Serum Testing for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease

Policy Number: AHS – G2110 – Serum Testing for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease	Prior Policy Name and Number, as applicable: • AHS – G2110 – Multianalyte Assays with Algorithmic Analysis for the Evaluation and Monitoring of Patients with Chronic Liver Disease • AHS – G2110 – Serum Marker Panels for Hepatic Fibrosis in the Evaluation and Monitoring of Chronic Liver Disease
Effective Date: 01/01/2023	

I. Policy Description

Chronic liver disease (CLD) refers to a wide range of liver pathologies that include inflammation (chronic hepatitis), liver cirrhosis, and hepatocellular carcinoma.

Hepatic fibrosis is associated with a cycle of extracellular matrix deposition and degradation. Biomarkers of extracellular matrix turnover are used to directly assess fibrosis and, theoretically, to monitor progression or regression (Valva, Rios, De Matteo, & Preciado, 2016). These markers include several glycoproteins, members of the collagen family, collagenases and their inhibitors, and a number of cytokines involved in the fibrogenic process (Valva et al., 2016). The markers may be utilized individually, as well as in panel combinations (Parikh, Ryan, & Tsochatzis, 2017).

II. Related Policies

Policy Number	Policy Title
AHS-G2036	Hepatitis C
AHS-G2124	Serum Tumor Markers for Malignancies
AHS-G2173	Gamma-glutamyl Transferase

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Medical Policy Statements do not ensure an authorization or payment of services. Please refer to the plan contract (often referred to as the Evidence of Coverage) for the service(s) referenced in the Medical Policy Statement. If there is a conflict between the Medical Policy Statement and the plan contract (i.e., Evidence of Coverage), then the plan contract (i.e., Evidence of Coverage) will be the controlling document used to make the determination. Specifications pertaining to Medicare and Medicaid can be found in Section VII of this policy document.

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website https://www.cms.gov/medicare-coverage-database/search.aspx or the manual website.

1) The use of multianalyte assays with algorithmic analysis (eg. FibroTest, FibroSure, ELF) to distinguish hepatic cirrhosis **MEETS COVERAGE CRITERIA** for individuals with one of the following conditions:
   a) Hepatitis C
   b) Hepatitis B
   c) Non-alcoholic fatty liver disease (NAFLD)
   d) Alcoholic hepatitis

2) Multianalyte assays with algorithmic analyses, such as NASH FibroSure, **DOES NOT MEET COVERAGE CRITERIA** for noninvasive assessment of hepatic steatosis and fibrosis.

3) Multianalyte assays with algorithmic analyses (eg. FibroTest, FibroSure, NASH FibroSure) to diagnose, evaluate, or monitor hepatic fibrosis **DO NOT MEET COVERAGE CRITERIA** in all other circumstances.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4) The use of the following serum biomarkers in immunoassays and/or immunohistochemistry assays to diagnose, evaluate, or monitor hepatic fibrosis of patients with chronic liver disease **DOES NOT MEET COVERAGE CRITERIA**:
   a) Signal-induced proliferation-associated 1 like 1 (SIPA1L1)
   b) microRNA (miRNA or miR) analysis, including but not limited to, the following:
      i) microRNA-21 (miRNA-21 or miR-21)
      ii) miRNA-29a (miR-29a)
      iii) miRNA-122 (miR-122)
      iv) miRNA-221 (miR-221)
      v) miRNA-222 (miR-222)
   c) Chitinase 3-like 1 (CHI3L1)
   d) Hyaluronic acid
   e) Type III procollagen (PCIII)
   f) Type IV collagen
   g) Laminin
   h) Plasma caspase-generated cytokeratin-18

i) Micro-fibrillar associated glycoprotein 4 (MFAP4)

IV. Scientific Background

Fibrosis is a wound healing response in which damaged regions are encapsulated by an extracellular matrix. This is common in individuals with chronic liver injury but may be seen in other organs such as the kidneys or lungs. Chronic liver injury may be caused by numerous conditions, such as hepatitis, and progressive fibrosis may lead to cirrhosis (Friedman, 2020). Liver biopsy remains the gold standard for evaluation of chronic liver disease to monitor treatment and disease progression. However, this invasive procedure has several drawbacks including pain, bleeding, inaccurate staging due to sampling error, and variability of biopsy interpretation (Chin, Pavlides, Moolla, & Ryan, 2016).

