory Board: Novo Nordisk, Intercept

Andres Duarte-Rojo: Research Grant to Institution: Echosens, Axcella Health. Consultant: Axcella Health. Advisory board for Mallinckrodt

Sumeet K Asrani: none

Mouaz Alsawas: none

Jonathan A. Dranoff: none

M. Isabel Fiel: none (in relation to this manuscript); otherwise Consultant for Progenity, Alexion, Q32

M. Hassan Murad: none

Daniel H. Leung: Research grant to institution: Abbvie, Gilead, Mirum, CF Foundation; Data Safety Monitoring Board: Merck; Advisory board: Gilead

Deborah Levine: none

Tamar H. Taddei: none

Bachir Taouli: none (in relation to this manuscript); otherwise Research support: Bayer, Regeneron, Takeda, Echosens, Siemens. Consultant: Bayer, Helio Health, Guerbet

Don C. Rockey: No conflict of interest related to the content of this manuscript. Advisory board consulting for the following: Merck, Takeda, AstraZeneca. Research funding provided to DCR's institution from AstraZeneca, Genfit, Gilead, Intercept, Novo Nordisk, Pfizer, and Viking

PURPOSE AND SCOPE

Chronic liver disease (CLD) leads to liver fibrosis; it is associated with approximately two million annual deaths worldwide and is an enormous health burden.[1, 2] The majority of liver-related outcomes such as hepatic decompensation and complications from portal hypertension (variceal bleeding, hepatic encephalopathy, and ascites) and hepatocellular carcinoma (HCC) occur almost exclusively in those with advanced fibrosis. Therefore, it is critical to identify patients with any fibrosis and, in particular, moderate-to-advanced fibrosis. Over the past few decades, multiple noninvasive blood biomarkers and imaging modalities or tests, termed here "noninvasive liver disease assessment(s) (NILDA)," have been developed to determine the presence and severity of liver fibrosis (F), steatosis (S), and clinically significant portal hypertension.

NILDA can be generally categorized as blood-based and imaging-based. The American Association for the Study of Liver Diseases (AASLD) Practice Guidelines Committee commissioned a diverse group of experts across multiple disciplines in the field of adult and pediatric liver disease to develop guidelines and guidance statements along with a systematic review covering blood-based NILDA to answer specific clinically focused questions ("patient, intervention, comparison, and outcome;" henceforth, PICO) (Table 1). This document focuses on the use of blood-based NILDA. The use of imaging-based NILDA[3, 4] in clinical practice and the use of blood and or imaging-based NILDA for assessment of clinically significant portal hypertension[5, 6] have been discussed elsewhere. These guidelines are intended primarily for

adult and pediatric health care providers who see patients with CLD to provide a guidance algorithm that is summarized at the end of this document.

METHODS

Overall approach

The guideline writing group consisted of a multidisciplinary panel of experts in both adult and pediatric hepatology, pathology, and radiology, including methodology experts. Two complementary approaches were taken to answer the PICO questions relevant to various CLDs. Autoimmune hepatitis (AIH) has been reviewed and discussed elsewhere.[7] The first approach depended on a commissioned systematic review conducted independently by the Mayo Clinic Evidence-Based Practice Center (suppl Fig. 1); this led to graded recommendations following the Grading of Recommendations, Assessment Development, and Evaluation system (GRADE) approach (Table 2).[8, 9] These recommendations are followed by a section that describes the quality of evidence, when applicable, and other considerations. The panelists monitored the literature for studies published during the systematic review's search date and included relevant studies through April 2022. Strength of recommendations was based on the quality of the evidence, balance of benefits and harms, the burden of testing (access and financial), and feasibility of the recommended action. The "strength of recommendation" determination assumed that performing tests with acceptable (>70%), excellent (>80%), or outstanding (>90%) diagnostic accuracy are associated with improved patient outcomes. The recommendations were graded as either strong (apply to most patients with minimal variation and can be adapted as policy in most situations) or conditional (apply to a majority of patients, but variation in care is acceptable). These recommendations are followed by a section that describes the quality of

evidence (if applicable) and other considerations. The panelists monitored the literature for studies published to included relevant studies through April 2022. Because of the rapid evolution of the field and predetermined quality of studies incorporated in our systematic reviews, we were not able to include every published study on the topic. In particular, studies with smaller sample sizes (<50 individuals) or those with mixed etiology were excluded.

In order to address several other important clinical questions that could not be answered by a systematic review due to sparse and/or indirect evidence, the second approach involved a thorough narrative review by the writing group to develop ungraded guideline statements. These ungraded statements considered additional sources and the clinical experience of the authors with regard to noninvasive assessments of hepatic fibrosis and steatosis. Technical remarks and supporting evidence for graded and ungraded statements are included with recommendations to help reconcile the level of the recommendation with the quality of the evidence and to facilitate implementation. For these guideline statements (below) on blood-based NILDA, adults are defined as being at least 18 years of age, and pediatrics are younger than age 18 years.

Consensus Process

For all guideline statements, we pursued a concensus approach to define the final set of recommendations using previously described methodology and also adapted by the AASLD practice metrics committee.[10] Statements with <75% agreement were rediscussed with the following: 1) review of the scores; 2) discussion to identify the reasons for variation; 3) revision of suboptimally worded statements for accuracy by consensus; 4) deletion of statements that

were deemed problematic or irrelevant by consensus; and 5) identification of additional statements deemed necessary for inclusion in the list of statements.

Rationale for NILDA

Accurate assessment of the degree of liver fibrosis and steatosis is essential in predicting prognosis and making treatment recommendations in patients with CLD. Although liver biopsy has long been the reference standard for assessing fibrosis and steatosis, it is costly, invasive, and carries a small, but important, risk of complications.[11, 12] Pain is the most common, whereas clinically apparent bleeding occurs in some one in every five hundred liver biopsies (rate of 0.2%), with severe bleeding in one out of every two thousand five hundred to one in ten thousand (rate of 0.04% to 0.01%).[13] The mortality rate associated with liver biopsy is estimated to be one per ten thousand to one per twelve thousand (rate of 0.01% to 0.0083%).[11] Biopsy complication rate varies based on operator experience, underlying comorbidities, size of the needle, number of passes, and underlying bleeding risk due to low platelets and/or increased prothrombin time.

Current noninvasive assessments rely on biochemical (blood) or physical (imaging) characteristics that are developed in relation to cross-sectional, histopathologic scores and do not account for the dynamic progression of fibrogenesis or variable disease etiology pathogenesis. In the last 20 years, noninvasive methods for assessing liver fibrosis and steatosis utilizing blood- and imaging-based methods have been developed to reduce the need for invasive liver assessment procedures.

Histopathological principles underlying NILDA

Fibrosis scores are generally disease-specific and technically cannot be unified across different CLDs. To achieve a cohesive approach for the purposes of NILDA, the writing group incorporated the various fibrosis staging systems into a single one and classified them into at least significant fibrosis (equivalent to at least fibrosis stage 2 or F2-4), at least advanced fibrosis (F3-4), and cirrhosis (F4). For simplicity, the Guidelines statements employ the generic "F" stages throughout the text. Various histologic scoring systems to stage fibrosis and grade inflammation and steatosis have been used as standard reference measures in studies validating NILDA biomarkers (Tables 3a, 3b).[14–22]

Although differences are subtle in most instances among different liver histologic scoring schemes for fibrosis, using scores interchangeably between and among different schemes is problematic (Table 3a). For example, Scheuer stage 3 is not equivalent to the meta-analysis of histological data in viral hepatitis (METAVIR) F3. The Ishak system has seven possible scores,[23–25] which allows for finer detail in fibrosis scoring; a challenge lies with scores five and six in that most treating physicians assume that score five is cirrhosis based on prognostic implications.[26] However, because Ishak 5 is defined as "marked bridging with occasional nodules" or "incomplete cirrhosis," and the definition of cirrhosis is diffuse parenchymal nodularity; Ishak 5 does not meet these criteria.[27] In adult patients with fatty liver disease, whether alcohol-associated or due to metabolic syndrome, fibrosis initially occurs in zone 3 (centrilobular area) with a perisinusoidal and pericellular pattern. In contrast, fibrosis in other types of CLD is largely portal-based. In children, fibrosis is often triggered by a genetic or persistent environmental insult or by biliary injury with duct obstruction. Thus, the patterns of fibrosis distribution depend on the etiology, susceptibility, and response to injury.

We acknowledge that there has been a recent multisociety endorsement of a nomenclature change from NAFLD to metabolic dysfunction–associated steatotic liver disease (MASLD). Although this is an important change that will impact of future of the study of this entity, all data utilized to develop these guideline statements were based on prior literature that utilized the previous NAFLD definition. Therefore, NAFLD is the term used throughout this document when referring to the existing literature. Current evidence indicates >98% overlap between patients who meet criteria for diagnosis of NAFLD/NASH and the new criteria for MASLD/metabolic dysfunction–associated steatohepatitis (MASH) in large cohort studies, indicating that the analyses and recommendations provided in these Guidelines for patients with NAFLD/NASH are likely to pertain to patients characterized by the new nomenclature of MASLD and MASH.

The two most commonly used scoring systems in steatototic liver disease (SLD) for steatosis and fibrosis in NAFLD are those by Brunt and the NASH Clinical Research Network (CRN), i.e., the NAFLD Activity Score (NAS).[21, 22]. The Brunt scoring system has four possible grades (0–3) and five possible stages (0–4). Both systems determine the degree of steatosis based on the percentage of steatotic hepatocytes involved: normal <5%, mild = 5% to 33%, moderate = 34% to 66%, and severe >66% (Table 3b). In children with NASH, steatosis is more profound, and the distribution of fibrosis and inflammation is found primarily and initially in zone 1 (periportal).[28]

Some experts have suggested that the grading and staging of NAFLD may also be applied to alcohol-associated liver disease (ALD) due to similarity and overlap in morphological

features.[29] Histologic scoring systems specifically for ALD have been proposed over the years,[30, 31] but none have been used in standard clinical practice. One scoring system has been proposed for alcoholic hepatitis, which correlates histological features with prognosis.[20] Although advanced fibrosis was identified as an independent predictor of short-term mortality, i.e., indicating chronicity and progression of disease, this was not the main outcome of the study; therefore, this histologic scoring system has not been applied in clinical practice.[20] Additionally, liver biopsies may not be routinely obtained in patients with suspected ALD, leading to challenges in correlating liver histology with outcome.

Although liver histology is considered the reference standard to which NILDA is assessed, several factors can bias liver histology, including sampling bias, classification bias, and spectrum bias. Liver biopsy specimen size and adequate number of portal tracts are very important to reduce sampling bias.[11, 32, 33] Unfortunately, most published studies have not adjusted for this bias.[34, 35] Quantitative techniques such as histomorphometry using collagen- or fat-specific stains have been introduced to overcome inherent problems encountered in semiquantitative histological staging systems.

Evidence using NILDA has suggested that fibrosis can regress (suggesting that the total amount of fibrosis in the liver becomes reduced; this does not, however, necessarily mean that the liver architecture becomes normal), particularly once the cause of liver injury is resolved.[36, 37] Unfortunately, there is no histopathological score that has been validated for use in regression of fibrosis, despite reports characterizing regression of fibrosis features, such as thinning and perforation of septa, isolated collagen fibers not attached to a portal tract/central vein, and

changes in baseline architectural distortion, including loss of zonation of vascular structures.[38, 39]

Assessment of diagnostic performance of noninvasive markers

We used several statistical tests and indices in our assessment of the performance of blood-based NILDA (Table 4). Although several studies have reported test characteristics such as sensitivity and specificity at a selected cutoff, the positive and negative predictive values of the test are dependent on the prevalence of the condition (e.g., fibrosis or steatosis).[40] The Likelihood Ratio (LR) is defined as the likelihood that a test result would be expected if the patient had the disease compared with the likelihood of this same result in a patient without the disease. Positive LR describes the odds of having fibrosis or steatosis among patients with a positive test, whereas negative LR describes the odds of having fibrosis or steatosis in patients with a negative test. Positive LR above 10 and negative LR below 0.1 suggest strong diagnostic evidence. The diagnostic odds ratio (DOR) is the ratio of the odds of disease in those who test positive to the odds of the disease in those who test negative (i.e., summarizing the odds of fibrosis in those with a positive test relative to those with a negative test) and provides a reliable estimate of a test's accuracy that is independent of the prevalence of the condition being tested. The area under the receiver operating characteristic curve (AUROC) analysis is another effective way to summarize the overall diagnostic accuracy of the test. The AUROC ranges from 0 to 1, where a value of 0 indicates a perfectly inaccurate test, and a value of 1 reflects a perfectly accurate test. In general, an AUROC of 0.5 suggests no discrimination (i.e., inability to diagnose patients with and without the disease or condition based on the test), 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered good, and more than 0.9 is considered excellent.

Blood-based biomarkers

Blood-based assessment of fibrosis takes advantage of the complex and dynamic interplay between the inflammatory response and fibrogenesis, including elements of extracellular matrix synthesis and degradation. Noninvasive blood-based biomarkers include combinations of tests of "direct" markers, which are mostly complex macromolecules derived from myofibroblasts and extracellular matrix remodeling, or "indirect" markers reflective of inflammation and/or portal hypertension. Although blood-based tests were initially developed for hepatitis C virus (HCV), many have been adopted to assess fibrosis in other CLDs, including NAFLD. Algorithms used are conceptually divided into the following: 1) simple, nonproprietary models that include routine blood tests; 2) those that combine routine tests with clinical variables; and 3) more complex proprietary models that include direct measurements of collagen synthesis or degradation with or without clinical variables (Table 5).[41–51]

Commonly used clinical variables are age, sex, body mass index (BMI), and the presence of diabetes mellitus (DM). Complex models include direct measurements of collagen synthesis and degradation (hyaluronic acid, N-terminal propeptide of type III procollagen, matrix metalloproteinase type 1 and 2, tissue inhibitors of matrix metalloproteinases type 1 and 2, α2-macroglobulin, apolipoprotein A1, transforming growth factor-β 1, procollagen type 1 carboxy-terminal peptide, chitinase-3-like protein 1 [YKL-40], and/or cytokeratin-18 fragments).[41–43, 45–50, 52] However, blood-based tests may be limited by clinical factors such as systemic inflammation or sepsis (Table 6).[53–62]

Unreliable classifications for blood-based biomarker algorithms that utilize bilirubin may occur in hemolysis, Gilbert's syndrome, or cholestasis. Other clinical disease states such as acute hepatitis, sepsis, and systemic inflammatory conditions may produce false-positive results in blood biomarker algorithms that incorporate aminotransferases or acute phase reactants such as hyaluronic acid, α-2 macroglobulin, platelets, N-terminal propeptide of procollagen type III, or false-negative results with elevated haptoglobin. Simple markers may have lower accuracy for advanced fibrosis in patients with HCV with end-stage renal disease and normal-range transaminases.[58] Hyaluronic acid levels may be influenced by age[63] or postprandial state.[59, 64] HIV co-infection may result in thrombocytopenia or may be associated with drug-induced elevations in bilirubin or γ-glutamyl transferase (GGT), which can also affect diagnostic accuracy of several blood-based marker panels.

Recommendations and Guideline Statements

PICO 1: In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, hepatitis B virus [HBV], HIV-HBV, NAFLD, and ALD) or cholestatic (primary sclerosing cholangitis [PSC] and primary biliary cholangitis [PBC]) disorders, are blood-based biomarker panels accurate in staging hepatic fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?

Guideline Statements

1. In adult patients with chronic HBV and HCV undergoing fibrosis staging prior to antiviral therapy, the AASLD recommends using simple blood-based NILDA such as APRI or Fibrosis-4 Index (FIB-4) as an initial test to detect significant (F2-4), advanced fibrosis (F3-4) or cirrhosis (F4) compared with no test (strong recommendation, moderate quality of evidence).

2. In adult patients with NAFLD undergoing fibrosis staging, the AASLD recommends using simple blood-based NILDA tests such as FIB-4 to detect advanced fibrosis (F3-4) compared to no test (strong recommendation, moderate quality of evidence).

3. In adult patients with ALD or chronic cholestatic liver disease undergoing fibrosis staging, there is insufficient evidence to recommend using blood-based NILDA for staging fibrosis (ungraded statement).

Technical Remarks

- Direct and indirect blood biomarkers include components (bilirubin, aminotransferases, platelets, and other acute-phase reactants) that may be associated with false-positive or false-negative test results in patients with certain disorders such as acute hepatitis, hemolysis, Gilbert's syndrome, human immunodeficiency virus (HIV)-induced thrombocytopenia, splenectomy, and disease or treatment-related elevation in bilirubin or aminotransferases (Table 6).
- Blood-based biomarkers have high sensitivity and negative predictive value (NPV) for "ruling out" advanced fibrosis in NAFLD but low positive predictive value (PPV) to "rule-in" in advanced fibrosis in low prevalence cohorts (suppl table 1, Figure 1, Table 7).[43, 54, 65–90]

- There are no validated blood-based biomarker thresholds that correlate with the fibrosis stage following sustained virologic response (SVR) in patients with HCV. Both indirect and direct blood biomarkers are associated with high false-negative rates for advanced fibrosis following antiviral therapy in patients with HBV or HCV.
- Although not included in the systematic review, NFS can be used to detect F3-4 in those with NAFLD.

Background

Although none of the current blood-based biomarkers are liver-specific, potential advantages include availability (for simple nonproprietary tests), interlaboratory reproducibility, and ease of use in routine clinical practice. However, an important consideration is the reliability of currently available blood-based markers to classify patients with CLD accurately. For example, prior modeling in HCV has indicated that because of sampling error, liver histology (the reference standard to which NILDA are compared with) is imperfect; therefore, the ideal biomarker performance usually does not exceed an AUROC of 0.9.[91] However, these performance measures do not overcome limitations related to disease heterogeneity and spectrum effect/bias in study cohorts.[92]

Evidence and Rationale

HCV

In the current era of direct-acting antiviral (DAA) therapies with high efficacy for HCV, excluding stage F0-1 prior to treatment is less clinically relevant than the detection of significant fibrosis (F3-4) or cirrhosis (patients with advanced disease should have ongoing post-treatment

HCC surveillance). A systematic review of 10 different simple and complex biomarker panels concluded that clinically relevant predictive values (PPV ≥ 90% and NPV ≥ 95%) for significant fibrosis (F2-4) could be obtained for only 35% of patients with HCV before therapy.[67] Aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and FIB-4 are the best validated of the simple, cheap, and readily available nonproprietary tests, but they are known to be associated with "indeterminate" range scores and unreliable diagnostic performance in some patients. FibroTest™ (BioPredictive, Paris, France) or in the United States, FibroSURE® (LabCorp, Burlington, North Carolina) are the most validated blood-based biomarkers with a proprietary algorithm. A meta-analysis of 172 studies evaluated several blood-based biomarkers in patients with HCV and indicated that blood-based NILDA tests had moderate diagnostic utility for the detection of F2-4 and F4.[93] Our systematic review[94] indicated that both simple and complex blood-based NILDA had acceptable diagnostic performance for detecting F2-4, F3-4, and F4 in patients with HCV prior to antiviral therapy (supplemental Table 1).

