# BIOMARKERS FOR DIAGNOSING AND STAGING NONALCOHOLIC FATTY LIVER DISEASE AND NONALCOHOLIC STEATOHEPATITIS:

## Current Perspectives and Potential Future Applications

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## FOREWORD

The nonalcoholic steatohepatitis (NASH) biomarkers landscape has evolved substantially over the years, with a growing body of evidence supporting the use of non-invasive modalities, often referred to as non-invasive tests or non-invasive diagnostics, to screen, diagnose, stage, and monitor patients with NASH. Through this lens, NASHNET, a consortium of hepatologists united under a common goal to advance NASH care delivery, convened a multidisciplinary task force to understand how NASH biomarkers are currently being operationalized, discuss the ideal application of NASH biomarkers within clinical care pathways, and brainstorm strategies to accelerate provider biomarker adoption with the aim to increase early identification of patients with NASH. Task Force members convened for a series of virtual meetings between December 2022 and January 2023, culminating in a Biomakers Summit, and included:

**Task Force Members:**

**S. Colby Danna, MD**

Associate Medical Director of Ochsner Primary Care & Senior Lecturer/Associate Professor Ochsner Health

**Mazen Noureddin, MD, MHSc**

Founder & Director of Houston Liver Institute

**Jeff McIntyre, MA**

Vice President, Liver Health Programs Global Liver Institute

**Michael Fuchs, MD, PhD**

Professor of Medicine, Virginia Commonwealth University; Chief of Gastroenterology, Central Virginia VA Health Care System; VISN 6 Lead Gastroenterologist; Vice-Chair, NAFLD FAB Subcommittee; Director of GI Research, Richmond Institute for Veterans Research

**Kathleen Wyne, MD, PhD**

Director of the Adult T1D Program, Director of the Adult CFRD Program, Director of the Diabetes Post-Discharge Transitions of Care Clinic, Medical Director of La Clinic Latina, and Professor Ohio State University/Wexner Medical Center

**Sonal Kumar, MD, MPH**

Director of Clinical Hepatology & Assistant Professor of Medicine Weill Cornell Medicine

**Kenneth Cusi, MD, FACP, FACE**

Chief of Division of Endocrinology, Diabetes, & Metabolism University of Florida

**Stephen Harrison, MD**

Founder & Chairman of the Board Pinnacle Clinical Research

Recognizing a need to advance awareness and education relating to NASH biomarkers, NASHNET has conducted a literature review and compiled key learnings within this white paper. Task Force participant consensus statements and considerations for adopting biomarkers within clinical care pathways are included throughout. Sponsorship support was provided by Novo Nordisk Inc.

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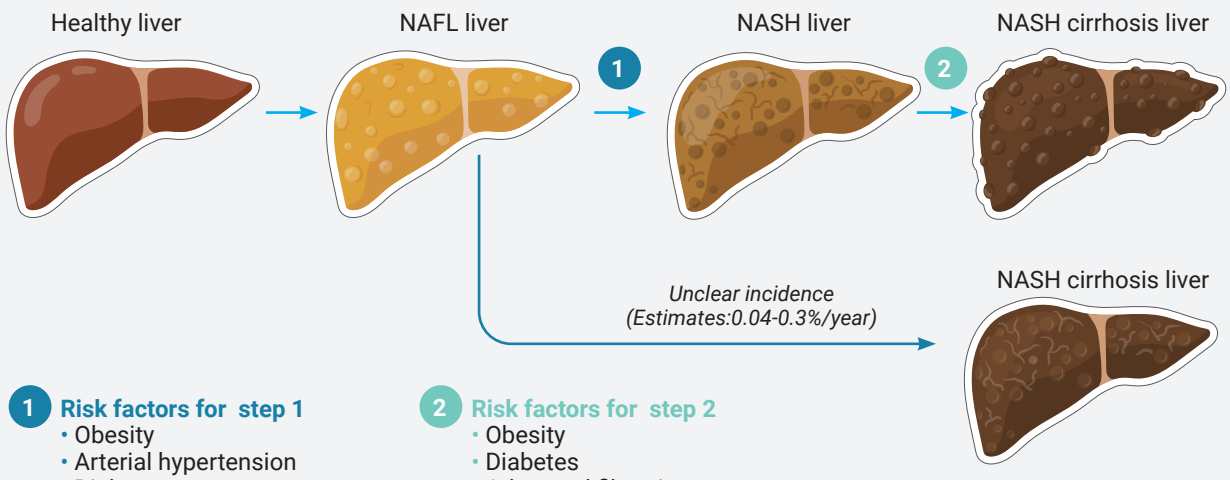
## INTRODUCTION

Globally, nonalcoholic fatty liver disease (NAFLD), which ranges from isolated hepatic steatosis or nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and NASH cirrhosis, is one of the most prevalent liver diseases.[1-4] NAFLD, which was first used as the overarching descriptor in 2002, is characterized by hepatic steatosis.

Hepatic steatosis is the macrovesicular accumulation of triglycerides in at least 5% of hepatocytes in the absence secondary causes such as medications, excessive alcohol consumption, and certain hepatic conditions.[3]

**NAFLD progression**




**1 Risk factors for step 1**
- Obesity
- Arterial hypertension
- Diabetes
- Dyslipidemia
- PNPLA3/TM6SF2 Polymorphisms
- Gut microbiota
- Dietary habits

**2 Risk factors for step 2**
- Obesity
- Diabetes
- Advanced fibrosis
- PNPLA3/TM6SF2 Polymorphisms
- Age

The two primary subtypes of NAFLD—NAFL and NASH—are distinguishable by the absence or presence of inflammation, respectively. In addition to presence of inflammation, NASH is characterized by hepatocyte injury, or ballooning, with or without fibrosis.[2-4] Of note, lobular inflammation accelerates progression of fibrosis.[5] In the cirrhotic liver, bands of fibrous septa lead to formation of cirrhotic nodules.[3] At this stage, the earlier features of NASH may not be evident on liver biopsy. NASH cirrhosis is associated with adverse outcomes, such as hepatic morbidity (including hepatocellular carcinoma) and mortality, the need for transplantation, cardiovascular disease, and overall mortality.[1,2,6-8] While individuals with NAFL typically experience slow disease progression—only 4% progress to cirrhosis—those with NASH are at greater risk for cirrhotic disease; more than 1 of every 5 NASH patients develops cirrhosis during his or her lifetime.[2]

The degree of fibrosis is the single most important predictor of liver-related mortality.[6]