Serum biomarkers, such as the aspartate aminotransferase (AST) to platelet ratio (APRI), have been proposed as measures of hepatic fibrosis assessment, and numerous panels exist (Curry & Afdhal, 2019). These markers (and corresponding panels) may be categorized as "direct" or "indirect." Direct markers of fibrosis evaluate extracellular matrix turnover, and indirect markers signify changes in hepatic function. Direct biomarkers may be further subdivided by markers associated with matrix deposition, matrix degradation, or cytokines (and chemokines) associated with fibrogenesis. Procollagen I peptide, procollagen III peptide, type I collagen, type IV collagen, YKL-40 (chondrex), laminin, and hyaluronic acid, MMP-2, TIMP-1, -2, TGF-beta, TGF-alpha, and PDGF have all been proposed as direct measures of fibrosis. Indirect markers include serum aminotransferase levels, platelet count, coagulation parameters, gamma-glutamyl transferase (GGT), total bilirubin, alpha-2-macroglobulin, and alpha-2-globulin (haptoglobin) (Curry & Afdhal, 2019). Other markers have been investigated to be used independently or as part of these panels. The human microfibrillar-associated protein 4 (MFAP4) is located in extracellular matrix fibers and plays a role in disease-related tissue remodeling. Bracht et al. (2016) evaluated the "potential" of MFAP4 as a biomarker for hepatic fibrosis. A total of 542 patients were included, and the authors focused on differentiation of no to moderate (F0–F2) and severe fibrosis stages and cirrhosis (F3 and F4). In the "leave-one-out cross validation," a sensitivity of 85.8% and specificity of 54.9% was observed and the multivariate model yielded 81.3 % sensitivity and 61.5 % specificity. The authors suggested that "the combination of MFAP4 with existing tests might lead to a more accurate non-invasive diagnosis of hepatic fibrosis and allow a cost-effective disease management in the era of new direct acting antivirals" (Bracht et al., 2016).

Plasma caspase-generated cytokeratin-18 fragments (CK-18) have been proposed as a biomarker in the diagnosis and staging of non-alcoholic steatohepatitis (NASH). Cusi et al. (2014) studied the clinical value of CK-18. The authors studied the adipose tissue, liver, and muscle insulin resistance of 424 patients as well as liver fat (n = 275) and histology (n = 318). The authors found that median CK-18 levels were elevated in patients with verses without non-alcoholic fatty liver disease (NAFLD) (209 U/L vs. 122 U/L) or with verses without NASH (232 U/L vs. 170 U/L). The CK-18 area under curve to predict NAFLD, NASH, or fibrosis were 0.77, 0.65, and 0.68, respectively. The overall sensitivity/specificity for NAFLD, NASH and fibrosis were 63%/83%, 58%/68% and 54%/85%, respectively. CK-18 correlated most strongly with ALT (r=0.57) and adipose tissue IR (insulin-suppression of FFA: r=-0.43), but not with ballooning, body mass index, metabolic syndrome, or type 2 diabetes. The authors concluded, "Plasma CK-18 has a high specificity for NAFLD and fibrosis, but its limited sensitivity makes it inadequate as a screening test for staging NASH. Whether combined as a diagnostic panel with other biomarkers or clinical/laboratory tests may prove useful requires further study" (Cusi et al., 2014).

Likewise, Chitinase 3-like 1 (CHI3L1) has been proposed to be a better serum biomarker than hyaluronic acid, type III procollagen, type IV collagen, and laminin. CHI3L1 is preferentially expressed in hepatocytes over any other body tissue. Huang et al. (2015) investigated CHI3L1 in 98 patients with

hepatitis B. The authors reported that CHI3L1 can be used to differentiate between early stages of liver fibrosis (S0-S2) from late stages (S3-S4) "with areas under the ROC curves (AUCs) of 0.94 for substantial (S2, S3, S4) fibrosis and 0.96 for advanced (S3, S4) fibrosis" (Huang et al., 2015). Wang, Liu, Zhou, You, and Jia (2018) also report that CHI3L1 is a useful marker for the assessment of liver fibrosis before treatment, and can also be used to monitor change during therapy.

MicroRNA (miRNA) sequences have also been proposed as a marker of liver function. MiRNA sequences often have roles in gene regulation and other cellular processes, so changes in these sequences may indicate a liver condition (Tendler, 2020). For example, Abdel-Al et al. (2018) investigated miRNA's association with Hepatitis C virus (HCV) patients. Forty-two patients with HCV and early-stage fibrosis, 45 patients with HCV and late-stage fibrosis, and 40 healthy controls were examined and the expression patterns of 5 miRNA sequences (miR-16, miR-146a, miR-214-5p, miR-221, and miR-222) were measured. The authors found miRNA-222 to have the highest sensitivity and specificity for both fibrosis groups, and all mi-RNA sequences except miRNA-214-5p were significantly upregulated in fibrosis. MiRNA-221 was also found to have significant positive correlations with miRNA-16 and miRNA-146a. The authors concluded that "the high sensitivity and specificity of miRNA-222 and miRNA-221 in late-stage fibrosis indicate promising prognostic biomarkers for HCV-induced liver fibrosis (Abdel-Al et al., 2018).