Liver biopsies are no longer performed routinely in patients with HCV who are post-SVR, and the diagnostic role of indirect and direct blood-based biomarkers for staging fibrosis in these patients has not been established. In general, routine use of blood-based biomarkers that include aminotransferases is likely to be associated with a high false-negative rate for advanced disease following viral clearance. A study in 115 patients with HCV and biopsy available 5-years post-SVR noted AUROC for APRI and FIB-4 of 0.81 to 0.88 for F2-4 and F3-4, although the selected biomarker thresholds were much lower post-SVR.[95] A smaller study of 38 patients with HCV stage F4 and biopsy 5-years post-SVR also noted lower scores for both indirect (APRI, FIB-4, King's score) and direct (European Liver Fibrosis [ELF], Siemens Healthineers AG,

Erlangen, Germany) biomarkers, with an AUROC of 0.58 to 0.63 for F4 post-SVR.[96] Thus, validation of post-SVR biomarker thresholds that correspond to fibrosis stages is required [97].

HBV

Management decisions in HBV infection consider not only fibrosis stage but also disease activity based on HBV DNA levels, alanine aminotransferase (ALT) elevation, and HBe-antigen (HBeAg) status, along with other variables.[98] Although blood-based biomarkers of fibrosis have not been routinely adopted for the management of HBV infection, detection of advanced fibrosis or cirrhosis has important prognostic implications. A meta-analysis of 30 studies with APRI, FIB-4, and FibroTest indicated a summary AUROC of 0.75 to 0.84 for F2-4 and 0.75 to 0.90 for F4 (99). Another meta-analysis of 16 studies that included 2494 patients with HBV (including 1754 with F4) indicated summary AUROC for FibroTest of 0.84 for F2-4 and 0.87 for F4.[100] Our systematic review,[94] which included 96 studies, indicated that APRI and FIB-4 had acceptable diagnostic performance for F2-4, F3-4, and F4 in patients with HBV and higher specificity (>0.80) at upper test cutoffs. A study in 510 patients with HBV or HCV indicated that optimal sensitivity cutoffs for F3-4 and F4 using FibroTest, FibroMeter®, and HepaScore were lower in HBV compared with HCV. These findings suggest that the use of thresholds established in HCV can result in higher false-negative rates for advanced fibrosis and cirrhosis in HBV.[101]

NAFLD

Increased fibrosis stage has important prognostic implications in NAFLD.[102, 103] Revised FIB-4 thresholds of ≤1.30 and ≥2.67 have been proposed as having higher predictive values for F3-4 in the NASH CRN cohort.[104] However, a prior meta-analysis that included six studies with

1910 patients noted that FIB-4 ≥ 2.67 and ≥3.25 both had a summary specificity of 0.96 to rule-in advanced fibrosis.[105] Our systematic review of 32 studies that reported these upper FIB-4 thresholds for NAFLD advanced fibrosis indicated similar pooled specificity of 0.94 for both FIB-4 ≥ 2.67 and ≥3.25.[94] Our results also indicated DOR of 7.81 and 10.19 for F3-4 at the lower FIB-4 thresholds of 1.3 and 1.45 and 10.76 and 7.01 for upper thresholds of 2.67 and 3.25, respectively. The NAFLD fibrosis score (NFS) was developed as a simple scoring algorithm to reduce the need for a liver biopsy to identify patients with NAFLD with advanced fibrosis.[43] Optimal test thresholds for selecting F3-4 using blood-based markers vary between studies due to differences in population characteristics and disease prevalence compared with the original test derivation cohort.[105] Our comprehensive review of NFS included 11,372 patients with NAFLD with advanced fibrosis on biopsy and assessed NFS performance at the original validated lower and upper thresholds of −1.455 and 0.676, respectively. At advanced fibrosis prevalence rates that varied from 3% to 80%, the summary median (95% confidence interval [CI]) sensitivity for excluding F3-4 at less than −1.455 was 0.75 (95% CI: 0.61–0.81), and specificity for diagnosing F3-4 at greater than 0.676 was 0.96 (95% CI: 0.93–0.98), with indeterminate rates of 33.5% (95% CI: 25.6–44.4; Table 7).

This is comparable with an individual patient meta-analysis of 3248patients with NAFLD that resulted in specificty of 0.91 for F3-4 at established cutoffs for NFS and indeterminate rates of 39%.[106] Consideration of disease prevalence in the target population is important because many of these simple and proprietary blood-based markers will be increasingly used to screen for advanced fibrosis in lower prevalence nontertiary cohorts at risk of NASH. A meta-analysis of 11 studies using ELF tests for F3-4 noted a high sensitivity (0.93) but limited specificity (0.34) at the lower recommended threshold of 7.7; higher thresholds and F3-4 prevalence of at least 30%

were required for increasing ELF PPV to >0.8 for advanced fibrosis.[107] Overall, both simple and complex blood-based marker algorithms have acceptable diagnostic accuracy for NAFLD advanced fibrosis in higher prevalence tertiary center cohorts. In community-based and other low prevalence cohorts, blood-based NILDA are useful for excluding advanced fibrosis with high NPV but require additional noninvasive tests to improve their PPV.

ALD

Assessment of the diagnostic utility of blood-based NILDA in ALD is limited due to small study cohorts with variable severity of alcoholic hepatitis, biopsy sampling, and histologic scoring systems. A study in 218 patients with ALD indicated that indirect markers such as APRI have low diagnostic accuracy for F2-4 or cirrhosis (AUROC 0.59–0.67), but proprietary tests such as FibroTest, FibroMeter, or HepaScore had better performance for detection of F2-4 (AUROC 0.83) and cirrhosis (AUROC 0.92–0.94).[108] A systematic review that included eight studies with blood-based marker panel assessment of advanced fibrosis or cirrhosis in patients with ALD also reported high accuracy for FibroTest, FibroMeter, HepaScore, and ELF for cirrhosis, but significant heterogeneity among studies precluded summary analysis.[109] Based on our systematic review,[94] there were too few studies to allow for recommendation regarding use of blood-based NILDA for ALD.

Other CLD

Similar to HCV mono-infection, NILDA tests are also important for the determination of liver disease severity in patients with HIV-HCV co-infection prior to DAA therapy. Our systematic review identified 12 studies, mostly reporting results for APRI and FIB-4.[94] In

general, blood-based markers appear to have similar diagnostic performance for significant fibrosis to patients who were HCV mono-infected, with fewer studies identified for the detection of advanced fibrosis and cirrhosis.

Post-SVR diagnostic limitations for blood-based NILDA also apply to HIV-HCV co-infection. Reduced blood-based NILDA accuracy due to associated thrombocytopenia, or potential antiretroviral therapy-related changes in bilirubin and GGT, need to be considered while interpreting these tests.[110]

Few studies have assessed the diagnostic role of blood-based biomarkers for staging fibrosis in chronic cholestatic diseases and have included mostly patients with PBC.[111] APRI and FIB-4 are the most frequently used simple nonproprietary tests. A study of 103 patients with PBC indicated AUROC of 0.77 to 0.93 for ≥F2 for APRI and FIB-4, with better performance for the detection of cirrhosis.[112] However, disease-specific diagnostic thresholds have not been established for blood-based tests.[112–114] In a study of 229 patients with PSC, ELF and FibroTest had AUROC > 0.8 for the detection of F4 but were comparable with simple tests.[115] In general, blood-based markers have acceptable accuracy for diagnosing cirrhosis related to chronic cholestatic disease; however, the clinical utility of blood-based NILDA tests for staging fibrosis, especially in less advanced stages of fibrosis, in these patients is less certain than for viral hepatitis or NAFLD.

PICO 2: In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HIV-HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, is any

blood-based biomarker panel superior to another blood-based biomarker panel in staging hepatic fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4 and F0-3 vs. F4) using histopathology as the reference?

Guideline Statements

4. In patients with chronic HCV who require fibrosis staging, the AASLD recommends using simple, less costly, and readily available blood-based NILDA such as FIB-4 over complex proprietary tests (strong recommendation, moderate quality of evidence).

5. In patients with NAFLD who require fibrosis staging, the AASLD recommends the use of simple, less costly, and readily available blood-based NILDA tests such as FIB-4 or NAFLD fibrosis score over complex proprietary tests for the detection of advanced fibrosis (F3-4; strong recommendation, moderate quality of evidence).

Technical Remarks

- Blood-based NILDA*:* Head-to-head studies comparing blood-based NILDA in the same patient population are limited in number. In comparing one study to another, the pooling of sensitivity and specificity may be suboptimal because different thresholds have been used across typically heterogeneous populations and settings. Other assessments (e.g., predictive values) depend on the clinical setting and prevalence of different fibrosis stages in the population being studied. Most of the research studies were developed in patient populations from tertiary or referral centers, which limits generalizability.

- In chronic HBV prior to therapy, there are limited data comparing simple with proprietary NILDA.
- There are limited data in diseases other than viral hepatitis and NAFLD that directly compare blood-based NILDA.

Background

Blood-based NILDA have been studied predominantly in patients with HCV and NAFLD. In addition, comparison is usually only between select blood-based markers and involves a variety of cutoffs. This makes recommending one marker over the other difficult, especially for intermediate stages. In general, all blood-based markers are more accurate at identifying the absence of fibrosis or the presence of cirrhosis than intermediate stages of fibrosis. The diagnostic performance of proprietary and nonproprietary tests is not significantly different in clinical practice. Although proprietary markers may be suitable in select situations, nonproprietary tests are readily available, repeatable, and less expensive than proprietary tests.

Several studies have compared APRI with an alternate blood-based NILDA with a paired liver biopsy across liver disease diagnoses.[94] The performance of proprietary and nonproprietary tests compared with APRI was not significantly different for F0-1 versus F2-4, F0-2 versus F3-4, and F0-3 versus F4 across select cutoffs. However, limitations include the following: 1) lack of comparison across all cutoffs; 2) few studies that do not have APRI as a comparator group; and 3) limited studies for proprietary markers in comparison to each other.

Evidence and Rationale

HCV

Studies have examined the role of blood-based NILDA predominantly in the pre-DAA era. Overall, proprietary and nonproprietary blood markers have comparable diagnostic accuracies for significant fibrosis.[116] Comparative data are largely limited to APRI, FIB-4, and FibroTest™ because these markers have the most complete data. Less comparative data are available for ELF™, Fibrometer™, Fibrospect II™, and Kings; however, sensitivities and specificities of these tests are not significantly different compared with the aforementioned tests. For the presence of significant fibrosis, the DOR range is from 5.44 to 13.35 and not significantly different among APRI (cutoff 0.5 or 1), FIB-4 (cutoff 1.45), Fibrometer (cutoff 0.5), and FibroTest (cutoff 0.48). APRI (cutoff 1) had the highest DOR 13.35 (6.7–26.57). For presence of advanced fibrosis, the DOR range is 6.87 to 21.49, with similar performance for APRI (cutoff 1.5), FIB-4 (cutoff 3.25), and FibroTest (0.48), as well as FIB-4 (cutoff 1.45 or 3.25) and ELF (cutoff 9.13–9.49). ELF had the highest DOR (21.49 [8.43–54.75]) [94]. In a large observational cohort (>2000 paired biopsy measurements), FIB-4 (0.83 [95% CI: 0.81–0.85]) and APRI (0.80 [95% CI: 0.78–0.82]) had equivalent performance.[117] In another study, FIB-4 correctly classified a higher proportion of patients even though the overall performance of APRI and FIB-4 was similar.[118] Single-center studies have suggested that there may be overestimation in fibrosis in African American individuals using FibroSpect II, FIB-4, and APRI[119] and inaccurate results in patients with normal transaminases, especially in the presence of end stage renal disease.[58, 120]

HBV

APRI and FIB-4 have the most complete data available, although proprietary markers (e.g., FibroTest™) may also have similar performance in predicting cirrhosis.[50, 121–123] For the presence of advanced fibrosis, the DOR ranged from 4.86 to 9.28 and was not significantly different for APRI (cutoff 0.5) and FIB-4 (cutoff 1.45). FIB-4 (cutoff 2.2) had the highest DOR. However, there are concerns that APRI and FIB-4 cutoffs may not be applicable across all populations, and there may be a high risk of misclassification, especially with current cutoffs.[123–126]

NAFLD

There are limited data comparing the DOR across the various tests. FIB-4 (using cutoff 1.45 to rule out or 2.67 to rule in) had a higher DOR than APRI (using cutoff 1.5), but data were not available to compare DOR for other tests.[94] There was insufficient data to compare DOR for other tests such as FibroTest (cutoff 0.70) or ELF (cutoff 9.8).

Nonproprietary tests such as FIB-4, APRI, and NFS help to rule-out advanced fibrosis.[126] Nonproprietary tests scores have generally similar performance in excluding advanced fibrosis, although, in select studies, NFS and FIB-4 may have better performance characteristics.[68, 104, 127] Cutoffs may need to be modified for select populations such as those who have class III obesity,[127] and scores do not have adequate performance characteristics across all demographics.[128–130] Performance also varied by age with increased sensitivity and decreased specificity of blood-based markers with age.[80, 86] There are conflicting data on the diagnostic accuracy of proprietary fibrosis panels (e.g., Fibrometer and ELF) compared with FIB-4 and NFS for the detection of fibrosis in NAFLD.[107, 131, 132]

Other CLD

In patients with HCV/HIV co-infection, the sensitivities and specificities of APRI, FIB-4, and FibroTest were not significantly different for significant fibrosis, advanced fibrosis, and cirrhosis.[94] The DOR was high for APRI for both significant fibrosis (DOR 3.9–5.5) as well as cirrhosis (DOR 15.24). Although smaller studies have shown that ELF and FibroTest performances were superior to nonproprietary tests (FIB-4 and APRI), there are not enough studies to recommend one test over the other.[133, 134] There are concerns that the performance of blood-based markers in individuals who are co-infected is not the same as compared with patients who are mono-infected with HCV.[135]

Comparative data using blood-based NILDA for ALD, PBC, and PSC are limited. In a prospective study in patients with ALD, ELF (cutoff 10.5), and FibroTest (cutoff 0.58) identified advanced liver fibrosis in both primary and specialty care with high diagnostic accuracy and outperformed nonproprietary markers (FIB-4 and APRI).[136] However, all tests (proprietary and nonproprietary) had an AUROC > 0.8. Proprietary markers slightly overestimated the probability of advanced fibrosis in patients from primary care, showing that the studies of accuracy likely had selection bias toward patients with more advanced fibrosis. In small studies in patients with PBC, both nonproprietary (FIB-4 and APRI) and proprietary markers (FibroTest and ELF) may have been comparable in staging fibrosis.[137, 138] APRI and FIB-4 have been studied in other liver diseases such as hemochromatosis. For example, a recent study in 181 C282Y homozygotes for the hereditary hemochromatosis gene showed both APRI and FIB-4 to have excellent performance (AUROC 0.86–0.88) with 81% accuracy in predicting advanced fibrosis.[139]

Quality of Evidence and Other Considerations

A meta-analysis supporting PICO 2 provided imprecise diagnostic estimates and was derived from studies that mostly had a low risk of bias.[94] The quality of evidence was judged to be moderate for sensitivity and specificity estimates.

PICO 3: In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HIV-HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, is the combination of two blood-based biomarker panels superior to a single one for staging fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?

Guidance Statements

6. In patients with chronic untreated HCV, the AASLD suggests a sequential combination of blood-based markers may perform better than a single biomarker for F2-4 or F4 (ungraded statement).

7. In patients with NAFLD, the AASLD suggests the sequential combination of blood-based NILDA may be considered for diagnosis of advanced fibrosis (F3-4) over using a single test alone (ungraded statement).

Technical Remarks

- Very few studies are available that have solely compared the combination of serum biomarkers to a single biomarker in assessing fibrosis with histopathology as reference.
- Because simple single blood-based NILDA such as APRI, FIB-4, and NFS with upper and lower cutoffs frequently have indeterminate results, adding a second blood-based test may help to better classify patients according to fibrosis severity.
- Analyses supporting PICO 3 provided imprecise diagnostic estimates and were derived from studies that mostly either had a high or unclear risk of bias. The quality of evidence was judged to be low for sensitivity and specificity estimates
- For identifying patients with NAFLD advanced fibrosis, the AASLD recommended a sequential approach with FIB-4 followed by imaging NILDA or ELF in FIB-4 $\geq$ 1.3 when available.[3, 4, 140]

Evidence and Rationale

HCV

In an international multicenter study involving 2035 untreated patients and using sequential algorithms that combined APRI and FibroTest™, the diagnostic accuracy was higher in detecting significant fibrosis F2-F4 (90%) and cirrhosis F4 (92%) compared with either test alone (65%–82%).[141] In HCV, when combined, APRI and FIB-4 have excellent NPV to exclude advanced fibrosis.[142]

HBV

Several studies have addressed various combinations of blood-based markers, but most of these have been performed in combination with imaging-based elastography. In one study, the

combination of FIB-4 and APRI had limited sensitivity (<64%) for F2-4 or F3-4.[143] A combination of five blood-based markers achieved an acceptable diagnostic accuracy of 76% in a small sample size of 70 patients with HBV. Sensitivity, specificity, PPV, and NPV were 87%, 70%, 60%, and 91%, respectively, for significant fibrosis.[144]

NAFLD

In a study using sequential analysis, the combination of FIB-4 and ELF did not achieve better diagnostic accuracy than FIB-4 alone.[131] Using various cutoffs, a meta-analysis showed that a combination of NFS and FIB-4 is better than BARD (a score derived from the BMI, AST/ALT ratio, and presence of type 2 diabetes mellitus [T2DM]) alone.[127] Another study in 407 patients with NAFLD indicated that the parallel combination of NFS+FIB-4 resulted in an AUC of 0.81 for F3-4 but with higher misclassification/indeterminate rate of 54%.[106] The sequential combination of FIB-4 and NFS resulted in a lower AUC of 0.77 but reduced misclassification/indeterminate rates to 28%.[127] Data from large NAFLD clinical trial cohorts have indicated that the simultaneous use of two noninvasive tests such as NFS or FIB-4 and ELF result in high sensitivity and specificity (0.89–0.99) but were associated with an increased proportion of patients (66%–92%) with nondiagnostic or indeterminate results.[86, 128] There are conflicting data on the diagnostic accuracy of proprietary fibrosis panels (e.g., Fibrometer and ELF) compared with FIB-4 and NFS for detection of fibrosis in NAFLD.[107, 129, 130] In a prospective study of patients with NAFLD in primary care, sequential testing using FIB-4 followed by ELF detected more advanced fibrosis/cirrhosis cases and reduced unnecessary referrals from primary care to secondary care by 80%. However, this pathway was only applicable to approximately one-half of the referrals. Sequential or two-tiered pathways also

improved resource utilization.[145, 146] Novel NASH biomarkers, including markers of apoptosis and cell death, metabolomic and lipidomic markers, oxidative markers, and several combinations, are currently being studied; however, none as yet are sufficiently accurate to be used clinically.[147]

Other CLD

For other chronic liver diseases such as ALD and PBC, no studies as of yet have addressed the question of whether the combination of serum markers is better than a single biomarker with liver histology being the reference.