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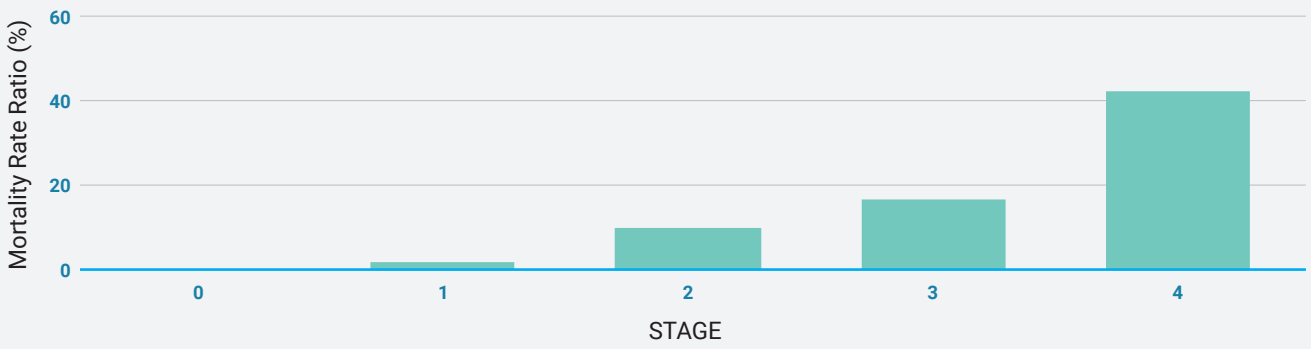
**Figure 2. Liver-related Mortality by Fibrosis Stage (multivariate analysis)[6]**

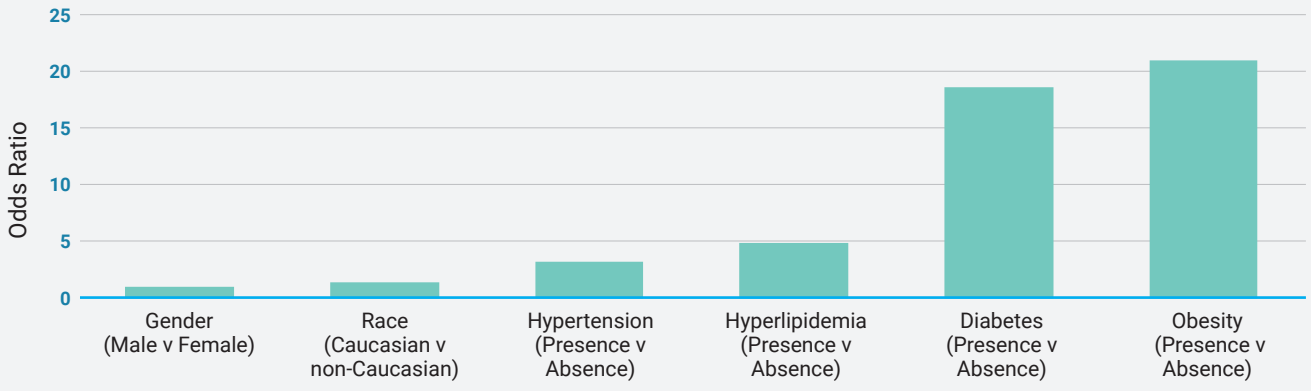



The global prevalence of NAFLD has increased considerably over the past years, comparable with the increased prevalence of obesity and metabolic comorbid disease (eg, insulin resistance, dyslipidemia, central obesity, hypertension, and metabolic syndrome).[1-3] Currently, NAFLD affects an estimated 30% of the US population, and ~5% of the US population is estimated to have NASH.[1] Individuals with risk factors (Figure 3) have increased prevalence of NAFLD. For example, 6 out of every 10 individuals with type 2 diabetes have NAFLD.[9]

Individuals with high-risk NASH—NAFLD activity score of >4 and fibrosis stage of >2 are at increased risk for adverse events. In the United States, the prevalence of age-adjusted high-risk NASH is 1.2%, affecting as many as 2 million individuals. The prevalence of this subtype among those with type 2 diabetes is higher, ranging from 8.7% to 22.5%.[7]

The prevalence of NASH varies according to clinical setting, race/ethnicity, presence/absence of NASH risk factors, and age.[3,10] Of note: NAFLD and its subtypes remains undiagnosed in many individuals.[3] A recent study analyzed data from patients with NASH from 2010 to 2020 in a large database that included electronic health records from 26 nationwide healthcare systems.[10] Study results confirm NASH disproportionately affects Caucasians, males, people aged 50-70, and individuals with diabetes, obesity, dyslipidemia, and hypertension. Multivariate analysis of risk factors for NASH confirmed increased odds ratios for risk factors associated with NASH, including diabetes hyperlipidemia, hypertension, and obesity (Figure 3).[10]

**Figure 3. Odds Ratio for NASH Based on Multivariate Analysis[10]**



Between 2010 and 2020, the prevalence of NASH prevalence rose by nearly 100%, and it is expected to continue to rise.[1,3,10] The prevalence of NASH is projected to double by 2030; correspondingly, the incidence of hepatic decompensation, hepatocellular carcinoma, and NASH cirrhosis-related mortality is estimated to increase by 2- to 3-fold.[3]

The burden of NASH is substantial. Compared with a matched general population, individuals with NASH had significantly lower[11]:

- Mental component score (43.19 v 46.22; P=0.010)
- Physical component score (42.04 v 47.10; P<0.001)
- Short Form Six Dimension (SF-6D) Health Index (0.63 v 0.69; P<0.001)
- EuroQoL 5 Dimension (EQ-5D) questionnaire (0.72 v 0.78; P<0.001)

Individuals with NASH experienced more anxiety and depression than those in the general population (37.5% v 25.5%; P=0.006 and 43.4% v 30.1%; P=0.004, respectively).[11] Healthcare resource utilization was higher in NASH patients, as reflected by more visits to a healthcare provider and emergency department visits, and hospitalizations (8.43 v 5.17, P<.001; 0.73 v 0.38, P-0.021; and 0.43 v 0.21, P=0.024, respectively). Specialist and nontraditional provider visits were also higher among those with NASH compared with the general population. The presence of NASH also impacted work productivity and activity compared with the general population[11]:

- Absenteeism (17.0% v 9.2%; P=0.041)
- Presenteeism (32.9% v 22.2%; P=0.019)
- Overall work impairment (39.6% v 26.2%; P=0.011)
- Activity impairment (44.7% v 30.8%; P<0.001)

A lifetime Markov model for all stages of NASH underscores the economic burden of the disease.[12] Lifetime costs of all NASH patients in the United States in 2017 were estimated at $222.6 billion; the cost for only the advanced NASH population was estimated to be $95.4 billion. Of note: the model did not include costs of comorbidities, nonmedical costs, or the societal costs of NASH. Direct medical costs of advanced fibrosis and hepatocellular carcinoma care associated with NASH had the most significant impact on the total health care costs. Importantly, the investigators found that increases in fibrosis regression would be impactful in reducing lifetime costs as 1 year in a fibrosis state is significantly less expensive than 1 year with advanced NASH.[12]

Early diagnosis of NASH and distinguishing it from simple steatosis is critical to mitigate the risk of disease progression to fibrosis.[5]

The high—and increasing—prevalence and associated personal, societal, and healthcare burden of NAFLD and its subtypes emphasize the need for early diagnosis and staging of fibrosis when present. Risk stratification is key. Experts in hepatology and gastroenterology recently attended a Biomarker Summit to discuss the status of available biomarkers, development of care pathways using biomarkers for optimal diagnosis, management, and prevention of disease progression, and ways to validate pathways through real-world evidence. The following highlights their discussion.