Multiple biomarkers may be combined into a panel. Panels may include a combination of direct markers, indirect markers, or markers from both categories. The most studied panels are the aspartate aminotransferase (AST) to platelet ratio (APRI), FibroTest/FibroSure, and Hepascore, although many more exist. FibroTest/FibroSure incorporates alpha-2-macroglobulin, alpha-2-globulin (haptoglobin), gamma globulin, apolipoprotein A1, GGT, and total bilirubin, age, and sex. HepaScore measures bilirubin, GGT, hyaluronic acid, alpha-2-macroglobulin, age, and sex. These panels have demonstrated some promising results, but Curry and Afdhal (2019) note that indeterminate outcomes are common. Furthermore, they state that no singular panel has emerged as the standard of care (Curry & Afdhal, 2019). Another test, known as the LIVERFAst™ by Fibronostics, utilizes a blood sample to measure 10 biomarkers; algorithm technology is used "to determine the fibrosis, activity and steatosis stages of the liver" (Fibronsotics, 2020).

Many combinations of biomarkers, and even combinations of panels, exist. For example, FibroMax combines FibroTest, SteatoTest, NashTest, ActiTest, and AshTest on the same result sheet and provides a more comprehensive estimation of the liver injury. This test measures 10 biomarkers which are as follows: GGT, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, alanine aminotransferase (ALT), AST Transaminase, triglycerides, cholesterol, and fasting glucose (BioPredictive, 2019). Fouad et al. (2013) analyzed samples from 44 patients and found that FibroMax results were positively correlated with viral load by quantitative polymerase chain reaction and histopathological findings. Further, body mass index was significantly higher in steatotic patients and was significantly associated with the results on FibroMax (Fouad et al., 2013).

Clinical Validity and Utility

Berends et al. (2007) performed a study assessing FibroTest's ability to detect methotrexate (MTX)-induced hepatic fibrosis. Twenty-four psoriasis patients that underwent a liver biopsy were included, and FibroTest identified 83 percent of the patients who had significant fibrosis. The authors suggested FibroTest may be used as part of monitoring MTX-induced fibrosis (Berends et al., 2007).

Kwok et al. (2014) performed a meta-analysis of non-invasive assessments of NASH. The authors identified 9 studies for transient elastography (TE) and 11 for cytokeratin-18 (CK-18). The pooled sensitivities and specificities for TE to diagnose $F \geq 2$, $F \geq 3$, and F4 disease were 79% and 75%, 85%

and 85%, and 92% and 92%, respectively. CK-18 was found to have a pooled sensitivity of 66% and specificity of 82% in diagnosing NASH. The authors concluded that "At present, serum tests and physical measurements such as TE come close as highly accurate non-invasive tests to exclude advanced fibrosis and cirrhosis in NAFLD patients. CK18 has moderate accuracy in diagnosing NASH, while other biomarkers have not been extensively studied (Kwok et al., 2014)."

Gao et al. (2018) compared aspartate amino transferase–to-platelet ratio index (APRI), the Fibrosis-4 index (FIB-4), transient elastography (TE), and two-dimensional (2D) shear-wave elastography (SWE). A total of 402 patients with chronic hepatitis B were included. 2D-SWE was found to have the highest area under the curve (AUC), with 0.87 compared to APRI's 0.70, TE's 0.80, and FIB-4's 0.73 (Gao et al., 2018).

Dong et al. (2018) compared the performance of several biomarkers (serum hyaluronan (HA), procollagen type III N-terminal peptide (PIIINP), type IV collagen (IVC), laminin (LN), ALT, AST) to transient elastography (FibroScan). Seventy patients with hepatitis B underwent a liver biopsy. Fibrosis was found in 24 patients. The correlation of serum levels with fibrosis stage are as follows: 0.468 (HA), 0.392 (PIIINP), 0.538 (IVC), 0.213 (LN), 0.350 (ALT), 0.375 (AST). The authors found that the combination of all five biomarkers yielded a superior diagnostic performance (area under curve: 0.861) compared to all five alone (Dong et al., 2018).

A pilot study of the FM-fibro index was performed with 400 patients enrolled, and the FM-fibro index, CA-fibro index, and European Liver Fibrosis panel (ELF) were compared with respect to estimating prognosis of patients with NAFLD. Three separate biomarkers comprise the FM-fibro index: type IV collagen 7S, hyaluronic acid, and vascular cell adhesion molecule-1. The area under the curve was 0.7093 for the CA-fibro index, 0.7245 for ELF, and 0.7178 (type IV collagen 7S)/0.7095 (hyaluronic acid)/0.7065 (vascular cell adhesion molecule-1) (Itoh et al., 2018). The sensitivity and specificity of the FM-fibro index for predicting NASH-related fibrosis was 0.5359/0.5210/0.4641 and 0.8333/0.8182/0.8788, respectively (Itoh et al., 2018). The accuracy of the FM-fibro index was not significantly different from that of the CA-fibro index and the ELF panel.