PICO 4: In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HIV-HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, do serial blood-based biomarker panels accurately predict the natural history of progression of fibrosis or regression of fibrosis in response to therapy relative to serial histopathology as the reference?

Guidance Statements

8. The AASLD suggests against the use of blood-based NILDA tests to follow progression, stability, or regression in histologic stage (as determined by biopsy) in chronic liver disease (ungraded statement).

Technical Remarks

- There are a limited number of blood-based biomarker/longitudinal biopsy studies in HCV from the interferon (IFN) era. There are no studies to assess changes in blood-based biomarkers and fibrosis stage, as determined by biopsy, with DAA therapy. As a result, the optimal interval for repeat measurements for blood-based biomarkers post-SVR is not established.
- There are a small number of longitudinal biopsy studies in HIV-HCV cohorts with variability in the interval among biopsy assessments, scoring systems, and the types of anti-retroviral and HCV antiviral therapy.
- A limited number of studies have assessed biomarker changes with histology following antiviral therapy in patients with HBV. There are no studies that have assessed both serial biomarkers and paired biopsy histologic assessment in other chronic hepatitis cohorts (such as HBeAg positive [immunotolerant phase] or negative [inactive carrier phase] infection).
- Very few paired biopsy studies have been done to assess NILDA in other CLD.

Background

Liver fibrosis can regress after therapy to reduce the precipitating factor (inflammation, necrosis, steatosis, and/or iron overload; Table 8).[95, 96, 115, 125, 126, 148–174]

The terms regression, reversion, and reversal are intended to indicate that fibrosis, even in the setting of histological cirrhosis, decreases. However, these terms are not intended to indicate that the liver returns to normal in architecture and/or fibrosis content, especially in the setting of histologic cirrhosis.[38, 173] Most of the evidence demonstrating fibrosis regression and/or cirrhosis comes from studies that have analyzed large cohorts of patients with HBV or HCV following antiviral therapy.[174–179] There is increasing evidence for the reversibility of fibrosis

in NAFLD, but there remains a relative paucity of longitudinal histologic data with blood-based biomarkers for other liver diseases. One of the major limitations of currently available blood-based biomarkers is that they often misclassify patients with intermediate stages of fibrosis[52, 180] and are not able to differentiate adjacent stage disease.[181] Importantly, extracellular matrix deposition and degradation is not a linear process and varies based on disease etiology.[182, 183] These factors limit the ability of blood-based biomarkers to follow the progression or regression of fibrosis across the spectrum of liver disease.

Evidence and Rationale

HCV

In the DAA era, there has been greater dependence on noninvasive tests, both pre- and post-treatment, to assess liver fibrosis stage. Blood-based biomarker scores appear to decline during treatment and immediately following SVR,[184–187] suggesting that biochemical responses may influence these indices during and immediately following antiviral therapy. Thus, routine use of blood-based biomarkers based on liver inflammation after SVR in patients with advanced fibrosis or cirrhosis is likely to be associated with a substantial underestimation for significant fibrosis,[95, 96] and there are no validated data on the degree of improvement in post-SVR biomarker thresholds that correlate with fibrosis regression.[28]

Although prior studies have assessed both histology and blood-based biomarkers following antiviral therapy in HCV, biomarker associations with fibrosis progression or regression are largely derived in the setting of IFN-based therapy[148–150, 153, 156, 161] or from maintenance IFN and other antifibrotic therapy in virologic nonresponders.[151, 152, 154, 155] We

could not identify large studies with long-term follow-up in patients receiving DAA therapy that included paired biopsy and biomarkers. Paired biopsy and biomarker studies in patients coinfected with HIV-HCV have included mixed cohorts with HCV monoinfection, various IFN-treatment regimens, and variable intervals of histological assessment.[157-162] Only a few studies have reported changes in biomarker indices with fibrosis stage. APRI, FIB-4, or FibroTest algorithms are the most frequently assessed biomarkers (Table 5). The fibrillary collagen formation marker procollagen type III (Pro-CIII) was associated with histologic fibrosis progression at 52 weeks in a chronic HCV nonresponder cohort receiving antifibrotic therapy, but this finding requires validation in other HCV paired-biopsy cohorts.[154] A recent study utilizing both baseline and follow-up FIB-4 after SVR with DAA along with baseline albumin and GGT had acceptable performance (time-dependent AUROC of 0.72–0.74) in excluding those who develop HCC within 3 years,[188] suggesting that blood-based NILDA may be used in the future to help risk-stratify patients for HCC surveillance after SVR.[188–194]

HBV

Antiviral therapy in HBV results in viral suppression and fibrosis regression, including reversal of cirrhosis.[175, 179] Despite the low cost, ease of interpretation, and access advantages in resource-limited settings, simple markers such as APRI and FIB-4 are not able to follow changes in fibrosis. In a cohort of 294 patients receiving antiviral therapy with paired-biopsy assessment, APRI and FIB-4 did not correlate with histologic fibrosis regression observed at 5 years.[124] Biomarkers incorporating transaminases or acute phase reactants will likely demonstrate early biochemical responses that may not reflect histologic regression following antiviral therapy in HBV, resulting in false-negative tests.

NAFLD

The current regulatory landscape requiring assessment of histologic efficacy endpoints in NAFLD therapeutic development has resulted in an increasing number of paired biopsy and biomarker studies reported from large clinical trials (Table 8). The most frequently assessed biomarkers include NFS, FIB-4, APRI, and ELF. Longitudinal data from the NASH CRN on 292 patients with paired biopsies over a median of 2.6 years indicated modest AUROCs (0.66–0.73) for predicting fibrosis progression using simple markers such as FIB-4, APRI, and NFS; fibrosis scores adjusted for baseline fibrosis stage were associated with progression, but not regression, of fibrosis.[126] The prevalence of significant fibrosis was 50% in this study, and the utility of these simple markers alone or in combination with other noninvasive tests, to follow fibrosis progression in lower prevalence settings, remains to be determined. A phase IIb study for NASH CRN stage 3 and 4 noted an improvement in histologic fibrosis by one stage in 18% to 23% of stage 3 patients and in 8% to 13% of patients with baseline cirrhosis.[195] Progression to cirrhosis was observed in 19% to 22% at 96 weeks across the treatment groups. Despite these histologic changes, there were no significant differences observed between the treatment and placebo groups through week 96 in liver biochemistry, ELF score, FibroTest, or NFS.[169] A 12-week clinical trial in 43patients with NAFLD (including 48% with advanced fibrosis) reported significant reductions in PRO-C3 and ELF in patients with histologic response (including improvement in NASH) compared with nonresponders, but a corresponding change in scores with change in fibrosis was not provided.[196] In an ongoing phase III study of 931 patients with NAFLD with stage F2 or F3, an interim analysis of biopsy and several blood markers (FIB-4, APRI, FibroTest, ELF, PRO-C3) indicated weak associations between change in markers and

improvement in fibrosis stage at 18 months.[140] Although multiple studies have noted improvement in NAFLD fibrosis stage following bariatric surgery for patients with class III obesity,[197] very few have incorporated blood-based biomarkers to evaluate for associations with histologic resolution. As with other CLDs, biomarkers that incorporate liver transaminases and acute phase reactants (Table 5) will need to be interpreted with caution following therapies that may improve necroinflammation, but not fibrosis, over a relatively short study duration.[198]

Other CLD

Although small studies in ALD and cholestatic disease have examined blood-based NILDA in cross-sectional assessments, for following disease progression or for determining prognosis, none have specifically evaluated blood-based biomarkers for following changes in fibrosis on biopsy. A recent phase II study in 234 patients with PSC evaluated FibroTest and ELF in relation to serial biopsy assessment at 96 weeks. Association and directional change in biomarker indices with observed fibrosis change at week 96 were not provided.[115]

PICO 5: In patients with NAFLD, are blood-based biomarker panels accurate in grading hepatic steatosis (S0 vs. S1-3, S0-1 vs. S2-3, and S0-2 vs. S3) using histopathology or magnetic resonance (MR) spectroscopy (MRS) or magnetic resonance imaging (MRI)-proton density fat fraction (PDFF) as the reference?

Guidance Statements

9. The AASLD suggests against the use of blood-based NILDA to detect steatosis in pateints with NAFLD (ungraded statement).

Technical Remarks

- In adult patients with CLD, ~~time to echo~~ Controlled attenuated parameter (CAP) and MRI can reliably quantify the degree of steatosis. MRI-PDFF and MRS have excellent correlation with histology for detecting and grading steatosis and can be used as reference standards.[3]
- Steatosis, independent of fibrosis, is associated with increased systemic inflammation and has prognostic importance as a predictor of cardiovascular disease, DM, and, in severe cases, liver-related mortality.
- Patients with chronic liver disease associated with steatosis other than NASH, such as chronic HCV genotype 3, have not been well-studied.
- The available evidence is insufficient to make a recommendation as to which noninvasive test(s) or algorithm(s) should be used, compared with others, to assess steatosis.
- There is insufficient evidence to recommend blood tests as clinical endpoints to monitor changes in steatosis, independent of fibrosis over time.
- There is insufficient evidence to make a recommendation regarding a specific blood-based test or algorithm to use in combination with imaging-based testing for the assessment of steatosis.
- Because BMI is included in many of the indices, caution is necessary when using NILDA to assess steatosis in patients who have undergone bariatric surgery.

Background

Although liver fibrosis assessment has been the focus of noninvasive tests in liver diseases, steatosis is also important in the assessment of disease severity in NAFLD.

Histologically, steatosis (S) is graded 0 to 3 based on the proportion of hepatocytes that contain fat as follows: S0 (<5%), S1 (5%–33%), S2 (34%–66%), and S3 (>66%) steatosis (Table 3b).[21, 22] In addition to liver-related outcomes in NASH (decompensation, HCC),[198, 199] steatosis is associated with systemic inflammatory markers,[200, 201] DM,[202–204] the metabolic syndrome,[205] cardiovascular disease,[203, 204, 206–209] and atherosclerosis.[210] Several noninvasive algorithms have been developed to assess steatosis using biochemical and clinical variables.[211, 212] Although many steatosis algorithms have been developed or validated based on ultrasound (US)[202, 213–217, 220, 221] several have utilized histologic[182, 217–221] or MR-based assessments[205, 222, 223] as the reference standard (Table 9). However, there are limited data to support longitudinal assessments of steatosis using these algorithms.[25]

Evidence and Rationale

Most algorithms include standard liver-related blood tests (AST, ALT, bilirubin, GGT), blood tests associated with hyperlipidemia (triglycerides [TG], cholesterol), and conditions associated with steatosis (DM, increased BMI, increased waist circumference [WC], and the metabolic syndrome) in some combination (Table 9). Of note, some algorithms differ by sex. Table 10 summarizes the performance and cutoffs for algorithms to assess steatosis.[202, 205, 217–223, 225–231]

Fatty liver index (FLI)

This algorithm utilizes TG, BMI, WC, and GGT. Although initially developed in comparison to conventional B-mode US,[214, 217] FLI has also been validated against liver histology and MRI.[205, 219–221, 228, 232] Depending on the cutoff, studies have shown sensitivity

ranges from 44% to 100%, whereas specificity ranged from 3% to 91% with AUROC 0.59 to 0.86. Furthermore, a FLI modified for North American patients (compared with non-North American patients) and including age, race and ethnicity, fasting insulin, and glucose seemed to perform better in a US population.[233]

Hepatic steatosis index (HSI)

This algorithm includes AST, ALT, BMI, and GGT. Although initially developed in a cohort compared with US,[213] HSI has also been validated against liver histology and MRI.[204, 219, 220, 228] Depending on the cutoff, HSI had a sensitivity ranging from 7% to 88%, specificity ranging from 9% to 93%, and AUROC 0.49 to 0.81. One advantage of HSI is its simplicity because it uses routine tests and does not require additional factors such as WC or insulin resistance to be measured. However, one limitation is that those with increased BMI, especially if over age 40 years, will have an increased HSI, which may explain its poor performance is some studies.[219, 230] Similar factors make HSI less reliable in the bariatric population.

Lipid accumulation product (LAP)

The lipid accumulation product was developed from the National Health and Nutrition Examination Survey to assess cardiovascular disease [200] and has been used to detect hepatic steatosis.[215]. The index includes only two variables: WC and TG. The index has been compared with both liver biopsy[218, 228] and MR,[216] with performance in assessing steatosis as a continuous variable with AUROC 0.68 to 0.73.

NAFLD liver fat score

The NAFLD liver fat score was developed against MRS and included the presence or absence of the metabolic syndrome and DM along with fasting insulin and AST and ALT.[222] Depending on the cutoff,[220, 222, 225] the sensitivity was 65% to 86%, specificity was 62% to 87%, and AUROC was 0.64 to 87.

Index of NAFLD

In a study of 152 patients with NAFLD from a cohort of 861 identified by increased echogenicity in the United States, the index of NAFLD (composed of waist-to-hip ratio, TG, ALT, and Homeostatic Model Assessment of Insulin Resistance) was developed and compared with FLI.[202] Depending on the cutoff, the sensitivity was 60% to 81%, specificity was 56% to 82%, and AUROC was 0.77.

SteatoTest®

This biomarker was developed based on the FibroTest™ and ActiTest® (AT), validated biomarkers for fibrosis and inflammation, respectively.[182, 218, 235] SteatoTest includes the six components of FibroTest-AT (ALT, α-2 macroglobulin, apolipoprotein A-1, haptoglobin, total bilirubin, GGT) and adds BMI, total cholesterol, TG, and glucose adjusted for age and sex.[214] This biomarker for steatosis has been used in those at high risk for NAFLD.[217, 225, 227, 236] One limitation of SteatoTest is the inclusion of total bilirubin, which can be increased in conditions such as Gilbert's syndrome. To overcome this, a modified version (SteatoTest-2®) has recently been developed that does not include BMI or bilirubin[230] for those with increased unconjugated bilirubin or inaccurate or unavailable BMI. Depending on the cutoff, SteatoTest-2 has a

sensitivity ranging from 38% to 90%, specificity ranging from 44% to 88%, and AUROC from 0.65 to 0.81.

TG-glucose index

The TG-glucose index was developed as a screening tool for insulin resistance.[239] When used to determine whether NAFLD was present,[220, 229, 238] it had an overall sensitivity of 70% to 94%, specificity of 60% to 92%, and AUROC of 0.68 to 0.90.

Visceral adiposity index

Increased visceral adiposity is associated with NAFLD.[239–241] There are limited studies in NAFLD using liver histology as the reference standard.[220] With a cutoff of 1.25, the visceral adiposity index showed a sensitivity of 79%, specificity of 92%, and AUROC of 0.92.

Dallas steatosis index

The Dallas steatosis index was developed from the Dallas Heart Study, a multiethnic, population-based, probability study of adults (age 18–65 years) to detect at least 5.5% steatosis by MRS.[242] The index, which includes ALT, BMI, age, sex, TG and glucose levels, DM, hypertension, and ethnicity, had a c-statistic of 0.824; it outperformed HSI (0.746) and overlapped with the FLI (0.810). However, the Dallas steatosis index has not been validated compared with liver histology as the reference standard.

PICO 6: In pediatric chronic liver disease (HCV, HBV, biliary atresia [BA], cystic fibrosis [CF] liver disease [CFLD], and NAFLD/NASH), are blood-based biomarkers accurate in

staging hepatic fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?

Guidance Statements

10. In the pediatric patients with chronic liver disease, the AASLD suggests the use of simple, cost-effective, and readily available blood-based NILDA, such as APRI or FIB-4, for the detection of advanced fibrosis (F3-4) (ungraded statement).

Technical Remarks

- Some blood-based NILDA in children have good accuracy in detecting advanced fibrosis but have difficulty discriminating earlier stages of fibrosis.
- FIB-4 does not perform as well in children as it does in adults, particularly very young children, due to the inclusion of age in the index.
- Rapid growth in children and attendant fluctuations in alkaline phosphatase can confound interpretation of blood or collagen-based NILDA tests in pediatric liver disease.
- There are insufficient biopsy validated data to recommend biomarkers for evaluating fibrosis in pediatric NASH and □1AT at this time.
- In the pediatric population with CLD, there is growing but insufficient evidence to recommend blood-based NILDA as endpoints to monitor changes in fibrosis over time.

Background

Inherited or acquired liver disorders of childhood such as BA, α1AT, and CFLD often and uniquely progress to cirrhosis and portal hypertension early in life. With the exception of NAFLD/NASH, HBV, and HCV, the majority of pediatric liver disorders that lead to advanced fibrosis and commonly require liver transplantation are hepatobiliary in nature. The rapid progression of liver disease in some children indicates a need to identify early markers of liver fibrosis to help facilitate early intervention. Markers empirically identified by genomic, proteomic, and metabolomic technologies, as well as targeted blood-based marker analysis, offer new strategies to predict outcomes in pediatric liver diseases. Putative growth-independent blood biomarkers reflecting matrix deposition, removal, and remodeling; hepatic stellate cell activation; collagen turnover; and chemoattractant expression in children with a variety of liver diseases have been identified.[243–245]

Most blood biomarker studies in children, even when validated by liver biopsy, are single-center investigations. Furthermore, many direct blood-based biomarkers are confounded by rapid somatic growth in children with liver disease. Although evolving anti-fibrogenic therapies and novel markers/endpoints for clinical trials are being studied, there are currently limited data to support longitudinal assessments of fibrosis using blood biomarkers in children. APRI, FIB-4, and FibroTest™ have been the most commonly studied NILDA tests in children; there is much less information regarding other NILDA tests such as ELF™, Fibrometer™, Fibrospect II™, eLIFT, King's fibrosis score, and Hepascore as surrogates of liver fibrosis, as validated by histology in pediatric populations.

Evidence and Rationale

Each pediatric liver disorder has a distinct pathophysiology with both genetic and epigenetic origins. These disorders are clinically heterogeneous; therefore, the performance of blood biomarkers as surrogates of liver fibrosis must be studied and compared within individual disease groups rather than in conglomerate or even by biomarker.

BA

BA is a neonatal liver disease characterized by rapidly progressive fibro-obliteration of the biliary tract and is the leading indication for pediatric liver transplantation.[246, 247] In BA, fibrosis typically develops early in life and leads to cirrhosis before age 6 months (without Kasai portoenterostomy) and would be an ideal target for newly developed anti-fibrotic pharmacotherapies.[247] The utility of APRI to assess or predict liver fibrosis in BA is mixed in the current literature.

In a study of 260 children with BA, an APRI > 1.22 was able to identify cirrhosis (at the time of presentation) with an AUROC of 0.83 (sensitivity 75% and specificity 84%).[248] In a much smaller Korean study of 35 infants with BA, the AUROC of APRI to distinguish F3-4 was 0.92 and F4, 0.91 using optimal cut-points of 1.01 and 1.41, respectively,[249] consistent with the thresholds proposed by Grieve et al.[248, 250] In a retrospective study of 91 infants with BA, METAVIR fibrosis was also significantly correlated with APRI ($R_s = 0.433$; $p < 0.05$).[251] The mean APRI value was 0.76 in METAVIR F0-F1, 1.29 in F2-3, and 2.51 in F4 ($p < 0.001$). The AUROC of APRI for diagnosing F2-3 and F4 was 0.75 and 0.81, respectively. The APRI cutoff of 0.95 was 61% sensitive and 76% specific for F2-3, and a threshold of 1.66 was 71% sensitive and 83% specific for F4.