## Biomarkers for Diagnosing and Staging Nonalcoholic Steatohepatitis

Presence of steatosis should be assessed whenever metabolic liver disease is suspected as a primary disease or coexisting condition.[13]

Liver biopsy has been the standard diagnostic and staging tool for NAFLD; however, its use is associated with several limitations including its invasiveness and complications (bleeding, pain, hospitalization, time away from work, and rarely, death), inter-and intra-observer variability, sampling error (a biopsy provides 1/50,000 of the liver for interpretation), and cost.[5,14-19] Thus, the ideal biomarker is one that[1,15,17]:

- Is noninvasive
- Is accurate
- Is reproducible
- Recapitulates normal physiologic or pathogenic processes and changes in parallel with disease improvement or worsening
- Can be used for both diagnosis and quantification of steatosis
- Determines presence and extent of NASH-related injury
- Identifies and quantifies fibrosis
- Has high accessibility
- Is cost-effective
- Is predictive of outcomes

Noninvasive biomarker panels can be categorized according to 1 of 2 approaches: a "biological" approach based on the quantification of biomarkers in serum samples or a "physical" approach based on the measurement of liver stiffness, using either imaging ultrasound- or magnetic resonance-based elastography (MRE) techniques.[5,17] Of note: serum biomarkers include several, not necessarily liver-specific, clinical and serum components that have been associated with NASH or fibrosis stage. As such, they may overlap with other conditions that must be given diagnostic consideration.

### Serum Biomarkers

Due to the lack of accuracy of a single biomarker for the diagnosis of NASH, biomarker panels combining clinical and laboratory components have been constructed.[5] Serum biomarker panels have broad application, from diagnosing or grading steatosis, staging fibrosis, and measuring hepatocellular damage to differentiating patients with NASH from those with simple steatosis and directly measuring fibrosis to identify patients with advanced fibrosis.[5,17] A few biomarkers, such as the Enhanced Liver Fibrosis (ELF) score, are proprietary formulas, but most are nonproprietary. Table 1 provides an overview of biomarker panels for diagnosing steatosis, while Table 2 outlines those for identifying NASH. Table 3 outlines fibrosis markers that are used to identify NAFLD fibrosis.

## Table 1. Biomarker Panels for Diagnosing Steatosis[5,17]

| Scoring System | Components, Interpretation | Sensitivity | Specificity | Limitations |
|---|---|---|---|---|
| Hepatic Steatosis Index (HSI) | ALT:AST ratio, BMI, T2DM, gender<br><br><30 rules out NAFLD; >36 detects NAFLD | Values <30 rule out NAFLD with a sensitivity of 93.1% | Values >36 detects NAFLD with a specificity of 93.1% | Poorly distinguishes mild steatosis from moderate-to-severe steatosis; accuracy decreases in obese children |
| Fatty Liver Index (FLI) | Triglycerides, BMI, GGT, waist circumference<br><br>Values <30 rule out NAFLD | 79.8% | 71.5% | Poorly distinguishes mild steatosis from moderate-to-severe steatosis; accuracy decreases in obese patients |
| SteatoTest[a] | Age, BMI, a-2 macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, AST, ALT, glucose, triglyceride, total cholesterol | 89.7%<br><br>PPV, 90.9% | 44.9%<br><br>NPV, 41.3% | Further studies needed for validation |
| NAFLD Fatty Liver Score (NFLS) | AST, AST:ALT ratio, fasting serum insulin<br><br>High accuracy; >5.56% detects liver fat | 86% | 71% | Lacks external validation |
| Lipid Accumulation Product | Gender, serum triglycerides, waist circumference<br><br>Assessment associated with severity of steatosis | | | Needs additional external validation<br><br>Results are gender-based |
| NAFLD Ridge Score | ALT, triglycerides, HDL, HbA1c, hypertension, leukocyte count<br><br>Effective for detecting NAFLD | | NPV, 96% | Limited to research setting<br><br>Fails to risk stratify different steatosis grades |

ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; GGT=γ-glutamyl transpeptidase; HbA1c=hemoglobin A1c; HDL=high density lipoprotein; NPV=negative predictive value; PPV=positive predictive value; T2DM=type 2 diabetes mellitus.

[a]The SteatoTest-2 omits BMI and total bilirubin as these two components can be associated with variability.[13] The SteatoTest-2 was found to be noninferior to the SteatoTest without variability of the two components and simpler to use.

## Table 2. Biomarkers Identifying NASH[5, 37-38]

| Scoring System (Company) | Parameters/Components | Target Condition |
|---|---|---|
| Gholam Score | AST, diabetes mellitus | Distinguishing NASH from NAFL |
| MAST Score | MRI, AST | Identifying patients higher risk for Fibro-NASH |
| MACK-3 | AST, HOMA, CK18 | NAS ≥4. F ≥2 |
| NASHnext (NIS-4) | mIR-34a-5p, a2-macroglobulin, YKL-40, glycated hemoglobin | NAS ≥4. F ≥2 |
| NASHMap | 14 variables: HbA1c, AST, ALT, total protein, AST/ALT, BMI, triglycerides, height, platelets, WBC, hematocrit, albumin, hypertension, gender | Predicting progression to NASH |

ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; CK=cytokeratin; GGT=g-glutamyl transferase; HOMA=homeostasis model assessment; mIR=micro RNA; NAS=NAFLD activity score.