Patel et al. (2018) performed a retrospective study focusing on fibrosis scoring systems to identify NAFLD. A total of 329 patients (296 NAFLD, 33 controls) were included. The following indices were studied: "NAFLD fibrosis score (NFS), fibrosis-4 calculator (FIB-4), aspartate aminotransferase-to-alanine aminotransferase ratio (AST/ALT ratio), AST-to-platelet ratio index (APRI), and body mass index, AST/ALT ratio, and diabetes (BARD) score by age groups" (Patel et al., 2018). NFS and FIB-4 were found to best predict advanced fibrosis with areas under curve of 0.71-0.76 and 0.62-0.80 respectively. However, the authors concluded that "While NFS and FIB-4 scores exhibit good diagnostic accuracy, FIB-4 is optimal in identifying NAFLD advanced fibrosis in the VHA. Easily implemented as a point-of-care clinical test, FIB-4 can be useful in directing patients that are most likely to have advanced fibrosis to GI/hepatology consultation and follow-up" (Patel et al., 2018).

Kim et al. (2017) evaluated the "association between plasma miR-122 [microRNA-122] and treatment outcomes following transarterial chemoembolization (TACE) in hepatocellular carcinoma patients." A total of 177 patients were included, and miR-122 levels were measured; the researchers found that 112 patients exhibited TACE refractoriness. Multivariate analyses showed that tumor number (hazard ratio [HR], 2.51) and tumor size (HR, 2.65) can independently predict overall TACE refractoriness. High miR-122 expression (> 100) was associated with early TACE refractoriness (within 1 year; HR, 2.77; 95% CI,) together with tumor number (HR, 22.73) and tumor size (HR, 4.90). Univariate analyses showed that high miR-122 expression tends to be associated with poor liver transplantation-free survival (HR, 1.42). However, this was statistically insignificant in multivariate analysis. The authors

concluded that "High expression levels of plasma miR-122 are associated with early TACE refractoriness in HCC patients treated with TACE" (Kim et al., 2017).

Suehiro et al. (2018) performed a study analyzing "the importance of serum exosomal miRNA expression levels in hepatocellular carcinoma (HCC) patients that underwent transarterial chemoembolization (TACE)." Seventy-five patients underwent TACE. Exosomal miR-122 expression levels significantly decreased after TACE. The expression levels of exosomal miR-122 before TACE were shown to correlate significantly with AST (r=0.31) and ALT (r=0.33) levels. According to the median relative expression of miR-122 after TACE/before TACE (miR-122 ratio) in liver cirrhosis patients (n=57), the patients with a higher miR-122 ratio had significantly longer disease-specific survival compared with that of the patients with the lower miR-122 ratio. A lower exosomal miR-122 ratio (HR 2.720) was associated with the disease-specific survival. The authors concluded that "the exosomal miR-122 level alterations may represent a predictive biomarker in HCC patients with liver cirrhosis treated with TACE" (Suehiro et al., 2018).

Kar, Paglialunga, Jaycox, Islam, and Paredes (2019) analyzed the performance of biomarkers implicated in hepatic inflammation. The authors enrolled 52 patients with NAFLD/NASH and evaluated the following biomarkers: IL-6, CRP, TNFα, MCP-1, MIP-1β, eotaxin, and VCAM-1. Serum IL-6 was found to be increased in patients with advanced fibrosis (2.71 pg/mL in fibrosis stages 3 and 4 compared to 1.26 pg/mL in stages 1-2 and 1.39 pg/mL in stage 0), but there were no other significant differences in CRP, TNFα, MCP-1, MIP-1β. VCAM-1 was noted to have increased by 55% over the mild fibrosis group and 40% over the no fibrosis group. VCAM-1 was also observed to have an area under curve of 0.87. The authors suggested that the "addition of biomarkers such as IL-6 and VCAM-1 to panels may yield increased sensitivity and specificity for staging of NASH" (Kar et al., 2019).

Srivastava et al. (2019) performed a cost-benefit analysis of non-invasive fibrosis tests (NILTS) for non-alcoholic fatty liver disease (NAFLD). The authors compared the current standard of care, FIB-4, and the Enhanced Liver Fibrosis (ELF) panel. The simulations consisted of 10000 NAFLD patients. Standard care (SC) was compared to the following four scenarios: "FIB-4 for all patients followed by ELF test for patients with indeterminate FIB-4 results; FIB-4 followed by fibroscan for indeterminate FIB-4; ELF alone; and fibroscan alone." The authors identified the following observations: "Introduction of NILT increased detection of advanced fibrosis over 1 year by 114, 118, 129 and 137% compared to SC in scenarios 2, 3, 4 and 5 respectively with reduction in unnecessary referrals by 85, 78, 71 and 42% respectively. Total budget spend [sic] was reduced by 25.2, 22.7, 15.1 and 4.0% in Scenarios 2, 3, 4 and 5 compared to £670 K at baseline." The authors suggested that the "use of NILT in primary care can increase early detection of advanced liver fibrosis and reduce unnecessary referral of patients with mild disease and is cost efficient" (Srivastava et al., 2019).