However, in another study of 29 patients with BA, APRI showed no significant correlations with METAVIR or Ishak global fibrosis scores.[251] In a Chinese study of 24 children with BA (mean age 6.6 years) with prior Kasai portoenterostomy early in life undergoing liver biopsy, participants with METAVIR F0-2 had a median APRI and FIB-4 of 0.82 (vs. 1.9, $p = 0.053$) and 0.4 (vs. 0.22, $p = 0.49$), respectively, compared with F3-4.[252] APRI had a positive correlation with fibrosis stage ($r = 0.583$) and showed significant differences between different fibrosis stages ($p = 0.035$), whereas FIB-4 did not. However, the AUROC of APRI for predicting F4 was only 0.56. Interestingly, in an Indian study of 48 children with neonatal cholestasis without BA, the mean APRI for METAVIR F0-3 was 1.38, whereas, for F4, it was 3.74. However, using an APRI threshold of 1.38, the AUROC to detect F4 among non-BA cholestatic infants was 0.75 with a sensitivity of 100% but a specificity of only 21.4%, thereby limiting its efficacy.

CFLD

CF is the most commonly inherited disease in Caucasian individuals manifesting in children. CFLD, with the development of portal hypertension, represents the third most common cause of death in CF, second only to pulmonary disease and lung transplant complications. Up to 7.5% of those with CF develop CFLD, and this typically becomes evident at a young age (median age 10.5 years). Liver biopsy is not essential to diagnose CFLD and thereby is not part of routine clinical care in the United States. However, a study comparing 51 Australian children with CFLD who underwent dual-pass liver biopsy with 104 age- and sex-matched children without CFLD demonstrated that APRI and FIB-4 not only identified those with CFLD but could

provide information about severity of fibrosis.[253] APRI had an AUROC of 0.8 for predicting advanced fibrosis, and a score >0.462 indicated sevenfold increased odds of advanced fibrosis.

HBV

Cirrhosis in children with HBV is rare given that the majority of children are immunotolerant, although finding some degree of fibrosis (i.e., F2-3) in pediatric patients with HBV is not uncommon. In a Polish study of 71 children (age 4–17 years; mean age 10 years; mean ALT 83 IU/L) with biopsy-proven chronic HBV (HBeAg positive) and confirmed HBV DNA replication prior to antiviral treatment, 34 (48%) had advanced fibrosis. An APRI of >0.59 differentiated children with significant fibrosis, with an AUROC of 0.75 PPV = 70% and NPV = 77%.[254]

In a cohort study of 36 pediatric patients (up to age 20 years) with chronic HBV or HCV, the AUROC of APRI was 0.71 for identifying patients with any fibrosis (METAVIR classification) and 0.52 for identifying patients with cirrhosis.[255] By disease, however, APRI had only modest performance characteristics when predicting fibrosis in patients with HBV and HCV (0.64 and 0.75, respectively) and in children age >13 years old (0.65).

FibroTest-ActiTest™ has been validated in adults with chronic HCV infection as a noninvasive alternative to liver biopsy, but there are few data of its use in children with HBV. In a Scandinavian study of FibroTest in 25 children with HBV, there was no correlation between FibroTest scores and histological stage of fibrosis.[256]

HCV

Cirrhosis is uncommon in children but has been reported. Studies examining the use of APRI or FIB-4 to assess fibrosis in children with HCV have been scarce. In an Egyptian study of 48 children with HCV, the AUROC curve for predicting significant fibrosis (F2-4 METAVIR) was 0.49 with APRI, which is not a clinically useful test.[257]

In a prospective study of 50 Egyptian children with chronic HCV who had FibroTest measurements at the time of liver biopsy, the median FibroTest level increased linearly with advancing fibrosis stage. FibroTest^{TM} values were 0.16 (0.07–0.25) in F0, 0.19 (0.18–0.24) in F1, 0.41 (0.20–0.66) in F2, 0.54 in F3, and 0.66 (0.43–0.77) in F4.[258] A significant correlation was also found between individual FibroTest^{TM} values and fibrosis stage, $r = 0.81$. At a FibroTest^{TM} cutoff of 0.25, and the AUROC to differentiate F2-4 from F0-1 was 0.97 with 92% sensitivity and 96% specificity. Utilizing a higher FibroTest^{TM} cutoff of 0.54, the AUROC was 0.92 to discriminate between F3-4 versus F0-2 with 71% sensitivity and 91% specificity.

There is also some limited evidence of discordance between FibroTest^{TM} and METAVIR scores in children with HCV. In a small Polish study of 10 children with chronic HCV with FibroTest^{TM}, there was no correlation of FibroTest^{TM} values with advancing METAVIR fibrosis staging.[259] There was also discordance between FibroTest^{TM} and METAVIR in 30% of cases, suggesting that FibroTest^{TM} values correlate poorly with histopathological stage.

In conclusion, blood-based NILDA tests in children vary widely in their accuracy, even in detecting F3-4 fibrosis, and have difficulty discriminating earlier stages of fibrosis. These tests

also have different disease-specific thresholds that correlate with histopathologic fibrosis and differ from adults. APRI and FIB-4 have been the most studied NILDA tests in children, but there is still insufficient evidence to recommend blood biomarkers as endpoints to monitor changes in fibrosis over time. Any blood-based NILDA that includes age (Table 5) should be used cautiously in children.

Quality of Evidence and Other Considerations

Analyses supporting PICO 6 were based on very few studies and meta-analysis was not feasible. The quality of evidence was judged to be low for sensitivity and specificity estimates due to severe imprecision.

A simplified blood-based NILDA algorithm for detection of fibrosis and steatosis

In an effort to facilitate the incorporation of blood-based NILDA into clinical practice, the AASLD NILDA Writing Group developed an algorithm intended to be used by clinicians in need of a readily available and simple decision support tool (Figure 1). This algorithm was developed with the summary NILDA evidence highlighted earlier. We recommend that fibrosis staging begin with simple blood-based NILDA, including simple nonproprietary tests because of their wide availability and performance compared to proprietary tests, although these can be used where available. The left side of the algorithm aims to rule out advanced fibrosis. Nonproprietary blood-based NILDA such as FIB-4 and NFS have sensitivities ranging from 60% to 75% for ruling out significant fibrosis and 75% to 85% for advanced fibrosis (depending on test cutoff and disease etiology) and the lowest negative likelihood ratios at proposed cutoff values across etiologies per our systematic review.[94] Of the three major nonproprietary NILDA (FIB-4, APRI,

and NFS in NAFLD), FIB-4 appears to have superior performance, particularly for the identification of F3-4 stages of fibrosis,[94] which is the spectrum of fibrosis for which the tests were designed.[42] NFS can be considered an equivalent to FIB-4 in patients with NAFLD in the assessment of advanced fibrosis.[45] Thus, in the appropriate clinical setting (i.e., low pre-test probability), these tests should suffice to rule out significant/advanced fibrosis. A FIB-4 cutoff threshold of 1.3 has been proposed as accurate to rule out F3-4 in NAFLD patients,[260] and our systematic review indicated a higher sensitivity, as expected for the lower FIB-4 cutoff 1.3, but higher DOR for the standard 1.45 threshold.[94] Confirmatory testing such as imaging-based NILDA should be performed for patients with values between the lower and upper thresholds. For those with blood-based values above the threshold for advanced fibrosis, imaging-based NILDA can be considered for confirmation and patients should be referred for HCC surveillance per AASLD guidelines.[261] These thresholds correspond to the highly specific cutoff values validated for the recognition of advanced fibrosis (FIB-4 and NFS, specificity of 91% to 97%) across etiologies (except for NFS, which is only for NAFLD) per our systematic review;[94] a revised upper FIB-4 cutoff value of 2.67 has been proposed to rule in F3-4 in NAFLD,[68] and although our systematic review indicated a lower DOR for the standard upper FIB-4 threshold of 3.25, both cutoff values had similar high specificity of 94% to “rule-in” advanced fibrosis in NAFLD patients.[94] Although imaging-based NILDA are more accurate than blood-based NILDA in some situations, elastography methods are not as not widely available. As imaging-based NILDA become more readily available in practice, their sequential incorporation with blood-based NILDA in clinical decision-making is expected to grow. Whenever more granularity is needed (i.e., start of antiviral treatment for a patient with HBV and significant fibrosis, initiating HCC surveillance), clinicians should refer to the associated NILDA Systematic

Reviews that have more detail on NILDA[4, 6, 94] or specific guidance documents.[3, 5] Per our systematic review, blood-based NILDA for steatosis are not accurate enough for daily practice,[94] and the AASLD NILDA Writing Committee recommends utilizing imaging-based NILDA for the identification of steatotic liver disease.[3]

Summary

NILDA has replaced liver biopsy in clinical practice in many situations. Because of the rapid evolution of the field and predetermined requirements for studies to be incorporated in our systematic reviews, we were not able to include every published study on the topic; in particular, studies with smaller sample sizes, those that did not have liver histology to assess fibrosis or, for fatty liver, did not have histology/MRS/MR-PDFF as the reference standard. Many studies with mixed etiologies or overlapping diseases were excluded. In blood-based NILDA with upper and lower thresholds to rule in or out fibrosis severity, up to one-third of patients can have indeterminate ranges that require additional diagnostic tests such as imaging-based NILDA (see *AASLD Practice Guideline: Imaging-Based Non-Invasive Liver Disease Assessments [NILDA] of Hepatic Fibrosis and Steatosis*).[3]

Future Research

Although substantial progress has been made in the area of NILDA, there are still many opportunities for future research. In the era of precision medicine, high-throughput technologies applied to experimental models will continue to generate a wealth of novel disease and injury-specific blood-based biomarkers for dynamic fibrosis assessment. Selection and validation of candidate biomarkers for fibrosis assessment from these multi-omics databases will be challenging. Progress in this field requires a paradigm shift from using a static and semi-

quantitative assessment of fibrosis as the reference standard, towards developing dynamic disease-specific models of clinical relevance that are associated with outcomes. Our writing group identified several major areas for future research that are needed, as detailed in Table 11.

Funding

Funding for the development of this Practice Guideline was provided by the American Association for the Study of Liver Diseases.

Acknowledgments

We thank Ruben Hernaez and Alfred Sidney Barritt IV and the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines Committee (PGC), for their expertise, patience, and editorial guidance, Elizabeth C. Verna, Chair, Cynthia Levy, Chair-Eelect, Saul Karpen, Goberning Board Liaison, Scott W. Biggins, Therese Bittermann, Po-Hung (Victor) Chen, Kathleen E. Corey, Albert Do, Juan F. Gallegos-Orozco, Lindsay Y. King, Christina C. Lindenmeyer, Jessica L. Mellinger, Anthony J. Michaels, Arpan Mohanty, Andrew Moon, Nadia Ovchinsky . Archita Parikh Desai, Jennifer C. Price, Elizabeth Rand, Adrienne Simmons, Ashwani K. Singal, Christopher Shubert, and Puneeta Tandon. We thank Audrey Davis-Owino at AASLD. We thank Marie Kreck at Virginia Commonwealth University for editorial assistance.

References

1. Moon AM, Singal AG, Tapper EB. Contemporary epidemiology of chronic liver disease and cirrhosis. *Clin Gastroenterol Hepatol* 2020;18(12):2650-2666.
2. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. *J Hepatol* 2019;70(1):151-171.
3. Sterling RK, Duarte-Rojo A, Patel K, Asrani SK, Alsawas M, Dranoff J, et al. AASLD Practice Guideline on imaging-based non-invasive liver disease assessments of hepatic fibrosis and steatosis. Hepatology. 2024; doi: 10.1097/HEP.0000000000000843.
4. Duarte-Rojo A, Taouli B, Leung DH, Levine D, Nayfeh T, Hasan B, et al. Imaging-based non-invasive liver disease assessment for staging liver fibrosis in chronic liver disease: A systematic review supporting the AASLD Practice Guideline. Hepatology. 2024; doi:10.1097/HEP.0000000000000852.
5. Sterling RK, Asrani SK, Levine D, Duarte-Rojo A, Patel K, Fiel MI, et al. AASLD Practice Guideline on non-invasive liver disease assessments of portal hypertension. Hepatology. 2024; doi: 10.1097/HEP.0000000000000844.
6. Rockey DC, Alsawas M, Rojo-Duarte A, Patel K, Levine D, Asrani SK, et al. Non-invasive liver disease assessment (NILDA) to identify portal hypertension – systematic and narrative reviews supporting the AASLD practice guideline. Hepatology. 2024. doi: 10.1097/HEP.0000000000000841.
7. Mack CL, Adams D, Assis DN, et al. Diagnosis and management of autoimmune hepatitis in adults and children: 2019 practice guidance and guidelines from the American Association for the Study of Liver Diseases. *Hepatology* 2020 Aug;72(2):671-722.
8. Schunemann HJ, Mustafa RA, Brozek J, et al. GRADE guidelines: 21 part 1. Study design, risk of bias, and indirectness in rating the certainty across a body of evidence for test accuracy. *J Clin Epidemiol* 2020;122:129-141.
9. Schunemann HJ, Mustafa RA, Brozek J, et al. GRADE guidelines: 21 part 2. Test accuracy: inconsistency, imprecision, publication bias, and other domains for rating the certainty of evidence and presenting it in evidence profiles and summary of findings tables. *J Clin Epidemiol* 2020;122:142-152.
10. Kanwal F, Tapper EB, Ho C, et al. Development of quality measures in cirrhosis by the practice metrics committee of the american association for the study of liver diseases. *Hepatology* 2019;69(4):1787-1797.
11. Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001;344(7):495-500.
12. Froehlich F, Lamy O, Fried M, Gonvers JJ. Practice and complications of liver biopsy. Results of a nationwide survey in Switzerland. *Dig Dis Sci* 1993;38(8):1480-1484.
13. Rockey D, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology* 2009;49(3):1017-1044.
14. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991;13(3):372-374.
15. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* 1995;19(12):1409-1417.
16. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1:431-435.
17. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22(6):696-699.

18. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994;20(1):15-20.
19. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol* 1978;379(2):103-112.
20. Altamirano J, Miquel R, Katoonizadeh A, et al. A histologic scoring system for prognosis of patients with alcoholic hepatitis. *Gastroenterology* 2014;146(5):1231-1239.e1231-1236.
21. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41(6):1313-1321.
22. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94(9):2467-2474.
23. Theise ND. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. *Mod Pathol* 2007;20 Suppl 1:S3-14.
24. Mannan R, Misra V, Misra SP, Singh PA, Dwivedi M. A comparative evaluation of scoring systems for assessing necro-inflammatory activity and fibrosis in liver biopsies of patients with chronic viral hepatitis. *J Clin Diagn Res* 2014;8(8):FC08-FC12.
25. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. *J Hepatol* 2003;38(2):223-229.
26. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA* 2012;308(24):2584-2593.
27. Germani G, Hytiroglou P, Fotiadu A, Burroughs AK, Dhillon AP. Assessment of fibrosis and cirrhosis in liver biopsies: an update. *Semin Liver Dis* 2011;31(1):82-90.
28. European Association for Study of Liver, Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. *J Hepatol* 2015;63(1):237-264.
29. Lackner C, Bataller R, Burt A, et al. Fibrosis evaluation by transient elastography in alcoholic liver disease: Is the histological scoring system impacting cutoff values? *Hepatology* 2017;65(5):1758-1761.
30. Yip WW, Burt AD. Alcoholic liver disease. *Semin Diagn Pathol* 2006;23(3-4):149-160.
31. Michalak S, Rousselet MC, Bedossa P, et al. Respective roles of porto-septal fibrosis and centrilobular fibrosis in alcoholic liver disease. *J Pathol* 2003;201(1):55-62.
32. Cholongitas E, Senzolo M, Standish R, et al. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006;125(5):710-721.
33. Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* 2003;39(2):239-244.
34. Guha IN, Myers RP, Patel K, Talwalkar JA. Biomarkers of liver fibrosis: what lies beneath the receiver operating characteristic curve? *Hepatology* 2011;54(4):1454-1462.
35. Duarte-Rojo A, Altamirano JT, Feld JJ. Noninvasive markers of fibrosis: key concepts for improving accuracy in daily clinical practice. *Ann Hepatol* 2012;11(4):426-439.
36. Rockey D. Liver fibrosis reversion after suppression of hepatitis b virus. *Clin Liver Dis* 2016;20(4):667-679.

37. Rockey DC, Friedman SL. Fibrosis regression after eradication of hepatitis c virus: from bench to bedside. *Gastroenterology* 2021;160(5):1502-1520.e1501.
38. Wanless IR, Nakashima E, Sherman M. Regression of human cirrhosis. Morphologic features and the genesis of incomplete septal cirrhosis. *Arch Pathol Lab Med* 2000;124:1599-1607.
39. Hytiroglou P, Theise ND. Regression of human cirrhosis: an update, 18 years after the pioneering article by Wanless et al. *Virchows Arch* 2018;473(1):15-22.
40. Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. *Acta Paediatr* 2007;96(3):338-341.
41. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;38(2):518-526.
42. 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(6):1317-1325.
43. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45(4):846-854.
44. Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007;45(2):297-306.
45. Cross TJ, Rizzi P, Berry PA, Bruce M, Portmann B, Harrison PM. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2009;21(7):730-738.
46. Boursier J, de Ledinghen V, Leroy V, et al. A stepwise algorithm using an at-a-glance first-line test for the non-invasive diagnosis of advanced liver fibrosis and cirrhosis. *J Hepatol* 2017;66(6):1158-1165.
47. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001;357(9262):1069-1075.
48. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004;127(6):1704-1713.
49. Patel K, Gordon SC, Jacobson I, et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004;41(6):935-942.
50. Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005;51(10):1867-1873.
51. Cales P, Oberti F, Michalak S, et al. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005;42(6):1373-1381.
52. Cales P, de Ledinghen V, Halfon P, et al. Evaluating the accuracy and increasing the reliable diagnosis rate of blood tests for liver fibrosis in chronic hepatitis C. *Liver Int* 2008;28(10):1352-1362.
53. Nguyen-Khac E, Thiele M, Voican C, et al. Non-invasive diagnosis of liver fibrosis in patients with alcohol-related liver disease by transient elastography: an individual patient data meta-analysis. *Lancet Gastroenterol Hepatol* 2018;3(9):614-625.