## Table 3. Biomarker Panels Identifying NAFLD-Associated Fibrosis and Direct Fibrosis Marker[5,17]

| Scoring System | Components | Strengths | Limitations |
|---|---|---|---|
| NAFLD Fibrosis Score (NFS) | Age, BMI, impaired fasting glucose and/or diabetes, AST:ALT ratio, platelets, albumin | High accuracy for identifying patients at low/high risk for AF or cirrhosis; cost-effective<br><-1.445 rules out AF; >0.676 detects AF | Low sensitivity; uses 2 cut-offs to rule out and detect AF, leading to inaccurate scoring of intermediate stages of fibrosis; influenced by age |
| Fibrosis 4 (FIB-4) Score | Age, platelet count, AST, ALT | Can identify patients at low/high risk for AF or cirrhosis; cost-effective<br>NPV >90%; PPV, 82%<br><-1.3 rules out AF; >2.67 detects AF | Poor accuracy associated with age; inaccurate scoring of intermediate stages of fibrosis |
| AST: Platelet Ratio Index (APRI) | AST:Platelet Ratio | High feasibility; cost-effective | Low sensitivity |
| BARD Score | BMI, AST:ALT ratio, diabetes | Useful model to predict SF; cost effective | Low accuracy to diagnose SF and cirrhosis; influenced by BMI among different ethnic groups |
| Direct Fibrosis Marker<br>Enhanced Liver Fibrosis (ELF) Score | TIMP-1, hyaluronic acid, PIIINP | High accuracy | Not optimal for diagnosing early fibrosis stages |
| Combination Serum + Imaging Noninvasive Tests | | | |
| FAST[3] | FibroScan + AST | Cut point (likely) ≥0.67, (unlikely) <0.35<br>≤0.35 (sensitivity 90%)<br>≥0.67 (specificity 90% | In validation studies, the PPV ranged between 0.33 and 0.81 |
| MAST[3] | MRI + AST | Cut point (likely) ≥0.242, (unlikely) ≤0.165 | 0.242 (specificity 90%); 0.165 (sensitivity 90%) |
| MEFIB[3] | MRE +FIB-4 | Cut point (likely) FIB-r ≥1/65 + MRE ≥3.3kPa, (unlikely) FIB-4 <1.6 + MRE <3.3 kPa | Sequential approach identifies patients with stage ≥2 fibrosis—90% PPV |
| cT1[33] | Iron corrected T1 mapping | Early study findings suggest cT1 is a useful NIT for identifying NAS ≥ 4 and fibrosis stage ≥ 2 | Needs further validation |

AF=advanced fibrosis; ALT=alanine aminotransferase; AST=aspartate aminotransferase; FIB-4= MRE=magnetic resonance elastography; MRI=magnetic resonance imaging; NAS=nonalcoholic fatty liver disease activity score; NPV=negative predictive value; PPV=positive predictive value; PIIINP= procollagen III amino-terminal peptide; SF=significant fibrosis; TIMP-1=tissue inhibitor of metalloproteinases 1.

## Imaging Biomarkers

Conventional ultrasonography is often used in clinical practice due to its accessibility, cost-efficiency, convenience, and ability to grade the degree of steatosis.[5] It is limited by poor sensitivity for detecting mild steatosis (<20% liver fat), which translates into a potentially large patient population with steatosis exceeding 5% yet less than 20% being missed.[5,20] Additionally, the accuracy of ultrasonography is lower in obese patients and those with severe fibrosis.[5] Similarly, the use of computed tomography is limited by insufficient grading of mild-to-moderate steatosis.

Fibrosis has no molecular signature that can be directly detected by conventional imaging techniques.[5]

Imaging tests measure fibrosis indirectly via detection of liver stiffness, which is associated with collagen deposition due to fibrosis. Of those available imaging tests, the most accurate for assessing liver stiffness are vibration controlled transient elastography (VCTE or Fibroscan), magnetic resonance elastography (MRE), acoustic radial force imaging (ARFI), and shear wave elastography (Table 4).[5,17,20 -23] New quantitative ultrasound-based techniques offer improved diagnostic performance. As an example, the controlled attenuation parameter (CAP), which is based on ultrasound signals, is used to assess liver fat and is measured by VCTE.[19,20] The technique employs one of two probes; the XL probe provides improved assessment in obese patients. Controlled attenuation parameter offers the advantages of point-of-care use and good sensitivity and specificity for fatty liver.

## Emerging biomarkers and their potential use

In addition to available serum and imaging biomarkers to detect steatosis, diagnose NASH, and grade fibrosis, others are emerging to address the limitations of those currently used in clinical practice. These might be new biomarkers or panels that use more than one approach. Using a combination of sequential serum biomarker panels and imaging techniques will likely improve risk stratification of patients with NAFLD. Such improvement may better define treatment, especially as new pharmacotherapies with differing targets come to market, and a care pathway. Ajmera and Loomba proposed one such combination for patients with NAFLD.[20]

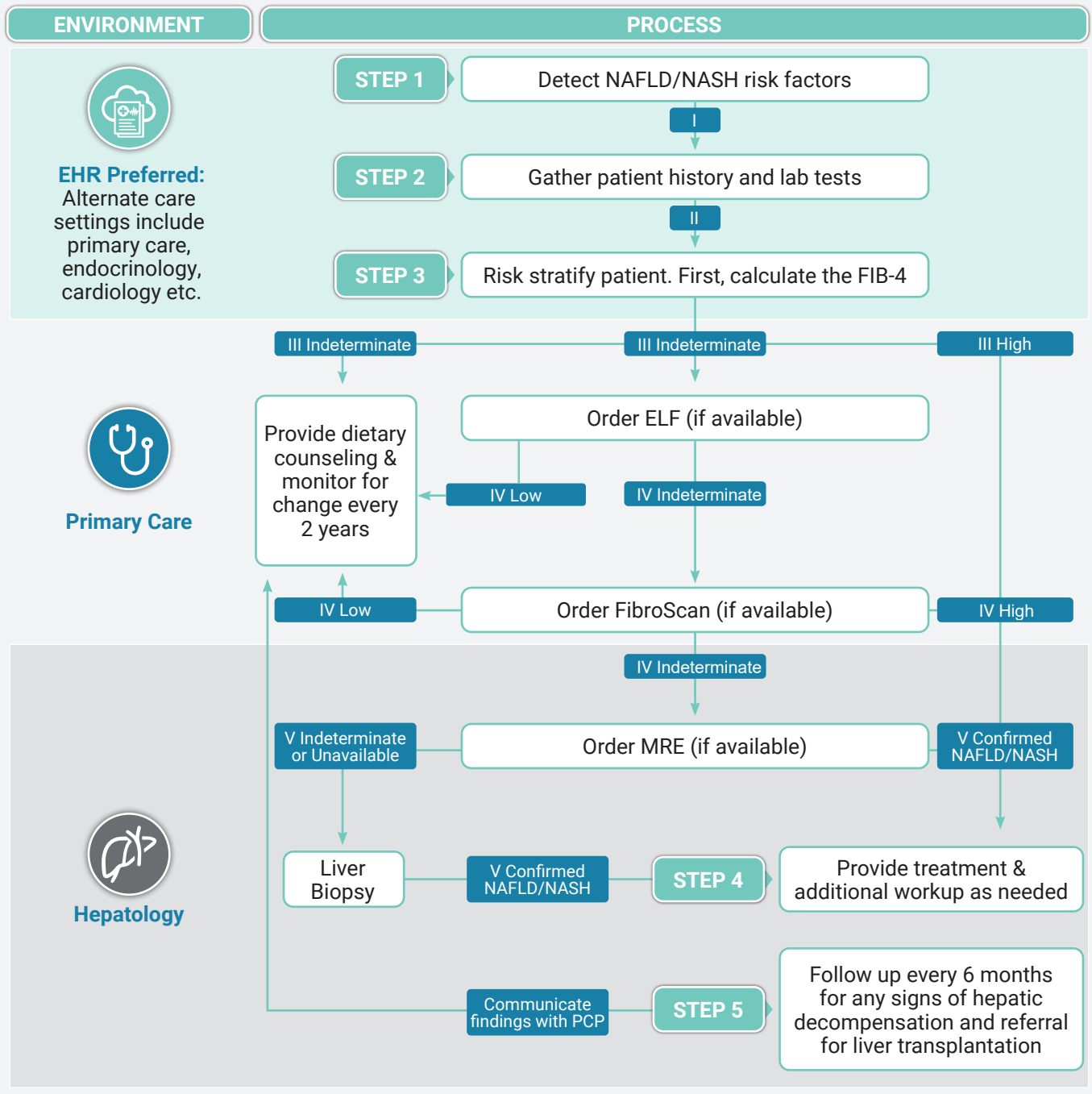
Procollagen-3 (PRO-C3), which detects a propeptide of the type III procollagen that is generated from procollagen cleavage during liver collagen deposition, is currently being used in clinical trials.[5] The PRO-C3 has shown to be useful as a biomarker for assessing severity of NASH and fibrosis stage.[24]