Weis et al. (2019) evaluated miRNA expression's ability to distinguish between HCC and cirrhosis. Sixty patients with chronic hepatitis C (CHC) were divided into three groups; 20 with fibrosis stages 0-2, 20 with cirrhosis, and 20 with cirrhosis and HCC. A total of 372 miRNA sequences were measured. The authors found that a theoretical panel consisting of miRNA-122-5p, miRNA-486-5p, and miRNA-142-3p distinguished HCC from cirrhosis (area under the curve [AUC]= 0.94; sensitivity = 80%, specificity = 95%) outperforming alpha-fetoprotein (AFP) (AUC = 0.64). Another theoretical panel of miRNA-122-5p and miRNA-409-3p distinguished cirrhosis from mild disease (AUC = 0.80; sensitivity = 85%, specificity = 70%). The authors concluded that "MicroRNAs have great potential as diagnostic biomarkers in CHC, particularly in HCC where they outperform the only currently-used biomarker, AFP" (Weis et al., 2019).

Both Parikh et al. (2017) and Kaswala, Lai, and Afdhal (2016) performed studies evaluating the diagnostic accuracy of non-invasive markers for liver conditions. Parikh et al. (2017) focused on

chronic hepatitis B virus (HBV) infections while Kaswala et al. (2016) studied nonalcoholic fatty liver. Tables detailing their summarized findings are listed below:

Diagnostic accuracy of most commonly used non-invasive fibrosis (≥F2) tests in chronic HBV infection from (Parikh et al., 2017)

Test	*Cut-off*	*AUROC*	*Sensitivity (%)*	*Specificity (%)*
Indirect markers				
FIB-4 index (high cut-off)	3.25	N/A	16.2	73.6
FIB-4 index (low cut-off)	1.45–1.62	0.78	65	77
APRI (low cut-off)	0.5	0.79	84	41
APRI (high cut-off)	1.5		49	84
Forns index (low cut-off)	3.11	0.68	91.4	31.5
Forns index (high cut-off)	5.11	N/A	42.5	75
Direct markers				
Hyaluronic acid	113–203	0.73	63–80	78–94
Hepascore	0.32	0.75	74	69
Fibrotest	0.38	0.77	65	78
Fibrometer	0.47	0.84	73	80
ELF	8.75	0.8	NA	NA

Diagnostic accuracy of most commonly used non-invasive fibrosis tests in nonalcoholic fatty liver (NAFL) from (Kaswala et al., 2016)

Test	*Cut-off*	*AUROC*	*Sensitivity (%)*	*Specificity (%)*
AST/ALT ratio	1	0.83	21	90

AST to platelet ratio index (low cutoff)	0.45	0.67–0.94	30	93
AST to platelet ratio index (high cutoff)	1.5			
BAAT score	2	0.84	71	80
BARD	2	0.8	86.8	32.5
ELF test	8.5–11.35	0.82–0.90	80	90
FibroMeter (low cutoff)	F3: 0.61	0.90–0.94	81	84
FibroMeter (high cutoff)	0.71			
FibroTest (low cutoff)	0.3	0.81–0.92	15–77	77–90
FibroTest (high cutoff)	0.7			
FIB-4 (low cutoff)	1.3–1.92	0.88	26–74	71–98
FIB-4 (high cutoff)	3.25			
Hepascore	0.37	0.81	75.5	84.1
	0.7	0.9	87	89
NAFLD (low cutoff)	−1.45	0.81	51	96
NAFLD (high cutoff)	0.67			

AST- aspartate aminotransferase; APRI- AST to platelet ratio; BAAT- body mass index (BMI), age, alanine aminotransferase (ALT), triglycerides; BARD- BMI, AST/ALT ratio, diabetes; ELF- Enhanced Liver Fibrosis panel; FIB-4- Fibrosis-4 index; NAFLD – Nonalcoholic fatty liver disease

Bril et al. (2019) assessed the performance of the FibroTest, along with other tests which measure steatosis, necrosis, and inflammation (the SteatoTest, ActiTest, NashTest), in a cohort of patients with type 2 diabetes. A total of 220 diabetic patients participated in this study. Plasma samples from each participant were used for the FibroTest. The researchers note that "Regarding the FibroTest score, its performance to identify patients with moderate or advanced fibrosis was 0.67" (Bril et al., 2019). The authors concluded that "Non-invasive panels for the diagnosis of steatosis, NASH and/or fibrosis, which were developed and validated in non-diabetic cohorts, underperformed when applied to a large cohort of patients with T2DM [type 2 diabetes mellitus]" (Bril et al., 2019)

In a metanalysis, 7 studies reported the accuracy of FibroTest™ in non-alcoholic fatty liver disease (NAFLD) patients. The mean AUC was 0.77, mean sensitivity was 0.72, and mean specificity was 0.69.