54. Petta S, Wai-Sun Wong V, Bugianesi E, et al. Impact of obesity and alanine aminotransferase levels on the diagnostic accuracy for advanced liver fibrosis of noninvasive tools in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol* 2019;114(6):916-928.
55. Wong GL, Wong VW, Choi PC, et al. Increased liver stiffness measurement by transient elastography in severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2009;24(6):1002-1007.
56. Liu CH, Liang CC, Huang KW, et al. Transient elastography to assess hepatic fibrosis in hemodialysis chronic hepatitis C patients. *Clin J Am Soc Nephrol* 2011;6(5):1057-1065.
57. Taneja S, Borkakoty A, Rathi S, et al. Assessment of liver fibrosis by transient elastography should be done after hemodialysis in end stage renal disease patients with liver disease. *Dig Dis Sci* 2017;62(11):3186-3192.
58. Schmoyer CJ, Kumar D, Gupta G, Sterling RK. Diagnostic accuracy of non-invasive tests to detect advanced hepatic fibrosis in patients with hepatitis c and end-stage renal disease. *Clin Gastroenterol Hepatol* 2020;18(10):2332-2339.e1.
59. Vuppalanchi R, Weber R, Russell S, et al. Is fasting necessary for individuals with nonalcoholic fatty liver disease to undergo vibration-controlled transient elastography? *Am J Gastroenterol* 2019;114(6):995-997.
60. Murawaki Y, Idobe Y, Ikuta Y, et al. Influence of a history of gastrectomy for gastric cancer on serum hyaluronan concentration in normal individuals and patients with chronic liver disease. *Hepatology Research* 1998;10(3):248-254.
61. Su Y, Gu H, Weng D, et al. Association of serum levels of laminin, type IV collagen, procollagen III N-terminal peptide, and hyaluronic acid with the progression of interstitial lung disease. *Medicine (Baltimore)* 2017;96(18):e6617.
62. Koh C, Turner T, Zhao X, et al. Liver stiffness increases acutely during sickle cell vaso-occlusive crisis. *Am J Hematol* 2013;88(11):E250-E254.
63. Lindqvist U, Laurent TC. Serum hyaluronan and aminoterminal propeptide of type III procollagen: variation with age. *Scand J Clin Lab Invest* 1992;52(7):613-621.
64. Fraser JR, Gibson PR. Mechanisms by which food intake elevates circulating levels of hyaluronan in humans. *J Intern Med* 2005;258(5):460-466.
65. Qureshi K, Clements RH, Abrams GA. The utility of the "NAFLD fibrosis score" in morbidly obese subjects with NAFLD. *Obes Surg* 2008;18(3):264-270.
66. Wong VW, Wong GL, Chim AM, et al. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. *Am J Gastroenterol* 2008;103(7):1682-1688.
67. 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(2):454-462.
68. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010;59(9):1265-1269.
69. Ruffillo G, Fassio E, Alvarez E, et al. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. *J Hepatol* 2011;54(1):160-163.
70. Xun YH, Fan JG, Zang GQ, et al. Suboptimal performance of simple noninvasive tests for advanced fibrosis in Chinese patients with nonalcoholic fatty liver disease. *J Dig Dis* 2012;13(11):588-595
71. Sumida Y, Yoneda M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012;12:2.

72. Cichoz-Lach H, Celinski K, Prozorow-Krol B, Swatek J, Slomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. *Med Sci Monit* 2012;18(12):CR735-C740.
73. Yoneda M, Imajo K, Eguchi Y, et al. Noninvasive scoring systems in patients with nonalcoholic fatty liver disease with normal alanine aminotransferase levels. *J Gastroenterol* 2013;48(9):1051-1060.
74. Lee TH, Han SH, Yang JD, Kim D, Ahmed M. Prediction of advanced fibrosis in nonalcoholic fatty liver disease: an enhanced model of BARD score. *Gut Liver* 2013;7(3):323-328.
75. Demir M, Lang S, Nierhoff D, et al. Stepwise combination of simple noninvasive fibrosis scoring systems increases diagnostic accuracy in nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2013;47(8):719-726.
76. Cui J, Ang B, Haufe W, et al. Comparative diagnostic accuracy of magnetic resonance elastography vs. eight clinical prediction rules for non-invasive diagnosis of advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease: a prospective study. *Aliment Pharmacol Ther* 2015;41(12):1271-1280.
77. Lykiardopoulos B, Hagstrom H, Fredrikson M, et al. Development of serum marker models to increase diagnostic accuracy of advanced fibrosis in nonalcoholic fatty liver disease: the new LINKI algorithm compared with established algorithms. *PLoS One* 2016;11(12):e0167776.
78. Rath MM, Panigrahi MK, Pattnaik K, et al. Histological evaluation of non-alcoholic fatty liver disease and its correlation with different noninvasive scoring systems with special reference to fibrosis: a single center experience. *J Clin Exp Hepatol* 2016;6(4):291-296.
79. Jun DW, Kim SG, Park SH, et al. External validation of the non-alcoholic fatty liver disease fibrosis score for assessing advanced fibrosis in Korean patients. *J Gastroenterol Hepatol* 2017;32(5):1094-1099.
80. 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(5):740-751.
81. Bertot LC, Jeffrey GP, de Boer B, et al. Diabetes impacts prediction of cirrhosis and prognosis by non-invasive fibrosis models in non-alcoholic fatty liver disease. *Liver Int* 2018;38(10):1793-1802.
82. Patel YA, Gifford EJ, Glass LM, et al. Identifying nonalcoholic fatty liver disease advanced fibrosis in the Veterans Health Administration. *Dig Dis Sci* 2018;63(9):2259-2266.
83. Chan WK, Treeprasertsuk S, Goh GB, et al. Optimizing use of nonalcoholic fatty liver disease fibrosis score, fibrosis-4 score, and liver stiffness measurement to identify patients with advanced fibrosis. *Clin Gastroenterol Hepatol* 2019;17(12):2570-2580.e2537.
84. Kaya E, Bakir A, Kani HT, Demirtas CO, Keklikkiran C, Yilmaz Y. Simple noninvasive scores are clinically useful to exclude, not predict, advanced fibrosis: a study in turkish patients with biopsy-proven nonalcoholic fatty liver disease. *Gut Liver* 2020:14(4);486-491.
85. Yang M, Jiang L, Wang Y, et al. Step layered combination of noninvasive fibrosis models improves diagnostic accuracy of advanced fibrosis in nonalcoholic fatty liver disease. *J Gastrointestin Liver Dis* 2019;28(3):289-296.
86. 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(5):1521-1530.

87. de Carli MA, de Carli LA, Correa MB, Junqueira G Jr, Tovo CV, Coral GP. Performance of noninvasive scores for the diagnosis of advanced liver fibrosis in morbidly obese with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2020;32(3):420-425.
88. Bril F, McPhaul MJ, Caulfield MP, et al. Performance of plasma biomarkers and diagnostic panels for nonalcoholic steatohepatitis and advanced fibrosis in patients with type 2 diabetes. *Diabetes Care* 2020;43(2):290-297.
89. Alkayyali T, Qutranji L, Kaya E, Bakir A, Yilmaz Y. Clinical utility of noninvasive scores in assessing advanced hepatic fibrosis in patients with type 2 diabetes mellitus: a study in biopsy-proven non-alcoholic fatty liver disease. *Acta Diabetol* 2020;57(5):613-618.
90. Pitisuttithum P, Chan WK, Piyachaturawat P, et al. Predictors of advanced fibrosis in elderly patients with biopsy-confirmed nonalcoholic fatty liver disease: the GOASIA study. *BMC Gastroenterol* 2020;20(1):88.
91. Mehta SH, Lau B, Afdhal NH, Thomas DL. Exceeding the limits of liver histology markers. *J Hepatol* 2009;50(1):36-41.
92. Mulherin SA, Miller WC. Spectrum bias or spectrum effect? Subgroup variation in diagnostic test evaluation. *Ann Intern Med* 2002;137(7):598-602.
93. Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Ann Intern Med* 2013;158(11):807-820.
94. AASLD writing group. Placeholder for SR1. 2023.
95. Tachi Y, Hirai T, Toyoda H, et al. Predictive ability of laboratory indices for liver fibrosis in patients with chronic hepatitis C after the eradication of hepatitis C virus. *PLoS One* 2015;10(7):e0133515.
96. D'Ambrosio R, Degasperi E, Aghemo A, et al. Serological tests do not predict residual fibrosis in hepatitis C cirrhotics with a sustained virological response to interferon. *PLoS One* 2016;11(6):e0155967.
98. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. *Hepatology* 2016;63(1):261-283.
99. Xu XY, Kong H, Song RX, et al. The effectiveness of noninvasive biomarkers to predict hepatitis B-related significant fibrosis and cirrhosis: a systematic review and meta-analysis of diagnostic test accuracy. *PLoS One* 2014;9(6):e100182.
100. Salkic NN, Jovanovic P, Hauser G, Brcic M. FibroTest/Fibrosure for significant liver fibrosis and cirrhosis in chronic hepatitis B: a meta-analysis. Am J Gastroenterol 2014;109(6):796-809.
101. Leroy V, Sturm N, Faure P, et al. Prospective evaluation of FibroTest®, FibroMeter®, and HepaScore® for staging liver fibrosis in chronic hepatitis B: comparison with hepatitis C. *J Hepatol* 2014;61(1):28-34.
102. 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(5):1557-1565.
103. 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(6):1611-1625 e1612.
104. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009;7(10):1104-1112.

105. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. *Hepatology* 2017;66(5):1486-1501.
106. 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(5):1006-1019.
107. 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(2):252-262.
108. Naveau S, Gaude G, Asnacios A, et al. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. *Hepatology* 2009;49(1):97-105.
109. Parkes J, Guha IN, Harris S, Rosenberg WM, Roderick PJ. Systematic review of the diagnostic performance of serum markers of liver fibrosis in alcoholic liver disease. *Comp Hepatol* 2012;11(1):5.
110. Matta B, Lee TH, Patel K. Use of non-invasive testing to stage liver fibrosis in patients with HIV. *Curr HIV/AIDS Rep* 2016;13(5):279-288.
111. Corpechot C. Utility of noninvasive markers of fibrosis in cholestatic liver diseases. *Clin Liver Dis* 2016;20(1):143-158.
112. Corpechot C, Carrat F, Poujol-Robert A, et al. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. *Hepatology* 2012;56(1):198-208.
113. Floreani A, Cazzagon N, Martines D, Cavalletto L, Baldo V, Chemello L. Performance and utility of transient elastography and noninvasive markers of liver fibrosis in primary biliary cirrhosis. *Dig Liver Dis* 2011;43(11):887-892.
114. Corpechot C, Gaouar F, El Naggar A, et al. Baseline values and changes in liver stiffness measured by transient elastography are associated with severity of fibrosis and outcomes of patients with primary sclerosing cholangitis. *Gastroenterology* 2014;146(4):970-e16.
115. Muir AJ, Levy C, Janssen HLA, et al. Simtuzumab for primary sclerosing cholangitis: phase 2 study results with insights on the natural history of the disease. *Hepatology* 2019;69(2):684-698.
116. Martinez SM, Crespo G, Navasa M, Forns X. Noninvasive assessment of liver fibrosis. Hepatology. 2011 Jan;53(1):325-35.
117. Holmberg SD, Lu M, Rupp LB, et al. Noninvasive serum fibrosis markers for screening and staging chronic hepatitis C virus patients in a large US cohort. *Clin Infect Dis* 2013;57(2):240-246.
118 Amorim TG, Staub GJ, Lazzarotto C, et al. Validation and comparison of simple noninvasive models for the prediction of liver fibrosis in chronic hepatitis C. *Ann Hepatol* 2012;11(6):855-861.
119. Tama M, Naylor P, Patel S, et al. Overestimate of fibrosis by FIBROSpect® II in African Americans complicates the management of their chronic hepatitis C. *J Clin Transl Hepatol* 2016;4(1):12-19.
120. Jiang Y, Huang E, Mehrnia A, et al. Can aminotransferase-to-platelet ratio index and other non-invasive markers effectively reduce liver biopsies for renal transplant evaluation of hepatitis C virus-positive patients? *Nephrol Dial Transplant* 2014;29(6):1247-1252.
121. Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with

chronic hepatitis B virus infection: a systemic review and meta-analysis. *Hepatology* 2015;61(1):292-302.
122. Kim BK, Kim DY, Park JY, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. *Liver Int* 2010;30(4):546-553.
123. Dong M, Wu J, Yu X, et al. Validation and comparison of seventeen noninvasive models for evaluating liver fibrosis in Chinese hepatitis B patients. *Liver Int* 2018;38(9):1562-1570.
124. Tan YW, Zhou XB, Ye Y, He C, Ge GH. Diagnostic value of FIB-4, aspartate aminotransferase-to-platelet ratio index and liver stiffness measurement in hepatitis B virus-infected patients with persistently normal alanine aminotransferase. *World J Gastroenterol* 2017;23(31):5746-5754.
125. Kim WR, Berg T, Asselah T, et al. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. *J Hepatol* 2016;64(4):773-780.
126. 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(9):1877-1885.e1875.
127. Sun W, Cui H, Li N, et al. Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with non-alcoholic fatty liver disease: a meta-analysis study. *Hepatol Res* 2016;46(9):862-870.
128. Kosick HM, Keyrouz A, Adeyi O, Sebastiani G, Patel K. A stepwise algorithmic approach and external validation study for noninvasive prediction of advanced fibrosis in nonalcoholic fatty liver disease. *Dig Dis Sci* 2021;66(11):4046-4057.
129. Meneses D, Olveira A, Corripio R, et al. Performance of noninvasive liver fibrosis scores in the morbid obese patient, same scores but different thresholds. *Obes Surg* 2020;30(7):2538-2546.
130. De Silva S, Li W, Kemos P, et al. Non-invasive markers of liver fibrosis in fatty liver disease are unreliable in people of South Asian descent. *Frontline Gastroenterol* 2018;9(2):115-121.
131. 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(8):1113-1123.
132. Guillaume M, Moal V, Delabaudiere C, et al. Direct comparison of the specialised blood fibrosis tests FibroMeter(V2G) and Enhanced Liver Fibrosis score in patients with non-alcoholic fatty liver disease from tertiary care centres. *Aliment Pharmacol Ther* 2019;50(11-12):1214-1222.
133. Abdel-Hameed EA, Rouster SD, Kottilil S, Sherman KE. The enhanced liver fibrosis (ELF)-Index predicts hepatic fibrosis superior to FIB4 and APRI in HIV/HCV infected patients. *Clin Infect Dis* 2021;73(3):450-459.
134. Castera L, Winnock M, Pambrun E, et al. Comparison of transient elastography (FibroScan), FibroTest, APRI and two algorithms combining these non-invasive tests for liver fibrosis staging in HIV/HCV coinfected patients: ANRS CO13 HEPAVIH and FIBROSTIC collaboration. *HIV Med* 2014;15(1):30-39.
135 Kliemann DA, Wolff FH, Tovo CV, et al. Biochemical non-invasive assessment of liver fibrosis cannot replace biopsy in HIV-HCV coinfected patients. *Ann Hepatol* 2016;15(1):27-32.

136 Thiele M, Madsen BS, Hansen JF, Detlefsen S, Antonsen S, Krag A. 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(5):1369-1379.
137. Friedrich-Rust M, Rosenberg W, Parkes J, Herrmann E, Zeuzem S, Sarrazin C. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. *BMC Gastroenterol* 2010;10:103.
138. Olmez S, Sayar S, Avcioglu U, et al. The relationship between liver histology and noninvasive markers in primary biliary cirrhosis. *Eur J Gastroenterol Hepatol* 2016;28(7):773-776.
139. Chin J, Powell LW, Ramm LE, Hartel GF, Olynyk JK, Ramm GA. Utility of serum biomarker indices for staging of hepatic fibrosis before and after venesection in patients with hemochromatosis caused by variants in HFE. *Clin Gastroenterol Hepatol* 2021;19(7):1459-1468 e1455.
140. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology* 2023;77(5):1797-1835.
141. Sebastiani G, Halfon P, Castera L., et al. SAFE biopsy: a validated method for large-scale staging of liver fibrois in chronic hepatitis C. *Hepatology* 2009;49(6):1821-1827.
142. Donepudi I, Paredes A, Hubbard S, Awad C, Sterling RK. Utility of evaluating HCV in an uninsured population. *Dig Dis Sci* 2015;60(4):1092-1097.
143. Yang L, Ding Y, Rao S, et al. Staging liver fibrosis in chronic hepatitis B with T1 relaxation time indx on gadoxetic acid-enhanced MRI: comparison with aspartate aminotransferase-to-platelet ratio index and FIB-4. *J Magn Reson Imaging* 2017;45(4):1186-1194.
144. Dong H, Xu C, Zhou W, et al. The combination of 5 serum markers compared to FibroScan to predict significant liver fibrosis in patients with chronic hepatitis B virus. *Clin Chim Acta* 2018;483:145-150.
145. 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(2):371-378.
146. Srivastava A, Jong S, Gola A, et al. Cost-comparison analysis of FIB-4, ELF and fibroscan in community pathways for non-alcoholic fatty liver disease. *BMC Gastroenterol* 2019;19(1):122.
147. Balakrishnan M, Loomba R. The role of noninvasive tests for differentiating NASH from NAFL and diagnosing advanced fibrosis among patients with NAFLD. *J Clin Gastroenterol* 2020;54(2):107-113.
148. Leroy V, De Traversay C, Barnoud R, et al. Changes in histological lesions and serum fibrogenesis markers in chronic hepatitis C patients non-responders to interferon alpha. *J Hepatol* 2001;35(1):120-126.
149. Poynard T, Imbert-Bismut F, Ratziu V, et al. Biochemical markers of liver fibrosis in patients infected by hepatitis C virus: longitudinal validation in a randomized trial. *J Viral Hepat* 2002;9(2):128-133.
150. Poynard T, McHutchison J, Manns M, Myers RP, Albrecht J. Biochemical surrogate markers of liver fibrosis and activity in a randomized trial of peginterferon alfa-2b and ribavirin. *Hepatology* 2003;38(2):481-492.