## Table 4. Imaging Modalities for Diagnosis of NAFLD and Fibrosis[5,17,20-23]

| Modality | Description | Performed By | Strengths | Limitations | Failure Rate (%) | Cost |
|---|---|---|---|---|---|---|
| VCTE | Low frequency (50 Hz) elastic shear wave is propagated through the liver; measurement of its velocity directly correlates to the stiffness of the liver parenchyma; results are defined in kPa (<7 kPa rules out fibrosis; higher values indicate an increased likelihood of severe fibrosis) | Hepatologist<br>Trained nurse or technician | Cost-effective, validated, short processing time, reproducible, low sampling error, excellent for ruling out AF and cirrhosis; can be widely used in the clinical setting; data from diverse populations; Use of the XL-probe (v the M-probe) can overcome discrepancies in obese patients | Uncertain optimal cut-off value; influenced by obesity; operator- and device-dependent; false-positive results; lack of availability | 3-27 | $ |
| MRE | Identifies the fibrosis stage by imaging the propagation of acoustic shear waves through the liver; available as 2-dimensional and 3-dimensional; further study is needed to validate 3-dimensional MRE in clinical practice | Radiologist | Accurate, reproducible, less affected by obesity and ascites, valuable to rule out AF and cirrhosis, low sampling error, low failure rate (<5%) | High cost, operator- and device-dependent, no validated optimal cut-offs, few prospective validation studies, influenced by hepatic iron overload | 0-2 | $$$ |
| ARFI (pSWE) | Ultrasound-based diagnostic tool that measures liver stiffness (via measurement of the shear wave generated from on sonographic frequency [meters/sec]); can detect severe and advanced fibrosis and cirrhosis | Radiologist or Ultrasonographer | Less affected by obesity and ascites, region of interest smaller than VCTE, reproducible | High cost, no well-defined optimal cut-offs, few prospective validation studies | 2 | $$ |
| 2D-SWE | Measures sonographic waves in multiple frequencies in real time, using 2-dimensional ultrasound (kilopascals [kPa]) | Radiologist or Ultrasonographer | Unaffected by obesity and ascites, reproducible; performs better for diagnosing mild fibrosis | High cost, operator- and device-dependent, no well-defined optimal cut-offs | 2 | $$ |

ARFI=acoustic radial force imaging; MRE=magnetic resonance elastography; SWE=shear wave elastography; VCTE= vibration controlled transient elastography.

**Figure 4. Sequential Use of Biomarkers for Risk Stratification of Patients with NAFLD[20]**



An "omics" approach—genomics, lipidomics, proteomics, and metabolomics, and glycomics—and using high-throughput technologies to detect thousands of different molecules may identify novel biomarkers of NAFLD, NASH, and advanced fibrosis.[5,17,25-27] A metabolomic approach might be used to evaluate disease severity, monitor disease progression, and identify therapeutic targets for patients. Another focus has been on the potential association between gut microbiota and NAFLD with the intent to find unique microbiome biomarkers to distinguish between NAFLD and NASH.[26,27] The use of artificial intelligence might help healthcare provider decision-making in diagnosing and risk stratifying NAFLD and NASH.[5] There are several clinical trials evaluating imaging techniques (Table 5). Many of these trials are focused on liver stiffness measurement. Validation of any biomarker is essential as is including cost as an important component. Each must also address an unmet need (Table 6).

### Table 5. Clinical Trials Involving Imaging Technologies

| Manufacturer | Device | Modality | Description | Clinical Trial Name | Enrollment | Status |
|---|---|---|---|---|---|---|
| Echosens | FibroScan | Imaging | Vibration controlled transient elastography (VCTE) | Prospective, Cross-sectional and Multicenter Study, Evaluating the Diagnosis Accuracy of the Controlled Attenuation Parameter(-CAP) Measured by FibroScan® (Either With M+ or XL+ Probe) in Patient With Non-Alcoholic Fatty Liver Disease Using Liver Biopsy as Reference. | 450 | Complete |
| E-Scopics | Ultrasound | Imaging | Ultrasound liver assessment | Point-of-care Ultrasound Screening and Assessment of Chronic Liver Diseases and NASH (POCUS-NASH) | 1,000 | Ongoing |
| Echosens | FibroScan | Imaging | Vibration controlled transient elastography (VCTE) | Prospective, Cross-sectional and Multicenter Study, Evaluating the Diagnosis Accuracy of the Controlled Attenuation Parameter(-CAP) Measured by FibroScan® (Either With M+ or XL+ Probe) in Patient With Non-Alcoholic Fatty Liver Disease Using Liver Biopsy as Reference. | 450 | Complete |
| E-Scopics | Ultrasound | Imaging | Ultrasound liver assessment | Point-of-care Ultrasound Screening and Assessment of Chronic Liver Diseases and NASH (POCUS-NASH) | 1,000 | Ongoing |
| Echosens | FibroScan | Imaging | Vibration controlled transient elastography (VCTE) | Prospective, Cross-sectional and Multicenter Study, Evaluating the Diagnosis Accuracy of the Controlled Attenuation Parameter(-CAP) Measured by FibroScan® (Either With M+ or XL+ Probe) in Patient With Non-Alcoholic Fatty Liver Disease Using Liver Biopsy as Reference. | 450 | Complete |