Due to poor AUC, sensitivity, and specificity values, FibroTest™ did not meet the minimally acceptable performance level in detecting significant, advanced, or any fibrosis. However, diagnostic accuracy of FibroTest™ was more promising in detecting cirrhosis, with an AUC of 0.92. The author states that in primary care settings which have a low disease prevalence, FibroTest™ can have a high negative predictive value, based on sensitivities between 0.90 and 0.98, demonstrating its ability to rule out advanced fibrosis in NAFLD patients. However, the test does have low specificity, leading to a considerable number of false positive results, which can lead to invasive and expensive follow-up tests. Overall, "this analysis showed that by optimizing sensitivity to values above 0.90, the test could result in high NPVs (>90%) in settings with low prevalence of disease, such as primary and secondary care settings, but with relatively low PPVs (11–61%)" (Vali et al., 2021).

V. Guidelines and Recommendations

American Association for the Study of Liver Diseases (AASLD) (AASLD-IDSA, 2015, 2018, 2019; Chalasani et al., 2017; Terrault et al., 2018)

The 2015 AASLD and Infectious Diseases Society of America (IDSA) recommendations for testing, managing, and treating adults infected with hepatitis C virus stated that "Recently, noninvasive tests to stage the degree of fibrosis in patients with chronic HCV infection include models incorporating indirect serum biomarkers (routine tests such as aspartate transaminase, alanine transaminase [ALT], and platelet count), direct serum biomarkers (components of the extracellular matrix produced by activated hepatic stellate cells), and vibration-controlled transient liver elastography. No single method is recognized to have high accuracy alone, and the results of each test must be interpreted carefully." The guidelines further stated that "although liver biopsy is the diagnostic standard, sampling error and observer variability limit test performance, particularly when inadequate sampling occurs. In addition, the test is invasive and minor complications are common, limiting patient and practitioner acceptance. Serious complications such as bleeding, although rare, are well recognized" (AASLD-IDSA, 2015).

The 2018 AASLD and Infectious Diseases Society of America (IDSA) recommendations for HCV testing stated that "evaluation for advanced fibrosis using liver biopsy, imaging, and/or noninvasive markers is recommended for all persons with HCV infection, to facilitate an appropriate decision regarding HCV treatment strategy and to determine the need for initiating additional measures for the management of cirrhosis (eg, hepatocellular carcinoma screening). Rating: Class I, Level A" (AASLD-IDSA, 2018).

The 2018 AASLD update (Terrault et al., 2018) on prevention, diagnosis and treatment of chronic hepatitis B state that:

For monitoring patients with a chronic HBV infection, who are not currently on treatment, "Alternative methods to assess fibrosis are elastography (preferred) and liver fibrosis biomarkers (e.g., FIB-4 or FibroTest). If these noninvasive tests indicate significant fibrosis (≥F2), treatment is recommended."

The 2018 AASLD practice guidelines (Chalasani et al., 2017) on the diagnosis and management of nonalcoholic fatty liver disease recommend:

- "In patients with NAFLD, metabolic syndrome predicts the presence of steatohepatitis, and its presence can be used to target patients for a liver biopsy."
- "NFS or FIB-4 index are clinically useful tools for identifying NAFLD patients with higher likelihood of having bridging fibrosis (stage 3) or cirrhosis (stage 4)."
- "Vibration controlled transient elastography or magnetic resonance elastography are clinically useful tools for identifying advanced fibrosis in patients with NAFLD."

The AASLD does not mention miRNA for assessment in liver disease.

A 2019 update from the AASLD and IDSA states that "Noninvasive tests using serum biomarkers or imaging allow for accurate diagnosis of cirrhosis in most individuals" and frequently used noninvasive methods to estimate liver disease severity include "serum fibrosis marker panels" (AASLD-IDSA, 2019). Further, regarding recommendations for counseling persons with an active HCV infection, the guideline recommend that "Evaluation for advanced fibrosis using noninvasive markers or liver biopsy, if required, is recommended for all persons with HCV infection to facilitate an appropriate decision regarding HCV treatment strategy, and to determine the need for initiating additional measures for cirrhosis management (eg, hepatocellular carcinoma screening)" (AASLD-IDSA, 2019).

In a 2021 update, AASLD discussed changes in liver biochemistry during normal pregnancy. AASLD states that an "elevation in aminotransferases, bilirubin, or bile acids in pregnancy is abnormal and requires investigation. Evaluation in pregnant patients must include a thorough history (including travel, environmental, and drug exposures), physical examination, and focused serologic testing. Hepatic ultrasonography (US) is the favored initial imaging modality. Diagnosis can usually be determined without liver biopsy" (Sarkar et al., 2021).