151. Fontana RJ, Dienstag JL, Bonkovsky HL, et al. Serum fibrosis markers are associated with liver disease progression in non-responder patients with chronic hepatitis C. *Gut* 2010;59(10):1401-1409.
152. Poynard T, Bruix J, Schiff ER, et al. Improved inflammatory activity with peginterferon alfa-2b maintenance therapy in non-cirrhotic prior non-responders: a randomized study. *J Hepatol* 2013;58(3):452-459.
153. Patel K, Remlinger KS, Walker TG, et al. Multiplex protein analysis to determine fibrosis stage and progression in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2014;12(12):2113-20.e1-3.
154. Nielsen MJ, Veidal SS, Karsdal MA, et al. Plasma Pro-C3 (N-terminal type III collagen propeptide) predicts fibrosis progression in patients with chronic hepatitis C. *Liver Int* 2015;35(2):429-437.
155. Patel K, Tillmann HL, Matta B, et al. Longitudinal assessment of hepatitis C fibrosis progression by collagen and smooth muscle actin morphometry in comparison to serum markers. *Aliment Pharmacol Ther* 2016;43(3):356-363.
156. Tanwar S, Trembling PM, Hogan BJ, et al. Noninvasive markers of liver fibrosis: on-treatment changes of serum markers predict the outcome of antifibrotic therapy. *Eur J Gastroenterol Hepatol* 2017;29(3):289-296.
157. Wilson LE, Torbenson M, Astemborski J, et al. Progression of liver fibrosis among injection drug users with chronic hepatitis C. *Hepatology* 2006;43(4):788-795.
158. Sulkowski MS, Mehta SH, Torbenson MS, et al. Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. *AIDS* 2007;21(16):2209-2216.
159. Halfon P, Carrat F, Bedossa P, et al. Effect of antiviral treatment on serum markers of liver fibrosis in HIV-hepatitis C virus-coinfected patients: the Fibrovic 2 Study - ANRS HC02. *Antivir Ther* 2009;14(2):211-219.
160. Sterling RK, Wegelin JA, Smith PG, et al. Similar progression of fibrosis between HIV/HCV-infected and HCV-infected patients: analysis of paired liver biopsy samples. *Clin Gastroenterol Hepatol* 2010;8(12):1070-1076.
161. Cales P, Zarski JP, Chapplain JM, et al. Fibrosis progression under maintenance interferon in hepatitis C is better detected by blood test than liver morphometry. *J Viral Hepat* 2012;19(2):e143-153.
162. Konerman MA, Mehta SH, Sutsiddiquecliffe CG, et al. Fibrosis progression in human immunodeficiency virus/hepatitis C virus coinfected adults: prospective analysis of 435 liver biopsy pairs. *Hepatology* 2014;59(3):767-775.
163. Schmid P, Bregenzer A, Huber M, et al. Progression of liver fibrosis in HIV/HCV co-infection: a comparison between non-invasive assessment methods and liver biopsy. *PLoS One* 2015;10(9):e0138838.
164. Poynard T, Ngo Y, Marcellin P, Hadziyannis S, Ratziu V, Benhamou Y. Impact of adefovir dipivoxil on liver fibrosis and activity assessed with biochemical markers (FibroTest-ActiTest) in patients infected by hepatitis B virus. *J Viral Hepat* 2009;16(3):203-213.
165. Surana P, Kapuria D, Broadwell C, et al. Longitudinal effects of Nucleos(t)ide analogue therapy in chronic hepatitis B patients and the utility of non-invasive fibrosis markers during treatment: a single-center experience for up to 17 years. *Antiviral Res* 2019;168:61-67.
166. Wong VW, Wong GL, Choi PC, et al. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. *Gut* 2010;59(7):969-974.

167. Moretto M, Kupski C, da Silva VD, Padoin AV, Mottin CC. Effect of bariatric surgery on liver fibrosis. *Obes Surg* 2012;22(7):1044-1049.
168. Vilar-Gomez E, Calzadilla-Bertot L, Friedman SL, et al. Serum biomarkers can predict a change in liver fibrosis 1 year after lifestyle intervention for biopsy-proven NASH. *Liver Int* 2017;37(12):1887-1896.
169. Harrison SA, Abdelmalek MF, Caldwell S, et al. Simtuzumab is ineffective for patients with bridging fibrosis or compensated cirrhosis caused by nonalcoholic steatohepatitis. *Gastroenterology* 2018;155(4):1140-1153.
170. Loomba R, Lawitz E, Mantry PS, et al. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial. *Hepatology* 2018;67(2):549-559.
171. 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(4):1198-1212.
172. Anstee AM, Harrison S, Sanyal AJ, et al. Obeticholic Acid (OCA) improves noninvasive markers of fibrosis in patients With nonalcoholic steatohepatitis (NASH): a secondary analysis of the phase 3 REGENERATE Study. Hepatology 2020;52(Suppl 1):E41-E42.
173. Rinella ME, Dufour JF, Anstee QM, et al. Non-invasive evaluation of response to obeticholic acid in patients with NASH: results from the REGENERATE study. *J Hepatol* 2022;76(3):536-548.
174. Younossi Z, Loomba. Noninvasive tests more accurately capture surrogates of clinical response than liver histology in NASH patients with advanced fibrosis. In: AASLD: Hepatology; 2019. p. 1045A.
175. Pérez-Tamayo R. Cirrhosis of the liver: a reversible disease? *Pathol Annu* 1979;14 Pt 2:183-213.
176. Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2002;122(5):1303-1313.
177. Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010;52(3):886-893.
178. Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000;132(7):517-524.
179. Marcellin P, Chang TT, Lim SG, et al. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2008;48(3):750-758.
180. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013;381(9865):468-475.
181. Sun Y, Zhou J, Wang L, et al. New classification of liver biopsy assessment for fibrosis in chronic hepatitis B patients before and after treatment. *Hepatology* 2017;65(5):1438-1450.
182. Poynard T, Lenaour G, Vaillant JC, et al. Liver biopsy analysis has a low level of performance for diagnosis of intermediate stages of fibrosis. Clin Gastroenterol Hepatol 2012;10(6):657-663 e657.
181. Poynard T, Morra R, Halfon P, et al. Meta-analyses of FibroTest diagnostic value in chronic liver disease. *BMC Gastroenterol* 2007;7:40.

182. Standish RA, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. *Gut* 2006;55(4):569-78.436.
183. Poynard T, Mathurin P, Lai CL, et al. A comparison of fibrosis progression in chronic liver diseases. *J Hepatol* 2003;38(3):257-265.
184. Fontana RJ, Bonkovsky HL, Naishadham D, et al. Serum fibrosis marker levels decrease after successful antiviral treatment in chronic hepatitis C patients with advanced fibrosis. *Clin Gastroenterol Hepatol* 2009;7(2):219-226.
185. Vergniol J, Foucher J, Castera L, et al. Changes of non-invasive markers and FibroScan values during HCV treatment. *J Viral Hepat* 2009;16(2):132-140.
186. Patel K, Friedrich-Rust M, Lurie Y, et al. FibroSURE and FibroScan in relation to treatment response in chronic hepatitis C virus. *World J Gastroenterol* 2011;17(41):4581-4589.
187. Patel K, Benhamou Y, Yoshida EM, et al. An independent and prospective comparison of two commercial fibrosis marker panels (HCV FibroSURE and FIBROSpect II) during albinterferon alfa-2b combination therapy for chronic hepatitis C. *J Viral Hepat* 2009;16(3):178-186.
188. Alonso Lopez S, Manzano ML, Gea F, et al. A model based on noninvasive markers predicts very low hepatocellular carcinoma risk after viral response in hepatitis C virus-advanced fibrosis. *Hepatology* 2020;72(6):1924-1934.
189. Ioannou GN, Beste LA, Green PK, et al. Increased risk for hepatocellular carcinoma persists up to 10 years after HCV eradication in patients with baseline cirrhosis or high FIB-4 scores. *Gastroenterology* 2019;157(5):1264-1278.e1264.
190. Kumada T, Toyoda H, Yasuda S, Tada T, Tanaka J. Usefulness of serial FIB-4 score measurement for predicting the risk of hepatocarcinogenesis after hepatitis C virus eradication. *Eur J Gastroenterol Hepatol* 2021;33(1S Suppl 1):e513-e521.
191. Petta S, Sebastiani G, Vigano M, et al. Monitoring occurrence of liver-related events and survival by transient elastography in patients with nonalcoholic fatty liver disease and compensated advanced chronic liver disease. *Clin Gastroenterol Hepatol* 2021;19(4):806-815.e805.
192. Tamaki N, Kurosaki M, Yasui Y, et al. Change in fibrosis 4 index as predictor of high risk of incident hepatocellular carcinoma after eradication of hepatitis C virus. *Clin Infect Dis* 2021;73(9):e3349-e3354.
193. Kanwal F, Kramer JR, Asch SM, Cao Y, Li L, El-Serag HB. Long-term risk of hepatocellular carcinoma in HCV patients treated with direct acting antiviral agents. *Hepatology* 2020;71(1):44-55.
194. Toyoda H, Tada T, Yasuda S, Mizuno K, Ito T, Kumada T. Dynamic evaluation of liver fibrosis to assess the risk of hepatocellular carcinoma in patients with chronic hepatitis C who achieved sustained virologic response. *Clin Infect Dis* 2020;70(6):1208-1214.
195. Wu X, Cai B, Su Z, et al. Aspartate transaminase to platelet ratio index and gamma-glutamyl transpeptidase-to-platelet ratio outweigh fibrosis index based on four factors and red cell distribution width-platelet ratio in diagnosing liver fibrosis and inflammation in chronic hepatitis B. *J Clin Lab Anal* 2018;32(4):e22341.
196. Harrison SA, Rinella ME, Abdelmalek MF, et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2018;391(10126):1174-1185.

197. Lee Y, Doumouras AG, Yu J, et al. Complete resolution of nonalcoholic fatty liver disease after bariatric surgery: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2019;17(6):1040-1060.e1011.
198. Cheung A, Neuschwander-Tetri BA, Kleiner DE, et al. Defining improvement in nonalcoholic steatohepatitis for treatment trial endpoints: recommendations from the liver forum. *Hepatology* 2019;70(5):1841-1855.
199. Unalp-Arida A, Ruhl CE. Noninvasive fatty liver markers predict liver disease mortality in the U.S. population. *Hepatology* 2016;63(4):1170-1183.
200. Fricker ZP, Pedley A, Massaro JM, et al. Liver fat is associated with markers of inflammation and oxidative stress in analysis of data from the Framingham Heart Study. *Clin Gastroenterol Hepatol* 2019;17(6):1157-1164.e1154.
201 Li Y, Liu L, Wang B, Wang J, Chen D. Simple steatosis is a more relevant source of serum inflammatory markers than omental adipose tissue. *Clin Res Hepatol Gastroenterol* 2014;38(1):46-54.
202. Otgonsuren M, Estep MJ, Hossain N, et al. Single non-invasive model to diagnose non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). *J Gastroenterol Hepatol* 2014;29(12):2006-2013.
203. Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. *BMC Cardiovasc Disord* 2005;5:26.
204. Balkau B, Lang C, Vol S, Fumeron F, Bonnet F, group study DESIR. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study. *BMC Gastroenterol* 2010;10:56.
205. Sviklāne L, Olmane E, Dzērve Z, Kupčs K, Pīrāgs V, Sokolovska J. Fatty liver index and hepatic steatosis index for prediction of non-alcoholic fatty liver disease in type 1 diabetes. *J Gastroenterol Hepatol* 2018;33(1):270-276.
206. Gastaldelli A, Kozakova M, Hojlund K, et al. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. *Hepatology* 2009;49(5):1537-1544.
207. Pais R, Giral P, Khan JF, et al. Fatty liver is an independent predictor of early carotid atherosclerosis. *J Hepatol* 2016;65(1):95-102.
208. Baratta F, Pastori D, Angelico F, et al. Nonalcoholic fatty liver disease and fibrosis associated with increased risk of cardiovascular events in a prospective study. *Clin Gastroenterol Hepatol* 2020;18(10):2324-2331.e2324.
209. Meyersohn NM, Mayrhofer T, Corey KE, et al. Association of hepatic steatosis with major adverse cardiovascular events, independent of coronary artery disease. *Clin Gastroenterol Hepatol* 2021;19:1480-1488.e1414.
210. Kozakova M, Palombo C, Eng MP, et al. Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. *Hepatology* 2012;55(5):1406-1415.
211. Stern C, Castera L. Non-invasive diagnosis of hepatic steatosis. *Hepatol Int* 2017;11(1):70-78.
212. Festi D, Schiumerini R, Marzi L, et al. Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther* 2013;37(4):392-400.
213. Lee JH, Kim D, Kim HJ, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010;42(7):503-508.

214. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;6:33.
215. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. *BMC Gastroenterol* 2010;10:98.
216. Cuthbertson DJ, Weickert MO, Lythgoe D, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. *Eur J Endocrinol* 2014;171(5):561-569.
217. Poynard T, Lassailly G, Diaz E, et al. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. *PLoS One* 2012;7(3):e30325.
218. Poynard T, Ratziu V, Naveau S, et al. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005;4:10.
219. Zhang Z, Wang G, Kang K, Wu G, Wang P. Diagnostic accuracy and clinical utility of a new noninvasive index for hepatic steatosis in patients with hepatitis B virus infection. *Sci Rep* 2016;6:32875.
220. Fedchuk L, Nascimbeni F, Pais R, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* 2014;40(10):1209-1222.
221. Borman MA, Ladak F, Crotty P, et al. The Fatty Liver Index has limited utility for the detection and quantification of hepatic steatosis in obese patients. *Hepatol Int* 2013;7(2):592-599.
222. Kotronen A, Peltonen M, Hakkarainen A, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology* 2009;137(3):865-872.
223. Cuthbertson DJ, Brown E, Koskinen J, et al. Longitudinal analysis of risk of non-alcoholic fatty liver disease in adulthood. *Liver Int* 2019;39(6):1147-1154.
224. Keating SE, Parker HM, Hickman IJ, et al. NAFLD in clinical practice: Can simple blood and anthropometric markers be used to detect change in liver fat measured by (1) H-MRS? *Liver Int* 2017;37(12):1907-1915.
225. Bril F, McPhaul MJ, Caulfield MP, et al. Performance of the SteatoTest, ActiTest, NashTest and FibroTest in a multiethnic cohort of patients with type 2 diabetes mellitus. *J Investig Med* 2019;67(2):303-311.
226. Chon YE, Jung KS, Kim SU, et al. Controlled attenuation parameter (CAP) for detection of hepatic steatosis in patients with chronic liver diseases: a prospective study of a native Korean population. *Liver Int* 2014;34(1):102-109.
227. Lassailly G, Caiazzo R, Hollebecque A, et al. Validation of noninvasive biomarkers (FibroTest, SteatoTest, and NashTest) for prediction of liver injury in patients with morbid obesity. *Eur J Gastroenterol Hepatol* 2011;23(6):499-506.
228. Ooi GJ, Earnest A, Kemp WW, et al. Evaluating feasibility and accuracy of non-invasive tests for nonalcoholic fatty liver disease in severe and morbid obesity. *Int J Obes (Lond)* 2018;42(11):1900-1911.
229. Petta S, Di Marco V, Di Stefano R, et al. TyG index, HOMA score and viral load in patients with chronic hepatitis C due to genotype 1. *J Viral Hepat* 2011;18(7):e372-e380.
230. Poynard T, Peta V, Munteanu M, et al. The diagnostic performance of a simplified blood test (SteatoTest-2) for the prediction of liver steatosis. *Eur J Gastroenterol Hepatol* 2019;31(3):393-402.

231. Xu L, Lu W, Li P, Shen F, Mi YQ, Fan JG. A comparison of hepatic steatosis index, controlled attenuation parameter and ultrasound as noninvasive diagnostic tools for steatosis in chronic hepatitis B. *Dig Liver Dis* 2017;49(8):910-917.
232. Sberna AL, Bouillet B, Rouland A, et al. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO) clinical practice recommendations for the management of non-alcoholic fatty liver disease: evaluation of their application in people with type 2 diabetes. *Diabet Med* 2018;35(3):368-375.
233. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther* 2015;41(1):65-76.
234. Ooi GJ, Mgaieth S, Eslick GD, et al. Systematic review and meta-analysis: non-invasive detection of non-alcoholic fatty liver disease related fibrosis in the obese. *Obes Rev* 2018;19(2):281-294.
235. Ratziu V, Giral P, Munteanu M, et al. Screening for liver disease using non-invasive biomarkers (FibroTest, SteatoTest and NashTest) in patients with hyperlipidaemia. *Aliment Pharmacol Ther* 2007;25(2):207-218.
236. Munteanu M, Tiniakos D, Anstee Q, et al. Diagnostic performance of FibroTest, SteatoTest and ActiTest in patients with NAFLD using the SAF score as histological reference. *Aliment Pharmacol Ther* 2016;44(8):877-889.
237. Guerrero-Romero F, Simental-Mendia LE, Gonzalez-Ortiz M, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab* 2010;95(7):3347-3351.
238. Simental-Mendia LE, Simental-Mendia E, Rodriguez-Hernandez H, Rodriguez-Moran M, Guerrero-Romero F. The product of triglycerides and glucose as biomarker for screening simple steatosis and NASH in asymptomatic women. *Ann Hepatol* 2016;15(5):715-720.
239. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346(16):1221-1231.
240. Thomas EL, Hamilton G, Patel N, et al. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 2005;54(1):122-127.
241. Price JC, Seaberg EC, Latanich R, et al. Risk factors for fatty liver in the multicenter AIDS cohort study. *Am J Gastroenterol* 2014;109(5):695-704.
242. McHenry S, Park Y, Browning JD, Sayuk G, Davidson NO. Dallas Steatosis Index identifies patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2020;18(9):2073-2080.e2077.
243. Nobili V, Alisi A, Torre G, et al. Hyaluronic acid predicts hepatic fibrosis in children with nonalcoholic fatty liver disease. *Transl Res* 2010;156(4):229-234.
244. Nobili V, Marcellini M, Giovannelli L, et al. Association of serum interleukin-8 levels with the degree of fibrosis in infants with chronic liver disease. *J Pediatr Gastroenterol Nutr* 2004;39:540-544.
245. Pereira TN, Lewindon PJ, Smith JL, et al. Serum markers of hepatic fibrogenesis in cystic fibrosis liver disease. *J Hepatol* 2004;41(4):576-583.
246. Asai A, Miethke A, Bezerra JA. Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes. *Nat Rev Gastroenterol Hepatol* 2015;12(6):342-352.
247. Ryckman FC, Alonso MH, Bucuvalas JC, Balistreri WF. Biliary atresia--surgical management and treatment options as they relate to outcome. *Liver Transpl Surg* 1998;4(5 Suppl 1):S24-S33.

248. Grieve A, Makin E, Davenport M. Aspartate aminotransferase-to-platelet ratio index (APRi) in infants with biliary atresia: prognostic value at presentation. *J Pediatr Surg* 2013;48(4):789-795.
249. Kim SY, Seok JY, Han SJ, Koh H. Assessment of liver fibrosis and cirrhosis by aspartate aminotransferase-to-platelet ratio index in children with biliary atresia. *J Pediatr Gastroenterol Nutr* 2010;51(2):198-202.
250. Yang LY, Fu J, Peng XF, et al. Validation of aspartate aminotransferase to platelet ratio for diagnosis of liver fibrosis and prediction of postoperative prognosis in infants with biliary atresia. *World J Gastroenterol* 2015;21(19):5893-5900.
251. Suominen JS, Lampela H, Heikkila P, Lohi J, Jalanko H, Pakarinen MP. APRi predicts native liver survival by reflecting portal fibrogenesis and hepatic neovascularization at the time of portoenterostomy in biliary atresia. *J Pediatr Surg* 2015;50(9):1528-1531.
252. Chen S, Liao B, Zhong Z, et al. Supersonic shearwave elastography in the assessment of liver fibrosis for postoperative patients with biliary atresia. *Sci Rep* 2016;6:31057.
253. Leung DH, Khan M, Minard CG, et al. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. *Hepatology* 2015;62(5):1576-1583.
254. Lebensztejn DM, Skiba E, Sobaniec-Lotowska M, Kaczmarski M. A simple noninvasive index (APRI) predicts advanced liver fibrosis in children with chronic hepatitis B. *Hepatology* 2005;41(6):1434-1435.
255. McGoogan KE, Smith PB, Choi SS, Berman W, Jhaveri R. Performance of the AST-to-platelet ratio index as a noninvasive marker of fibrosis in pediatric patients with chronic viral hepatitis. *J Pediatr Gastroenterol Nutr* 2010;50(3):344-346.
256. Sokucu S, Gokce S, Gulluoglu M, Aydogan A, Celtik C, Durmaz O. The role of the non-invasive serum marker FibroTest-ActiTest in the prediction of histological stage of fibrosis and activity in children with naive chronic hepatitis B infection. *Scand J Infect Dis* 2010;42(9):699-703.
257. El-Sayed R, Fahmy M, El Koofy N, et al. Can aspartate aminotransferase to platelet ratio index replace liver biopsy in chronic hepatitis C? *Trop Gastroenterol* 2011;32(4):267-272.
258. El-Shabrawi MH, Mohsen NA, Sherif MM, et al. Noninvasive assessment of hepatic fibrosis and necroinflammatory activity in Egyptian children with chronic hepatitis C virus infection using FibroTest and ActiTest. *Eur J Gastroenterol Hepatol* 2010;22(8):946-951.
259. Pokorska-Śpiewak M, Kowalik-Mikołajewska B, Aniszewska M, Pluta M, Marczyńska M. Non-invasive evaluation of the liver disease severity in children with chronic viral hepatitis using FibroTest and ActiTest - comparisonS with histopathological assessment. *Clin Exp Hepatol* 2017;3(4):187-193.
260. Younossi ZM, Corey KE, Alkhouri N, et al. Clinical assessment for high-risk patients with non-alcoholic fatty liver disease in primary care and diabetology practices. *Aliment Pharmacol Ther* 2020;52(3):513-526.
261. Marrero J, Kulik L, Sirlin C, et al. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018;68(2):723-750.