## Table 5. Clinical Trials Involving Imaging Technologies (Continued)

| Manufacturer | Device | Modality | Description | Clinical Trial Name | Enrollment | Status |
|---|---|---|---|---|---|---|
| E-Scopics | Ultrasound | Imaging | Ultrasound liver assessment | Point-of-care Ultrasound Screening and Assessment of Chronic Liver Diseases and NASH (POCUS-NASH) | 1,000 | Ongoing |
| Multiple Sponsors | MRE | Imaging | Magnetic resonance (MR) biomarkers | Technical Validation of MR Biomarkers of Obesity-Associated NAFLD (NAFLD) | 145 | Ongoing |
| Perspectum | LiverMultiScan | Imaging | Contrast free MRI scan | Repeatability and Reproducibility of Multiparametric MRI | 61 | Ongoing |
| Perspectum | LiverMultiScan | Imaging | Contrast free MRI scan | Assessing Kids for Liver Inflammation and Fibrosis Using Non-invasive MRI (Kids4LIFe) | 35 | Complete |
| Perspectum | LiverMultiScan | Imaging | Contrast free MRI scan | Non-invasive Rapid Assessment of NAFLD Using Magnetic Resonance Imaging With LiverMultiScan (RADIcAL1) | 801 | Complete |
| Perspectum | LiverMultiScan | Imaging | Contrast free MRI scan | Characterization and Technical Evaluation of cT1 for NASH (CATE-NASH) | 60 | Ongoing |
| Perspectum | LiverMultiScan | Imaging | Contrast free MRI scan | Multi-Parametric MRI Assessment of the Liver in the Dallas-FortWorth Metroplex Population (DFWRegistry) | 100 | Ongoing |
| Perspectum | LiverMultiScan | Imaging | Contrast free MRI scan | Multi-Parametric MRI Assessment of the Liver in Diabetic Volunteers (Partners Registry) | 200 | Ongoing |
| Philips | Investigational Software | Imaging | Investigational Liver Fat Quantification Software | Pediatric Liver Fat Quantification (LFQ) Phase 2 Pilot Study | 30 | Ongoing |
| Philips | Investigational Software | Imaging | Investigational Liver Fat Quantification Software | Ultrasound-Based Liver Fat Quantification (LFQ) Pilot Study (LFQ) | 60 | Ongoing |
| Samsung Medison | Quantitative Ultrasound | Imaging | Quantitative ultrasound imaging parameters | Quantitative US for Evaluation of Hepatic Steatosis in NAFLD | 173 | Complete |
| Samsung Medison | Quantitative Ultrasound | Imaging | Quantitative ultrasound imaging parameters | Quantitative US for Hepatic Steatosis | 124 | Complete |
| Siemens | MRI | Imaging | MRI | Ultrasound Quantification of Liver Fat | 56 | Ongoing |
| Sonic Incytes | Liver Incytes System | Imaging | Shear Wave Absolute Vibro-Elastography (S-WAVE) | Quantitative Ultrasound With Liver Incytes for Evaluation of Non-Alcoholic Fatty Liver Disease | 100 | Ongoing |
| Sonic Incytes | Liver Incytes System | Imaging | Shear Wave Absolute Vibro-Elastography (S-WAVE) | The Sonic Incytes Liver Incytes System, Evaluation of Liver Fibrosis and Steatosis Versus MRE and MRI PDFF | 100 | Ongoing |

**Table 6: Unmet Needs in Diagnosing and Risk Stratifying NAFLD and NASH and Research Priorities[17]**

- Determination of cut points for each modality in the context of use (eg, screening in primary care, screening of patients with diabetes)
- Validation of quality criteria for each modality
- Cost-effectiveness of sequential use of clinical prediction rules (eg, FIB-4), followed by VCTE, SWE, ARFI followed by MRE
- Clinically meaningful increase/decrease in liver stiffness that is linked to a clinical outcome in NAFLD
- Clinically meaningful increase in liver stiffness that is associated with a 1-stage increase in liver fibrosis
- Clinically meaningful decrease in liver stiffness that is associated with a 1-stage decrease in liver fibrosis
- Cut point for liver stiffness for each modality that is associated with a need to treat varices in patients with NAFLD
- Clinically meaningful decrease in liver stiffness that is linked to clinical outcome in NAFLD
- Address whether reduction in liver stiffness in cirrhosis is associated with reduction in the risk for liver decompensation despite no change in fibrosis stage

## Guideline-directed use of biomarkers to diagnose or stage NASH

Inclusion of biomarkers in guidelines is important for awareness of biomarker utilization and widespread adoption. Ideally, diagnostic biomarker recommendations will be integrated into health management electronic health records (EHRs) to encourage screening and NAFLD/NASH population building. Several guidelines associations currently include recommendations for the use of biomarkers for individuals with NAFLD or NASH (Table 7). Notably, some associations and organizations have not yet developed guidelines, including the American Association of Family Physicians and the United States Preventive Services Taskforce. Table 7 delineates NAFLD and NASH guidance from six major associations and organizations.[3,28-32]

## Table 7. Guidelines Recommendations for Screening for NAFLD and Use of Biomarkers in Adults[3,28-32]

| AASLD | Guidance for Screening for NAFLD | Biomarkers for the Diagnosis and Assessment of NAFLD | |
|---|---|---|---|
| | General population-based screening for NAFLD is not advised.<br><br>All patients with hepatic steatosis or clinically suspected NAFLD based on the presence of obesity and metabolic risk factors should undergo primary risk assessment with FIB-4.<br><br>High-risk individuals, such as those with T2DM, medically complicated obesity, family history of cirrhosis, or more than mild alcohol consumption, should be screened for advanced fibrosis.<br><br>In patients with pre-DM, T2DM, or two or more metabolic risk factors (or imaging evidence of hepatic steatosis), primary risk assessment with FIB-4 should be repeated every 1–2 years.<br><br>Patients with NASH cirrhosis are at the highest risk for liver-related outcomes and require routine surveillance for HCC, esophageal varices, and monitoring for decompensation.<br><br>Patients with suspected advanced NASH or discordant noninvasive tests should be referred to a specialist for evaluation, management, and/or further diagnostic evaluation.<br><br>Aminotransferase levels are frequently normal in patients with advanced liver disease due to NASH and should not be used in isolation to exclude the presence of NASH with clinically significant fibrosis.<br><br>First degree relatives of patients with NASH cirrhosis should be counselled regarding their increased individual risk and offered screening for advanced hepatic fibrosis | Although ultrasound can detect hepatic steatosis, it is **not** recommended as a tool to identify hepatic steatosis due to low sensitivity across the NAFLD spectrum.<br><br>CAP as a point-of-care technique may be used to identify steatosis. MRI-PDFF can additionally quantify steatosis.<br><br>If FIB-4 is ≥1.3, VCTE, MRE, or ELF may be used to exclude advanced fibrosis.<br><br>**Key Point**<br><br>Highly elevated liver stiffness, FIB-4, and ELF scores can predict an increased risk for hepatic decompensation and mortality. | |