American Gastroenterological Association (AGA) (Lim, Flamm, Singh, & Falck-Ytter, 2017)

The 2017 guidelines (Lim et al., 2017) on the Role of Elastography in the Evaluation of Liver Fibrosis state that:

- "In patients with chronic hepatitis C, the AGA recommends vibration controlled transient elastography, if available, rather than other nonproprietary, noninvasive serum tests (APRI, FIB-4) to detect cirrhosis."
- "In patients with chronic hepatitis B, the AGA suggests vibration controlled transient elastography (VCTE) rather than other nonproprietary noninvasive serum tests (ie, APRI and FIB-4) to detect cirrhosis."
- "The AGA makes no recommendation regarding the role of VCTE in the diagnosis of cirrhosis in adults with NAFLD."

World Health Organization (WHO) (WHO, 2015, 2018)

In March 2015, the WHO released Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. In the section titled "Non-invasive Assessment of Liver Disease Stage at Baseline and during Follow up," the following is noted: aspartate aminotransferase (AST)-to-platelet ratio index (APRI) is recommended as the preferred non-invasive test (NIT) to assess for the presence of cirrhosis (APRI score >2 in adults) in resource-limited settings. Transient elastography (e.g., FibroScan) or FibroTest may be the preferred NITs in settings where they are available and cost is not a major constraint (WHO, 2015).

The WHO also published guidelines for management of patients with Hepatitis C. In it, they suggest "that aminotransferase/platelet ratio index (APRI) or FIB-4 be used for the assessment of hepatic fibrosis rather than other non-invasive tests that require more resources such as elastography or FibroTest." However, they do note that "FibroScan, which is more accurate than APRI and FIB-4, may be preferable in settings where the equipment is available and the cost of the test is not a barrier to testing."

The WHO does not mention miRNA as a tool for assessment of hepatitis (WHO, 2018).

US Preventive Services Task Force (USPSTF) (USPSTF, 2020)

The USPSTF published their final recommendation statement on Hepatitis C screening in adolescents and adults in 2020. THE USPSTF recommends "screening for hepatitis C virus (HCV) in adults aged 18 to 79" (grade B recommendation) (USPSTF, 2020).

National Institute for Health and Care Excellence (NICE) (NICE, 2016)

NICE has released guidelines regarding chronic liver conditions. They note that the enhanced liver fibrosis test (ELF) may be considered in patients with NAFLD to test for advanced liver fibrosis. The ELF test should be offered to adults every 3 years and to children and young people every 2 years. (NICE, 2016).

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 (EASL, 2015, 2016, 2017, 2018)

These joint guidelines include recommendations for fibrosis, mentioning ELF, FibroTest, NFS, and FIB-4. Their recommendations include the following:

- "Biomarkers and scores of fibrosis, as well as transient elastography, are acceptable non-invasive procedures for the identification of cases at low risk of advanced fibrosis/cirrhosis (A2). The combination of biomarkers/ scores and transient elastography might confer additional diagnostic accuracy and might save a number of diagnostic liver biopsies (B2)."
- "Monitoring of fibrosis progression in clinical practice may rely on a combination of biomarkers/scores and transient elastography, although this strategy requires validation (C2)."
- "The identification of advanced fibrosis or cirrhosis by serum biomarkers/scores and/or elastography is less accurate and needs to be confirmed by liver biopsy, according to the clinical context (B2)."
- The guidelines observe that due to non-invasive tests' high negative predictive values, they "may be confidently used for first-line risk stratification to exclude severe disease." Still, they state that "There is no consensus on thresholds or strategies for use in clinical practice when trying to avoid liver biopsy. Some data suggest that the combination of elastography and serum markers performs better than either method alone. Importantly, longitudinal data correlating changes in histological severity and in non-invasive measurements are urgently needed."
- For non-alcoholic steatohepatitis (NASH), the guidelines state that "to date, non-invasive tests are not validated for the diagnosis of NASH" and addresses CK-18 as a proposed biomarker.
- For monitoring of NAFLD, the guidelines state that "Monitoring should include routine biochemistry, assessment of comorbidities and non-invasive monitoring of fibrosis" (EASL, 2016).

The EASL also released guidelines on management of Hepatitis C. In it, they recommend that "Fibrosis stage must be assessed by non-invasive methods initially, with liver biopsy reserved for cases where there is uncertainty or potential additional aetiologies." Non-invasive methods include FibroScan, ARFI, Aixplorer, FibroTest, APRI, and FIB-4 (EASL, 2018).

Guidelines for Hepatitis B were also published. In it, EASL remarks that "the diagnostic accuracy of all non-invasive methods is better at excluding than confirming advanced fibrosis or cirrhosis." Non-invasive methods include assessment of serum biomarkers of liver fibrosis (EASL, 2017).