Table 1. PICO Questions in NILDA

Blood-based testing for fibrosis or steatosis in adults	
PICO 1	In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HCV/HBV, HIV/HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, are blood-based biomarker panels accurate in staging hepatic fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?
PICO 2	In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HIV-HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, is any blood-based biomarker panel superior to another blood-based biomarker panel in staging hepatic fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?
PICO 3	In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HIV-HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, is the combination of two blood-based biomarker panels superior to a single one for staging fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?
PICO 4	In adult patients with chronic liver disease, including hepatocellular (HCV, HIV-HCV, HBV, HIV-HBV, NAFLD, and ALD) or cholestatic (PSC and PBC) disorders, do serial blood-based biomarker panels accurately predict the natural

	history of progression of fibrosis or regression of fibrosis in response to therapy relative to serial histopathology as the reference?
PICO 5	In patients with NAFLD, are blood-based biomarker panels accurate in grading hepatic steatosis (S0 vs. S1-3, S0-1 vs. S2-3, and S0-2 vs. S3) using histopathology or MR-spectroscopy or MRI PDFF as the reference?
Blood-based testing in children	
PICO 6	In pediatric chronic liver disease (HCV, HBV, BA, CFLD, and NAFLD/NASH), are blood-based biomarkers accurate in staging hepatic fibrosis (F0-1 vs. F2-4, F0-2 vs. F3-4, and F0-3 vs. F4) using histopathology as the reference?
ALD = alcohol-associated liver disease; BA = biliary atresia; CFLD = cystic fibrosis liver disease; F = fibrosis; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; MR = magnetic resonance; MRI PDFF = magnetic resonance imaging proton density fat fraction; PBC = primary biliary cholangitis; PICO = Patient, Intervention, Comparison and Outcome; PSC = primary sclerosing cholangitis.	

Table 2. GRADE Approach*

1. Rating the quality of evidence			
Study design RCT Observational	**Initial rating of quality of evidence** High Moderate Low Very low	**Rate down when:** Risk of bias Inconsistency Imprecision Indirectness Publication bias	**Rate up when:** Large effect size (e.g., RR = 0.5) Very large effect (e.g., RR = 0.2) Dose-response gradient All plausible confounding would increase the association
2. Determinants of strength of a recommendation Quality of evidence Balance of benefits and harms Patient values and preferences Resources and costs			
3. Implications of the strength of a recommendation Strong Population: Most people in this situation would want the recommended course of action, and only a small proportion would not. Health care workers: Most people should receive the recommended course of action. Policy makers: The recommendation can be adopted as policy in most situations.			

Conditional
Population: The majority of people in this situation would want the recommended course of action, but many would not.
Health care workers: Be prepared to help patients make a decision that is consistent with their values using decision aids and shared decision-making.
Policy makers: There is a need for substantial debate and involvement of stakeholders.

*Modified from references 8 and 9.

Abbreviations: GRADE = Grading of Recommendations, Assessment Development, and Evaluation system; RCT = randomized controlled trial; RR = relative risk.

Table 3a. Staging of Fibrosis Across Multiple Liver Diseases and Corresponding Classification Scores

	Fibrosis stage				
	0	**F1**	**F2**	**F3**	**F4**
			Significant fibrosis		
				Advanced fibrosis	
					Cirrhosis
Scheuer/Batts-Ludwig (Viral and autoimmune hepatitis)[14, 15]	No fibrosis	Enlarged, fibrotic portal tracts	Periportal or P-P septa but intact architecture	Fibrosis with architectural distortion but no obvious cirrhosis	Probable or definite cirrhosis
Knodell (Viral and autoimmune hepatitis)[16]	No fibrosis	Fibrous portal expansion	N/A	Bridging fibrosis	Cirrhosis
Ishak (Various etiologies)[17]	0: No fibrosis	1: Fibrous expansion of some portal areas, with or	2: Fibrous expansion of most portal areas, with or without short fibrous septa	4: Fibrous expansion of portal areas with marked bridging	6: Cirrhosis (probable or definite)

		without short fibrous septa	3: Fibrous expansion of most portal areas with occasional portal to portal bridging	5: Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	
Meta-analysis of histologic data in viral hepatitis (METAVIR) (Various etiologies)[18]	No fibrosis	Stellate enlargement of portal tract but without septa formation	Enlargement of portal tract with rare septa formation	Numerous septa without cirrhosis	Cirrhosis
Ludwig (PBC and PSC)[19]	N/A	N/A	N/A	Bridging fibrosis	Cirrhosis
Alcohol-associated liver disease (alcohol hepatitis histological score)[20]	No fibrosis or portal fibrosis		Expansive periportal fibrosis	Bridging fibrosis	Cirrhosis

Brunt-Kleiner (NAFLD)[21, 22]	No fibrosis	1°: Delicate perisinusoidal 1B: Dense perisinusoidal 1C: portal-only fibrosis	Perisinusoidal and portal/periportal fibrosis	Bridging fibrosis	Cirrhosis

Abbreviations: NAFLD = nonalcoholic fatty liver disease; N/A = not applicable; P-C = port-central; P-P = portal-portal; PBC = primary biliary cholangitis; PSC = primary sclerosing cholangitis.

Table 3b. Assessment and Grading of Steatosis Based On the Percent of Hepatocytes Affected

Degree of steatosis			
0 (Normal or minimal)	1 (Mild)	2 (Moderate)	3 (Severe
<5%	5%−33%	34%–66%	>66%

Based on references Kleiner et al.[21] and Brunt et al.[22]

Table 4. Diagnostic Performance Indices used in NILDA

Diagnostic index	Calculation	Comments
Sensitivity	TP/(TP + FN)	Not dependent on the prevalence of the condition in the population. High sensitivity helps rule out the disease (few FNs).
Specificity	TN/(TN + FP)	Not dependent on the prevalence of the condition in the population. High specificity helps ruling in disease (few FPs).
Accuracy	(TP +TN)/(P+N)	
PPV	TP/(TP+FP)	The probability that a person with a positive test indeed has the disease or condition of interest. Affected by the prevalence of the disease in the population.
NPV	TN/(TN+FN)	The probability that a person with a negative test does NOT have the disease or condition of interest. Affected by the prevalence of the disease in the population.
Positive LR	Sensitivity/(1-Specificity) OR TP/P	Positive LR greater than 10 suggests strong test to predict outcome.

Negative LR	(1-Sensitivity)/Specificity OR TN/N	Negative LR less than 0.1 suggests strong diagnostic evidence for not having the outcome.
DOR	Positive LR/Negative LR	The ratio of odds of positivity of those with disease relative to odds of positivity in those without disease. The higher the DOR, the better the test.
AUROC	Graph values of test performance from 0 (a perfectly inaccurate test) to 1 (a perfect test). Plots the diagnostic ability of a binary classifier system as its discrimination threshold is varied.	Summarizes the overall diagnostic accuracy of a test. In general, an AUROC of 0.5 suggests no discrimination (i.e., ability to diagnose patients with and without the disease or condition based on the test), 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered excellent, and more than 0.9 is considered outstanding
Abbreviations: AUROC = area under the receiver operating characteristic curve; DOR= diagnostic odds ratio; FP = false-positive; FN = false-negative; LR = likelihood ratio; N = all negative tests; NILDA = noninvasive liver disease assessments; NPV = negative predictive value; P = all positive tests; PPV = positive predictive value; TP = true positive; TN = true negative.		

Table 5. Components of Blood-Based Biomarker Algorithms for Fibrosis*

Blood-marker panel, year (reference)	Disease cohort	Clinical variables	Indirect markers	Direct markers	Model algorithm
Simple blood-based NILDA with or without clinical data					
APRI, 2003[41]	HCV	-	AST, platelets	-	[(AST level/ULN)/platelet count (10^9/L)] × 100
FIB-4, 2006[42]	HIV-HCV	Age	AST, ALT, platelets	-	$\frac{\text{age (years)} \times \text{AST (U/L)}}{\text{platelet count } (10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}}$
NFS, 2007[43]	NAFLD	Age, BMI, IFG/diabetes	AST, ALT, platelets, albumin	-	-1.675 + (0.037 × age) + (0.094 × BMI) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × (AST/ALT ratio) − (0.013 ×

					platelets) − (0.66 x albumin)
Fibroindex (2007)[44]	HCV		AST, platelets, gamma globulin		1.738 − 0.064(platelet [$\times 10^4/mm^3$]) + 0/005(AST IU/L) + 0.463(gamma globulin[g/Dl])
King's Score, 2009[45]	HCV	Age	AST, INR, platelets		Age × AST × INR/[platelet count (109/L)]
Easy Liver Fibrosis Test (Elift), 2017[46]	Mixed	Age, sex	GGT, AST, platelets, Prothrombin Index	-	Component weighted scores (0-4)
Complex, proprietary blood-based NILDA					
FibroSure™/FibroTest®, 2001[47]	HCV	-	α2M, GGT, total bilirubin, haptoglobin,Apo A-I[1]	-	Proprietary

ELF™, 2004[48]	Mixed	Age	-	HA, PIIINP, TIMP-1	Proprietary
FibroSpect II™, 2004[49]	HCV	-	α2M	HA, TIMP-1	Proprietary
HepaScore™, 2005[50]	HCV	Age, sex	Total bilirubin, α2M, GGT	HA	Proprietary
FibroMeter™, 2005[51]	Mixed	Age	Platelets, Prothrombin Index, urea, AST, α2M	HA	Proprietary

*Original study cohorts are referenced. Abbreviations: A2M = α2-macroglobulin; ALT = alanine aminotransferase; ApoA-1 = apolipoprotein A-1; APRI = AST-to-platelet Ratio Index; AST = aspartate aminotransferase; BMI = body mass index; ELF = enhanced liver fibrosis; Elift = easy liver fibrosis; FIB-4 = Fibrosis-4 index; GGT = gamma-glutamyl transferase; IFG = impaired fasting glucose; INR = international normalized ratio (also known as prothrombin time); HA = hyaluronic acid; L = liter; NAFLD = nonalcoholic fatty liver disease; NFS = NAFLD fibrosis score; PIIINP = amino-terminal propeptide of type III procollagen; PT = prothrombin time; TIMP-1 = tissue inhibitor matrix metalloproteinase 1; U = units; ULN = upper limit of normal common blood tests (includes the following: AST, ALT, platelet count, albumin, gamma-globulin, GGT, haptoglobin, PT, and total cholesterol).

Table 6. Clinical Factors Affecting Performance of Blood-Based Noninvasive Assessment of Fibrosis

Clinical condition	Tools affected	Comments
Age	FIB-4 NFS King's eLift ELF Hepascore™ FibroMeter™	In the age extremes (both very young and very old), may not perform as well.
Splenectomy	APRI FIB-4 Fibroindex FibroMeter™ NFS	Because these tools use platelets as a biomarker of portal hypertension, attenuated thrombocytopenia from splenectomy gives a falsely lower estimation.
Thrombocytopenia (not related to portal hypertension)	APRI FIB-4 Fibroindex FibroMeter™ NFS	Because these tools use platelets as a biomarker of portal hypertension, thrombocytopenia from other conditions gives a falsely higher estimation.
Active alcohol use[53]	FibroTest™ HepaScore™	Increases GGT, leading to falsely elevated estimation.
Elevated ALT and/or AST (inflammatory hepatitis) [53-55]	APRI FIB-4 Fibroindex FibroMeter™ NAFLD fibrosis score	Elevated aminotransferases occurring in relation to acute or acute-on-chronic hepatitis lead to falsely elevated estimation.

Chronic kidney disease[56-58]	Fibroindex APRI FIB-4 FibroMeter™	Elevated urea levels can result in falsely lower estimation. Hemodialysis patients tend to have lower ALT and AST levels, resulting in falsely lower estimation. Hemofiltration can result in lower stiffness in patients with baseline fluid overload.
Malnutrition	NAFLD fibrosis score	Albumin reduction that is disproportionate to liver dysfunction results in falsely elevated estimation.
Inflammatory condition	FibroTest™ Fibroindex HepaScore™ FibroMeter™	Can result in increased α2-macroglobulin levels and falsely elevated Fibrotest, and increased α-globulin and falsely elevated Fibroindex.
Hemolysis	FibroTest™ Hepascore™	Decreases haptoglobin levels and increases total bilirubin leading to falsely elevated estimation.
Gilbert syndrome and other cholestatic diseases	FibroTest™ Hepascore™	Can result in increased total bilirubin and falsely elevated estimation.
Postprandial[59]	NFS	Liver stiffness increases up to 26% have been described for TE-LSM 2 h after a meal. A rise in postprandial glucose (>110 mg/Dl) falsely elevates NAFLD fibrosis score.
Gastrectomy[60]	Fibrospect™ HepaScore™ ELF™	Increases hyaluronic acid resulting in falsely elevated estimation.
Extra-hepatic fibrosing conditions[61]	FibroMeter™ Fibrospect™ ELF™	Conditions such as interstitial lung disease can increase collagen turnover markers resulting in elevated estimation.
Acute sickle cell crisis[62]	FibroTest™	Related to hemolysis (as aforementioned); Decreases haptoglobin levels and increases total bilirubin leading to falsely elevated estimation.

Abbreviations: ALT = alanine aminotransferase; APRI = AST-to-platelet ratio index; AST = aspartate aminotransferase; BMI = body mass index; ELF = enhanced liver fibrosis; FIB-4 = Fibrosis-4 index; GGT = gamma glutamyl transferase; NAFLD = nonalcoholic fatty liver disease; NFS = nonalcoholic fatty liver disease fibrosis score.

Table 7. NAFLD Fibrosis Score for Diagnosis of Advanced Fibrosis

Author, year (reference)	Number of patients (% F3-4)	AUROC F3-4	Sensitivity/specificity ≤1.455*	Sensitivity/specificity >0.676**	Number of Indeterminates (%)	Comments and subgroups
Angulo, 2007[43]	480 (26%) 253 (29%)	0.88 0.82	0.82/0.77 0.77/0.71	0.51/0.98 0.43/0.96	114 (24%) 70 (28%)	LR+ 11-26 (high cutoff) −LR 0.23–0.32 (low cutoff)
Qureshi, 2008[65]	331 (14%)	N/A	0.96/N/A	N/A/0.84	154 (46%)	
Wong, 2008[66]	162 (11%)	0.64	0.39/0.81	0/0.99	32 (20%)	
Wong, 2010[67]	228 (23%)	0.75	0.73/0.69	0.18/0.96	N/A	

McPherson, 2010[68]	145 (19%)	0.81	0.78/0.58	0.33/0.98	N/A	
Ruffillo, 2011[69]	138 (27%)	0.68	0.23/N/A	N/A/1.0	42 (30%)	
Xun, 2012[70]	154 (16%)	0.65	0.37/0.86	0.08/1.0	25 (16%)	
Sumida, 2012[71]	576 (11%)	0.86	0.92/0.63	0.33/0.96	206 (36%)	
Cichoz-Lach, 2012[72]	126 (21%)	0.92	0.96/N/A	N/A/0.84	39 (31%)	
Yoneda, 2013[73]	235 (16%)	0.84	N/A	0.68/0.88	N/A	Normal ALT cohort
Lee, 2013[74]	107 (32%)	0.88	0.82/0.77	N/A	N/A	
Demir, 2013[75]	Aqsw` daZ	0.96	0.75/0.93	0.19/1.0	16 (13%)	
Cui, 2015[76]	102 (19%)	0.82	0.84/0.69	0.21/0.96	N/A	
Lykiardopoulos, 2016[77]	158 (24%)	0.79	0.44/N/A	N/A/0.37	84 (53%)	

Rath, 2016[78]	60 (3%)	0.47	0.05/N/A	N/A/1.0	8 (13%)	
Jun, 2017[79]	328 (18%)	0.64	0.53/0.67	0.09/0.98	N/A	
McPherson, 2017[80]	74 (11%) 96 (19%) 197 (22%) 191 (34%) 76 (40%)	0.52 0.86 0.81 0.83 0.81	0/0.91 0.78/0.80 0.81/0.65 0.95/0.44 0.93/0.20	0/1.0 0.22/1.0 0.22/0.97 0.31/1.0 0.57/0.85	N/A	Age (years) ≤35 36–45 46–55 56–64 ≥65
Bertot, 2018[81]	241 (31%)	0.72	N/A	0.76/0.85	N/A	
Patel, 2018[82]	115 (10%) 154 (34%)	0.72 0.76 0.71	0.09/0.35 0.02/0.62 0.04/0.84	0.45/0.98 0.68/0.83 0.74/0.68	N/A	Age (years) <50 years 50–64 ≥65

	60 (46%)					
Chan, 2019[83]	753 (24%)	0.69	N/A	0.16/0.99	215 (29%)	
Kaya, 2019[84]	463 (17%)	0.71	0.71/0.63	0.15/0.96	173 (37%)	
Yang, 2019[85]	453 (28%)	0.53	N/A	0.19/0.92	N/A	
Anstee, 2019[86]	2417 (80%)	0.74	0.89/0.37	0.38/0.89	1208 (51%)	Clinical trial cohort
Petta, 2019[54]	968 (28%)	0.76	0.74/0.70	0.16/0.97	348 (36%)	
De Carli, 2020[87]	246 (9%)	N/A	N/A	0.12/0.96	N/A	Bariatric surgery cohort
Bril, 2020[88]	213 (17%)	0.64	N/A	0.91/0.40	144 (68%)	
Alkayyali, 2020[89]	166 (29%) 183 (10%)	0.73 0.72	0.75/0.47 0.85/0.60	0.25/0.93 0/0.97	79 (47%) 77 (42%)	DM Non-DM

Pitisuttithu m, 2020[90]	472 (6%) 131 (17%)	0.68 0.65	0.67/0.65 0.74/0.41	0.10/0.94 0.26/0.86	N/A N/A	Age (years) <60 ≥60

*Lower cutoff to rule-out F3-4, **higher cutoff to rule-in F3-4.