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| AACE[a] | Blood tests (eg, diagnostic panels and specific biomarkers) to diagnose NAFLD with clinically significant fibrosis (stages F2-F4) in adults | Imaging studies to diagnose NAFLD with clinically significant fibrosis (stages F2-F4) in adults | |
| | Clinicians should use liver fibrosis prediction calculations to assess the risk of NAFLD with liver fibrosis. The preferred noninvasive initial test is the FIB-4<br><br>Clinicians should consider persons belonging to the "high-risk" groups who have an indeterminate or high FIB-4 score for further workup with an LSM (transient elastography) or ELF test, as available | To stage the risk of fibrosis in persons with NAFLD, clinicians should prefer the use of VCTE as best validated to identify advanced disease and predict liver-related outcomes<br><br>Alternative imaging approaches may be considered, including shear wave elastography (less well validated) and/or magnetic resonance elastography (most accurate but with a high cost and limited availability; best if ordered by liver specialist for selected cases) | |
| AGA | Staging of Liver Fibrosis in Patients With NAFLD | | |
| | Combination of at least 2 noninvasive tests, each coming from 1 of 3 groups of tests (point of care; serum-based specialized test; imaging-based)<br><br>For risk stratification for HCC screening, a higher cut-point threshold should be used to maximize specificity (90%); for VCTE and MRE, consider16.1 kPa and 5 kPA, respectively as cut-off points for detection of cirrhosis for HCC screening<br><br>Patients in whom both tests are concordant for advanced fibrosis or cirrhosis should be considered for HCC screening | | |

| APASL | Noninvasive Score to Use in MAFLD[b] | Monitoring Treatment | |
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| | Abdominal ultrasonography is the recommended first-line diagnostic modality for imaging of MAFLD and is usually sufficient for the detection of hepatic steatosis<br><br>If available, controlled attenuation parameter (CAP) measurement by VCTE may be used as a more sensitive tool than ultrasonography. If imaging modalities are not available or feasible such as in very large epidemiologic studies, serum biomarkers and scores such as the fatty liver index (FLI) may be used as an alternative method for the diagnosis of steatosis<br><br>There is no robust biomarker for steatohepatitis and liver biopsy remains the test of choice for assessment of steatohepatitis<br><br>The exclusion of high risk of significant or advanced fibrosis is acceptable using non-invasive tools, liver stiffness measurement by VCTE or shear wave elastography and blood biomarkers and scores of fibrosis or their sequential combination<br><br>The confirmation of significant or advanced fibrosis by liver stiffness measurement and/or serum biomarkers/scores is less accurate and would require further confirmation by liver biopsy as per the clinical context | Patients without fibrosis can be monitored at intervals of 2 or 3 years in the absence of worsening of metabolic risk factors using a combination of noninvasive scores and liver stiffness measurement<br><br>Patients with fibrosis should be monitored on an annual basis using a combination of noninvasive scores and liver stiffness measurement<br><br>Patients with cirrhosis should undergo monitoring at 6-month intervals including surveillance for hepatocellular carcinoma | |

| EASL | Use of Noninvasive Scores for Diagnosing Steatosis in Patients with Metabolic Risk Factors and/or Suspected NAFLD | Screening in at-Risk Patients: Identification of advanced Fibrosis | Use of Noninvasive Scores and Imaging Methods for Evaluation of NAFLD Severity |
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| | Non-invasive scores are not recommended for the diagnosis of steatosis in clinical practice<br><br>Noninvasive fibrosis tests should be used for ruling out rather than diagnosing advanced fibrosis in low prevalence populations<br><br>Noninvasive fibrosis tests should be preferentially used in patients at risk for advanced liver fibrosis (such as patients with metabolic risk factors and/or harmful use of alcohol) and not in unselected general populations<br><br>ALT, AST and platelet count should be part of the routine investigations in primary care in patients with suspected liver disease, so that simple non-invasive scores can be readily calculated<br><br>The automatic calculation and systematic reporting of simple noninvasive fibrosis tests such as FIB-4, in populations at risk of liver fibrosis (individuals with metabolic risk factors and/or harmful use of alcohol) in primary care, is recommended to improve risk stratification and linkage to care | Individuals at risk of advanced fibrosis due to metabolic risk factors and/or harmful use of alcohol should be entered into appropriate risk stratification pathways using noninvasive fibrosis tests<br><br>The selection of noninvasive tests and the design of diagnostic pathways for testing low-prevalence populations for advanced fibrosis should be performed in consultation with a liver specialist<br><br>**Screening to Predict Liver-Related Outcomes in Patients With NAFLD**<br><br>Serum scores (APRI, FIB-4, NFS, ELF™) and LSM by VCTE should be used to stratify the risk of liver-related outcomes in NAFLD<br><br>Repeated measurements of noninvasive tests can be used to refine stratification of risk of liver-related events in patients with NAFLD/NASH.<br><br>Despite the lack of evidence regarding the optimal timeframe between subsequent LSM assessment, it seems reasonable to repeat noninvasive tests every 3 years in patients with early stage and every year in patients with advanced stage NAFLD | For ruling out advanced fibrosis in clinical practice:<br><br>–LSM by TE <8 kPa<br><br>–Patented tests: ELF™ <9.8 or FibroMeter™ <0.45 or FibroTest® <0.48<br><br>--Non-patented tests: FIB-4 <1.3 or NFS <-1.455<br><br>Upon referral of a patient with FIB-4 over 1.3, the use of VCTE and/or patented serum tests should be used to rule out/in advanced fibrosis<br><br>MRE is the most accurate noninvasive method for staging liver fibrosis. However, it is only marginally better than other noninvasive tests for F3–F4 fibrosis and it is not recommended as a first-line noninvasive test given its cost and limited availability |
| NICE | **Identifying Individuals With Advanced Liver Fibrosis** | | |
| | Consider using the enhanced liver fibrosis (ELF) test in people who have been diagnosed with NAFLD<br><br>An ELF score ≥ 10.51 and NAFLD indicates advanced liver fibrosis<br><br>Offer retesting for advanced liver fibrosis for adults with an ELF score below 10.51 every 3 years<br><br>Consider using ELF for retesting people with advanced liver fibrosis | | |

AACE=American Association of Clinical Endocrinology; AASLD= American Association of the Study of Liver Disease; AGA=American Gastroenterological Association; ALT= alanine aminotransferase; APASL= Asian Pacific Association for the Study of the Liver; APRI= AST to platelet ratio index; AST= aspartate aminotransferase; CAP=controlled attenuation parameter; DM=diabetes mellitus; EASL= European Association for the Study of the Liver; ELF= enhanced liver fibrosis; FIB-4=fibrosis-4 index; HCC=hepatocellular carcinoma; LSM=liver stiffness measurement; MRE=magnetic resonance elastography; NFS=nonalcoholic fatty liver disease fibrosis score; NICE=National Institute for Health and Care Excellence; TE=transient elastography; T1DM=type 1 diabetes mellitus; T2DM=type 2 diabetes mellitus; VCTE=vibration-controlled transient elastography.

[a]Co-sponsored by AASLD. [b]The APASL uses MAFLD (metabolic-associated fatty liver disease) in lieu of NAFLD.