The EASL also published guidelines titled "Non-invasive tests for evaluation of liver disease severity and prognosis." In it, they state the following:

- "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests."
- "Serum biomarkers of fibrosis are well validated in patients with chronic viral hepatitis (with more evidence for HCV than for HBV and HIV/HCV coinfection). They are less well validated in NAFLD and not validated in other chronic liver diseases."

- "Their performances are better for detecting cirrhosis than significant fibrosis."
- "FibroTest®, APRI and NAFLD fibrosis score are the most widely used and validated patented and nonpatented tests."
- "Among the different available strategies, algorithms combining TE and serum biomarkers appear to be the most attractive and validated one."
- "HCV patients who were diagnosed with cirrhosis based on non-invasive diagnosis should undergo screening for HCC and PH and do not need confirmatory liver biopsy."
- "Non-invasive assessment including serum biomarkers or TE can be used as first line procedure for the identification of patients at low risk of severe fibrosis/ cirrhosis."
- "The identification of significant fibrosis is less accurate with non-invasive tests as compared to liver biopsy and may necessitate, according to the clinical context, histological confirmation."
- "Follow-up assessment by either serum biomarkers or TE for progression of liver fibrosis should be performed among NAFLD patients at a 3 year interval (EASL, 2015)."

EASL released guidelines on non-invasive tests for evaluation of liver disease severity and prognosis (EASL, 2020). The following recommendations were made:

- "Serum biomarkers can be used in clinical practice due to their high applicability (>95%) and good interlaboratory reproducibility. However, they should be preferably obtained in fasting patients (particularly those including hyaluronic acid) and following the manufacturer's recommendations for the patented tests. In addition, transient electrography (TE) and serum biomarkers have equivalent performance for detecting significant fibrosis in patients with untreated viral hepatitis.
- In patients with viral hepatitis C, when TE and serum biomarkers results are in accordance, the diagnostic accuracy is increased for detecting significant fibrosis but not for cirrhosis. In cases of unexplained discordance, a liver biopsy should be performed if the results would change the patient management.
- All HCV patients should be screened to exclude cirrhosis by TE if available. Serum biomarkers can be used in the absence of TE.
- TE and serum biomarkers have equivalent performance for detecting significant fibrosis in patients with untreated viral hepatitis.
- In patients with viral hepatitis C, when TE and serum biomarkers results are in accordance, the diagnostic accuracy is increased for detecting significant fibrosis but not for cirrhosis. In cases of unexplained discordance, a liver biopsy should be performed if the results would change the patient management" (EASL, 2020).

VI. State and Federal Regulations, as applicable

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

A search for "fibrosis" on the FDA website on July 18, 2021, did not yield any results relevant to hepatic conditions. Although several of these panels are patented, none are FDA approved. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-

complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

Code Number	Code Description
81596	Infectious disease, chronic hepatitis c virus (HCV) infection, six biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, and haptoglobin) utilizing serum, prognostic algorithm reported as scores for fibrosis and necroinflammatory activity in liver Proprietary test: HCV FibroSURE™, FibroTest™ Laboratory/Manufacturer: BioPredictive S.A.S
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
0002M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, haptoglobin, AST, glucose, total cholesterol and triglycerides) utilizing serum, prognostic algorithm reported as quantitative scores for fibrosis, steatosis and alcoholic steatohepatitis (ASH) Proprietary test: ASH FibroSURE™ Laboratory/Manufacturer: BioPredictive S.A.S
0003M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein A-1, total bilirubin, GGT, haptoglobin, AST, glucose, total cholesterol and triglycerides) utilizing serum, prognostic algorithm reported as quantitative scores for fibrosis, steatosis and nonalcoholic steatohepatitis (NASH) Proprietary test: NASH FibroSURE™ Laboratory/Manufacturer: BioPredictive S.A.S
0014M	Liver disease, analysis of 3 biomarkers (hyaluronic acid [HA], procollagen III amino terminal peptide [PIIINP], tissue inhibitor of metalloproteinase 1 [TIMP-1]), using immunoassays, utilizing serum, prognostic algorithm reported as a risk score and risk of liver fibrosis and liver-related clinical events within 5 years Proprietary test: Enhanced Liver Fibrosis™ (ELFTM) Test Lab/Manufacturer: Siemens Healthcare Diagnostics Inc/Siemens Healthcare Laboratory LLC
0166U	Liver disease, 10 biochemical assays (α2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT, AST, triglycerides, cholesterol, fasting glucose) and biometric and

	demographic data, utilizing serum, algorithm reported as scores for fibrosis, necroinflammatory activity, and steatosis with a summary interpretation Proprietary test: LiverFASt™ Lab/Manufacturer: Fibronostics

VIII. Evidence-based Scientific References

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IX. Revision History

Revision Date	Summary of Changes
01/01/2023	Initial Effective Date