ALT = alanine aminotransferase; AUROC = area under receiver operating characteristic curve;

DM = diabetes mellitus;

LR = likelihood ratio; N/A = not available/not applicable

Table 8. Serum Biomarkers for Fibrosis Progression and Regression

Serum biomarker, year of study (reference)	Etiology and baseline fibrosis prevalence	Paired biopsy (n)	Sampling interval	Fibrosis change from baseline	Change in index biomarker scores with change in fibrosis stage	Comments
PIIINP and HA, 2001[148]	HCV (F2-4 = 38% for *n* = 105 NR)	239	16–26 months	No significant change in Knodell/METAVIR stage	No change in fibrosis or serum markers	Data based on response to IFN-based therapy
FibroTest™, 2002[149]	HCV (F3 = 32%, F4 = 0%)	134	72 weeks	Progression (*n* = 28) No change (*n* = 83) Regression (*n* = 23)	Progression: 0.04 No Change: −0.02 Regression: −0.03	IFN-based therapy; Knodell score (no stage F2)
FibroTest™, 2003[150]	HCV	352	72 weeks	Progression (*n* = 61)	Progression:	IFN-based therapy; *N*

	(F2 = 17%, F3 = 6%, F4 = 6%)			No change (n = 193) Regression (n = 98)	+1 stage = −0.06, +2 = 0.02, +3 = −0.01 No change: −0.07 Regression: −1 stage = −0.09, −2 =−0.15,−3=−0.25	= 32 F4; FT decline significant in 17/32 ≥ 1 stage decrease. No change in FT for n = 15/32 with F4 at follow-up
HA, TIMP-1, PIIINP, YKL-40, 2010[151]	HCV (Ishak 4 = 30%)	209	24–48 months	Progression n = 70 (34%)	Not provided	HALT-C IFN-based therapy. Baseline HA and platelets significant in multivariate model

						for fibrosis progression
FibroTest™, 2013[152]	HCV (F2 = 46%, F3 = 54%)	258	3.6–3.9 years	Progression (*n* = 97) No change (*n* = 111) Regression (50)	Progression: +1 stage = 0.04, +2 = 0.07, +3 = 0.23 No change = 0.03 Regression: −1 stage = 0.01, −2 = 0.01,−3 = −0.01	EPIC-3 IFN-based therapy. No association between FibroTest and differences in fibrosis stage
FibroSURE®, 2014[153]	HCV (F2-4 = 48%)	133	72 weeks	No change *n* = 80 (60%)	Change in FT/FS was not associated with change in	IFN-based therapy

					fibrosis stage	
FibroTest™, 2014[154]	HCV (Ishak 2 = 40%, 3 = 45%, 4 = 15%)	194	52 weeks	Progression *n* = 34 (18%)	Not provided	HCV non-IFN Antifibrotic study; Pro-CIII associated with fibrosis progression in multivariate model
FIB-4, APRI, Forns Index, 2015[95]	HCV (F0-1 = 60%, F2 = 27%, F3-4 = 13%)	115	5.9 ± 1.8 years	Progression (*n* = 5) No change (*n* =1 06) Regression (*n* = 4)	Lower index scores for all markers at post-SVR biopsy	All patients with SVR Optimal lower cutoffs associated with accuracy

						71%-79% for F2-4, and 70%-83% for F3-4
FibroTest™, 2016[155]	HCV (Ishak 2 = 39%, 3 = 44%, 4 = 15%, 5 = 1%)	201	52 weeks	Progression (*n* = 42) No change (*n* = 122) Regression (*n* = 31)	Progression: +1 stage = −0.04, +2 = 0.00 No change = −0.03 Regression: −1 stage = 0.02	HCV in non-IFN antifibrotic study No association with FibroTest index and changes in fibrosis stage
FIB-4, APRI, King score, ELF®, 2016[96]	HCV (F4 = 100%)	38	61 (48–104) months	Regression (*n* = 23) No change (*n* = 15)	Lower index scores for all markers at post-	All patients with SVR No difference in scores

					SVR biopsy. AUROC for post-SVR F4 APRI = 0.58, FIB-4 = 0.59, King score = 0.59, ELF = 0.63	between regressors and non-regressors at post-SVR biopsy (AUROC 0.52-0.75)
ELF®, 2017[156]	HCV (Ishak 3 = 14%, 4 = 14%, 5/6 = 26%)	70	24 months	Progression (*n* = 21) No change (*n* = 25) Regression (*n* = 24)	ELF at baseline/12 months to predict 1-stage progression (AUROC 0.72) and regression (0.64)	IFN-based therapy

FibroSURE®, APRI, 2006[157]	Mixed (HCV and HIV-HCV) (F2 = 28%, F3 = 7%, F4 = 3%)	119	4.2 (2.8–6) years	Progression *n* = 25 (21%)	FibroSure PPV 0.31 and APRI 0.375 for predicting F2-4 on second biopsy	IDU cohort; HIV-HCV = 27%
APRI, 2007[158]	HIV-HCV (Ishak 3 = 11%, 4 = 1%	174	2.9 years	Progression in *n* = 41 (24%)	AST but not APRI associated with fibrosis progression	
FibroTest™ Forns Index, APRI, FIB-4, HepaScore™, FibroMeter™, 2009[159]	HIV-HCV (F2 = 46%, F3 = 23%, F4 = 11%)	114	72 weeks	Progression (*n* = 37) No change (*n* = 49) Regression (*n* = 28)	Significant decline in all biomarker index scores with	Data based on IFN-based therapy response

					SVR, except HepaScore	
FIB-4, APRI, 2010[160]	HIV-HCV (Ishak 3 = 15%, 4 = 9%)	66	4.7 years	Progression (*n* = 21) No change (*n* = 26) Regression (19)	No difference in FIB-4 and APRI between progressors (Ishak ≥ 2) and no fibrosis change	
FibroMeter™, FibroTest™, HepaScore™, 2012[161]	HCV and HIV-HCV (F3 = 25%, F4 = 27%)	101 (HCV n=62, HIV-HCV	96 weeks	Progression (mean 0.2 METAVIR units)	Not provided	IFN-based therapy Progression in area of fibrosis, FibroMeter, and CirrhoMeter

		n=39)				
FIB-4, APRI, 2014[162]	HIV-HCV (F2 = 11%, F3 = 3%)	282	2.5 years	Progression *n* = 97 (34%)	Not provided	AST and ALT >2.5 ULN between biopsies associated with fibrosis progression in multivariate model
FIB-4, APRI, FibroTest™, 2015[163]	HIV-HCV (F0-F3)	38	3 years	Progression (*n* = 10) No change (*n* = 27) Regression (*n* = 1)	Progression: FIB-4 +0.75, APRI +0.36, FT +0.04 No change/regressor:	Only *N* = 5 with HCV treatment; differences between progressors and non-progressors for APRI

					FIB-4: −0.06, APRI: −0.30, FT: −0.03	and FIB-4 (p = 0.03); FT= not significant
FibroTest™, 2009[164]	HBV (F2-4 = 44%)	462	48 weeks	Regression (0.16-0.30 mean METAVIR units)	Not provided	Antiviral therapy/placebo treatment; FibroTest improved in virologic responders with F2-4, and placebo
APRI, FIB-4, 2016[125]	HBV (Ishak 3 = 23%, 4 = 10%, 5-6 = 24%)	294	240 weeks	Regression in F4-6 from 34% to 12%)	No correlation with regression	On antiviral therapy; 81%-89% baseline advanced fibrosis or cirrhosis missed by

						simple scores
APRI, FIB-4, 2019[165]	HBV (median Ishak 3)	80	2.06 years to second biopsy	Regression 0.18 Ishak Units/year	Not provided	Multiple biopsies over 17 years, variable treatment, Greater relative decline FIB-4 (-17%) and APRI (−43%) in year 1
APRI, FIB-4, NFS, BARD, 2010[166]	NAFLD (F3-4 = 4%)	52	36 months	Progression (*n* = 14) No change (*n* = 25) Regression (*n* = 13)	Progression: APRI = +0.003, FIB-4 = +0.079,	Prospective study; No significant correlation between change in fibrosis

					NFS = +0.06, BARD = 0 No change/ Regression : APRI = −0.029, FIB−4 = −0.019, NFS = −0.017, BARD = 0	stage and markers
APRI, 2012[167]	NAFLD (Any fibrosis = 45%)	78	Variable	Not provided Any fibrosis *n* = 22 (31%)	Baseline APRI = 0.29 After weight loss APR1 = 0.29	Bariatric surgery cohort with morbid obesity. Variable biopsy interval after weight loss. No

						change in APRI
APRI, FIB-4, NFS, 2017[168]	NAFLD (F3-4 = 10%)	261	52 weeks	Progression (*n* = 45) No change (*n* = 165) Regression (*n* = 51)	Progression: APRI = −0.16, FIB-4 = −0.05, NFS = +0.02 No change: APRI = −0.14, FIB-4= −0.08, NFS = −0.42 Regression: APRI = −0.25, FIB-4 = −0.23, NFS = −1.00	Lifestyle intervention study
ELF™, FibroTest™, NFS, 2018[169]	NAFLD (NASH CRN F3	427	96 weeks	F3 : Progression (*n* = 41)	No significant change in	Phase Iib study

	= 46%, F4 = 54%)			Regression (*n* = 40) ; F4: Regression (*n* = 22)	serum markers with fibrosis stage	
ELF™, FibroTest™/FibroSure®, 2018[170]	NAFLD (F2 = 35%, F3 = 65%)	72	24 weeks	Progression (*n* = 23) No change (*n* = 34) Regression (*n* = 23)	No change in serum markers across treatment groups	Phase II study for NAFLD stage F2-3
APRI, FIB-4, NFS, 2019[125]	NAFLD (F3-4 = 26%)	292	2.6 years	Progression (*n* = 92) No change (*n* = 126) Regression (*n* = 74)	Progression: APRI = +0.2, FIB-4 = +0.5, NFS = +0.7 No change: APRI = −0.2, FIB-4 = +0.1, NFS = +0.4	NASH CRN cohort. APRI, FIB-4, and NFS associated with progression, but not regression

					Regression : APRI = −0.3, FIB-4 = 0.0, NFS = +0.5	
ELF™, 2020[171]	NAFLD (F3 = 44%, F4 = 4%)	43	12 weeks	Regression (*n* = 14)	Decline in ELF (−7% vs. −3%) and Pro−CIII (−56% vs. −9%) for histologic responders vs. non-responders	Phase II study
FIB-4, APRI, FibroSURE®, ELF™ 2019, 2022[172, 173]	NAFLD (F3 = 56%)	931	18 months	Progression (*n* = 130) No change (*n* = 412) Regression (*n* = 223)	AUROC 0.58-0.61 for 10% decrease in markers at month 18 to predict	Phase III study Data provided by treatment groups indicate

					fibrosis regression	greater decline in markers with regression. Overall weak association between improvement in markers and fibrosis stage
ELF™, FibroTest™, 2019[174]	NAFLD (F3 = 52%, F4 = 47%)	1527	48 weeks	Regression (*n* = 207) No histologic response (*n* = 1324)	Response (regression): ELF = −0.2%; FT not provided No response: ELF =	Pooled Phase III data. Data provided as fibrosis regression and no worsening NASH

					1.3%; FT not provided	(histologic response)
ELF™, FibroTest™/FibroSURE®, 2019[115]	PSC (Ishak 4-6 = 26%)	234	96 weeks	Progression (*n* = 80) No change (*n* = 74) Regression (*n* = 79)	Not provided	Phase II study. Baseline ELF associated with progression to cirrhosis

Abbreviations: APRI = AST-to-platelet Ratio Index; AUROC = Area Under Receiver Operating Characteristic Curve; BARD = body mass index, AST/ALT ratio, and presence of type 2 diabetes mellitus; ELF = enhanced liver fibrosis; FT/FS = FibroTest/FibroSURE; HA = hyaluronic acid; HALT-C = hepatitis c antiviral long-term treatment against cirrhosis; HBV = Hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IFN = interferon; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NFS = NAFLD fibrosis score; PIIINP = amino-terminal propeptide of type III procollagen; PBC = primary biliary cholangitis; PSC = primary sclerosing cholangitis; Pro-C3 = N-terminal pro-peptide of type III procollagen; TIMP-1 = tissue inhibitor matrix metalloproteinase 1; ULN = upper limit of normal.

Table 9. Noninvasive Algorithms to Assess Hepatic Steatosis Compared With Histology or MR Spectroscopy or MR PDFF

Algorithm	Formula or Components
FLI	Log(0.953 × ln TG) + 0.139 × BMI + 0.718 + ln(GGT) + 0.053 × WC − 15.745 × 100
HSI	8 × ALT/AST + BMI + 2 (if DM) + 2 (if female)
LAP	(WC [cm] − 65) × TG (mmol/L) male individuals (WC [cm] − 58) × TG (mmol/L) female individuals
NLFS	−2.89 + 1.18 × MS + 0.45 × DM + 0.15 × insulin + 0.04 × AST − 0.94 × AST/ALT
ION	1.33 × waist-to-hip ratio + 0.03 TG (mg/dL) + 0.18 ALT (U/L) + 8.53 HOMA-IR − 13.93 in male individuals 0.02 TG (mg/dL) + 0.24 ALT (U/L) + 9.61 HOMA-IR − 13.99 in female individuals
Steatotest™	ALT, A2M, ApoA1, haptoglobin, total bilirubin, GGT, total cholesterol, TG, glucose, age, gender, BMI
TyG	Log(TG [mg/dL]) × glucose (MG/dL)/2
VAI	(WC/39.68 + 1.88 BMI) × (TG/1.03 × 1.31/HDL) for male individuals (WC/36.58 + 1.89 BMI) × (TG/0.81 × 1.52/HDL) for female individuals
DSI	ALT, BMI, age, sex, triglyceride and glucose levels, diabetes, hypertension, and ethnicity

Abbreviations: ALT = alanine aminotransferase; ApoA1 = apolipoprotein A; AST = aspartate aminotransferase; A2M = α-2 macroglobulin; BMI = body mass index; DM = diabetes mellitus; DSI = Dallas steatosis index; FLI = fatty liver index; GGT = gamma-glutamyl transferase; HDL = high-density lipoprotein; HOMA-IR = Homeostasis Model of Assessment For Insulin Resistance; HSI = hepatic steatosis index; ION = index of NALFD; LAP = lipid accumulation product; MS = metabolic syndrome; NAFLD = nonalcoholic fatty liver disease; NLFS, NAFLD liver fat score; PDFF = proton density fat fraction; TG = triglyceride; TyG = triglyceride index; VAI = visceral adiposity index; WC = waist circumference.

Table 10. Performance of Blood-Based Algorithms for Diagnosis of Hepatic Steatosis

Test	Reference	*N*	Cutoffs (if provided)	Comparator	Sensitivity	Specificity	AUROC
FLI	224	182	<30	LB	100	3	0.59
			≥60		97	13	
	201	40	<30 or ≥60	MR	90	74	0.86
	214	264	<30 or ≥60	LB			0.75
	216	324	>60	LB	76	87	0.83
	219	336	>30	MR	75	69	0.79
			>60		44	91	
	217	250	≥79	LB	81	49	0.67
	199	4458	<30	LB	80		
	222	135		LB		80	0.74
~~his~~HSI	224	182	<30-45	LB	88	10	0.41
			≥36-67		7	90.	
	201	40	<30 or ≥36	MR	86	66	0.75
	215	364		LB			0.63
	217	324	>41.6	LB	61	93	0.81
	227	366	35.6	LB	61	63	0.66
	209	10,724		LB	78	69	0.77
	222	135					0.71
LAP	224	182	Continuous	LB			0.63
	215	364		LB			0.70
	219	336		MR			0.78
NFLS	218	470	−0.640	MR	86	71	0.87
	224	182	−06.40	LB	71	62	0.64
	226	324	>0.16	LB	65	87	0.80
ION	199	4458	<11	LB	81	56	0.77
			≥22		60	82	
Steato-Test™	218	310	≥0.3	LB	90	54	0.79
			≥0.7		46	88	
	227	288	0.38	LB	86.9	50	0.65
			0.69		42	79	0.81
	217	494	0.38	LB	89	44	
			0.69		38	81	0.80

	225	220	0.52	MR	73	72	0.73
SteatoTest-2™	227	2997	0.40	LB	79	50	0.77
TyG	220	324	>8.38	LB	80	92	0.90
	238	50	4.235	LB	94	69	0.86
	229	340	4.515	LB	70	60	0.68
VAI	220	324	>1.25	LB	79	92	0.92

Abbreviations: AUROC = area under receiver operator characteristic curve; FLI = fatty liver ihisx; HSI = hepatic steatosis index; ION = index of NAFLD; LAP = lipid accumulation product; LB = liver biopsy; MR = magnetic resonance; NAFLD = nonalcoholic fatty liver disease; NFLS= NAFLD liver fat score; TyG = triglyceride index; VAI = visceral adiposity index.

Table 11. Blood-Based NILDA: Major Areas for Future Research

Comparative studies of proprietary and nonproprietary blood-based NILDA are needed in the primary care population, with lower expected prevalence of advanced fibrosis and with attention to cost-effectiveness to generalize the application of NILDA.
Studies on NILDA should include diverse populations and children.
All findings among patients with NAFLD in this guideline will need to be confirmed among patients with the new MASLD and SLD nomenclature.
Confirmation that novel markers such as PRO-C3, a serologic biomarker that detects formation of type III collagen from activated myofibroblasts, especially when combined with age, presence of T2DM, and platelet count, are superior to APRI, and FIB-4 in MASLD and NASH is needed.
Emerging data with newer biomarkers such as ELF™ may improve the accuracy of blood-based NILDA in NAFLD and MASLD.
Comparative studies combining both blood-based and imaging-based tests synchronously and sequentially are needed to reflect clinical practice, with recognition of test utility by insurance and third-party payors.
Blood-based algorithms have the potential to help identify those with steatosis, but, to enhance clinical utility, they need to differentiate simple steatosis from MASLD and NASH.
Utilization of artificial intelligence and machine-learning tools should allow for incorporation of demographics and a wide array of clinical data to improve diagnosis and management of CLD.

Longitudinal studies of NILDA to assess the natural history of chronic liver diseases, clinical outcomes, and changes with therapy are needed.
Abbreviations: APRI = AST-to-platelet ratio index; AST = aspartate aminotransferase; CLD = chronic liver disease; ELF = enhanced liver fibrosis; FIB-4 = Fibrosis-4 Index; NASH = nonalcoholic steatohepatitis; NILDA = noninvasive liver disease assessments; MASLD = metabolic dysfunction-associated steatotic liver disease; PRO-C3: N-terminal propeptide of type III collagen; SLD = steatotic liver disease; T2DM = type II diabetes mellitus.

Acknowledgements: We thank Audrey Davis-Owino from the American Association for the Study of Liver Diseases (AASLD) for her untiring support and Marie Kreck at Virginia Commonwealth University for editorial assistance. We also thank Ruben Hernaez and the AASLD Practice Guidelines Committee for their expertise, patience, and editorial guidance.

Figure 1