## Barriers to Implementation of Biomarkers in Care Pathways

The experts who attended the Biomarker Summit also pinpointed barriers to implementation in care pathways and ultimately in routine clinical practice. These include:

- Cost of the biomarker
- Payer coverage
- Available approved therapeutic options
- Patient and provider acceptance

Upon Food and Drug Administration (FDA) approval of a NASH therapeutic, diagnostic biomarkers adoption is anticipated to accelerate. Care pathways that include screening, diagnosis, grading of fibrosis, and optimal management will begin to be developed and implemented in EHRs (Figure 4). Of note in terms of care pathways: a lengthy step-by-step process using sequential/combination biomarkers and/or requiring multiple visits is not realistic for primary care physicians who have limited time and currently have few management options from which to choose. For endocrinologists whose patients are at high risk, a multi-step process might be more feasible. Education—for providers and patients—is key for adoption of any pathway.

## Challenges Associated with Optimal Use of NASH Biomarkers

In addition to addressing barriers to implementation of NAFLD/NASH care pathways that include biomarkers, health system management must face and resolve challenges. One of these is acquiring real-world evidence from health system implementation. Case studies and results from clinical trials are emerging.

Younossi and colleagues used EHRs to identify patients who had type 2 diabetes plus one at least one of the following factors: 1) one component of metabolic syndrome (hypertension, hyperlipidemia, or body mass index (BMI) greater than 29.9), 2) type 2 diabetes with elevated levels (1.5 X upper limit of normal [ULN]) of aspartate aminotransferase (AST) or alanine aminotransaminase (ALT), 3) history of fatty liver identified by any imaging modality, 4) or no type 2 diabetes but the presence of at least three components of the metabolic sydrome.[33] They then used three noninvasive tests—AST: platelet ratio index (APRI), NAFLD Fibrosis Score (NFS), and FIB-4 index. At least two of the tests had to be above certain thresholds to identify patients with high-risk NASH. These patients were referred to a gastroenterologist or hepatology clinic for clinical assessment and transient elastography. Sixty percent of patients whose noninvasive tests met the threshold and who underwent transient elastography were at low risk for adverse outcomes as demonstrated by liver stiffness measurement of less than 6 kPa. Notably, 8% of patients had liver stiffness of 12 kPa or higher, suggesting the presence of cirrhosis. These patients were more likely to have higher BMI, elevated liver enzyme levels and noninvasive test scores, as well as such comorbidities as diabetes and cardiovascular disease. While the investigators acknowledge that further study is needed for the development of algorithms using noninvasive tests, they reiterate that prospective application of a stepwise algorithm using noninvasive tests and transient elastography in the primary care setting may lead to identification of high-risk NAFLD patients.

The use of noninvasive tests for patients with NAFLD or NASH must be cost-effective. Investigators conducted a cost-effective analysis of noninvasive testing (NFS and/or VCTE) compared with standard care (liver biopsy) according to setting (primary care physician [PCP] office v specialty clinic).[34] Findings confirmed the cost-effectiveness of noninvasive tests over liver biopsy as well as initial patient assessment in the PCP office in contrast to referral to a specialist. The NFS performed in the PCP office produced a cost-savings of $13,585 per patient when compared with referral for liver biopsy. When examining the cost per quality adjusted life-years (QALY), NFS in the PCP office outperformed both NFS after specialty referral and referral for liver biopsy ($5,985, $6138, and $7,229, respectively). The second most cost-effective strategy was the combination of NFS with VCTE, also performed in the PCP office. The investigators concluded that these strategies should be explored in a broader clinical context.

A more recently completed study used a decision model that quantified the accuracy and costs of 9 single or combination strategies for the detection of cirrhosis in NAFLD patients.[35] These strategies included 3 noninvasive tests—FIB-4, vibration-controlled transient elastography (VCTE), and magnetic resonance elastography (MRE)—and liver biopsy. The study population was a hypothetical cohort of middle-aged NAFLD patients who were seen in 3 different settings: general population (prevalence of NAFLD cirrhosis, 0.27%); a specialty clinic (prevalence of NAFLD cirrhosis, 2%); and tertiary referral center (prevalence of NAFLD cirrhosis, 4%). In each setting, FIB-4 plus VCTE had the highest accuracy and lower per-patient cost. For the general population, the diagnostic accuracy was 89.3% compared with 88.5% and 87.5% for the specialty clinic and tertiary referral setting, respectively. Costs per person were $401, $690, and $1,024, respectively. For all three settings, the combination of FIB-4 and MRE was second in costs person: $491, $781, and $1,114, respectively. Diagnostic accuracy for this combination was 92.4%, 91.6%, and 90.6%, respectively. The incremental cost-effectiveness ratios (ICERs) were lower for FIB-4 plus MRE for all three settings ($2,864, $2,918, and $2,921) compared with FIB-4 plus liver biopsy ($4,454, $5,156, and $5,956, respectively. Similarly, the ICERs for FIB-4 plus VCTE were lower compared with FIB-4 plus liver biopsy. The study findings suggest the combination of FIB-4 plus VCTE is the preferred strategy for screening for cirrhosis across healthcare settings and among the general population due to its lower costs, high accuracy, and accessibility.

Payer coverage differs between government and commercial payers. One payer (Aetna) has identified tests that are “medically necessary.” These are posted on Aetna's website, as are those still considered “experimental and investigational.” Those relevant to NAFLD/NASH that are considered medically necessary include:[36]

- Transient elastography (eg, FibroScan) for distinguishing hepatic cirrhosis from non-cirrhosis in persons with NAFLD and NASH; no more than twice annually or within 6 months of liver biopsy
- FibroTest-ActiTest/ for distinguishing hepatic cirrhosis from non-cirrhosis in persons with NAFLD and NASH; no more than twice annually or within 6 months following a liver biopsy or transient elastography
- Magnetic resonance elastography for patients with NASH; no more than twice annually or within 6 months following a liver biopsy
- Enhanced Liver Fibrosis (ELF) test for the detection and prognosis of liver fibrosis in persons with chronic liver diseases; no more than twice annually or within 6 months following a liver biopsy (or other test for liver fibrosis)

While coverage will vary across settings and payers, the recognition of medical necessity by this one example is encouraging for the implementation of noninvasive testing within care pathways for the diagnosis of NAFLD and NASH and staging of fibrosis.

## Summary

Identification of biomarkers for NAFLD and NASH and assessment of fibrosis are essential to avoid liver biopsy, which has several limitations including cost, risk for complications, sampling error, as well as inter- and intra-observer variability. That the prevalence of NAFLD will increase substantially in coming years and several therapeutics will be FDA-approved in the near future underscore the importance of development and implementation of care pathways that include biomarkers, either as panels and/or in combination. External validation of a number of both serum and imaging biomarkers is needed as is education for healthcare providers and their patients. Cost, including the expenditure for devices, is a key factor in the adoption of a care pathway. Coverage of biomarkers by Medicare, Medicaid, and commercial payers will be essential for improving the detection of steatosis, diagnosing NAFLD and NASH, asssessing fibrosis, and monitoring patient response to treatment. Early identification of at-risk patients eligible for treatment will improve outcomes associated with NASH/NAFLD.

Biomarkers can be used for the diagnosis and quantification of steatosis, to determine the presence and extent of NASH-related liver injury, and to identify and quantify fibrosis.[1]

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