Medical Policy Manual Laboratory, Policy No. 47

Multianalyte Assays with Algorithmic Analysis for the Evaluation and Monitoring of Patients with Chronic Liver Disease

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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Multianalyte serum assays with algorithmic analysis are being evaluated as a substitute for biopsy in the screening, evaluation, and monitoring of patients with chronic liver disease.

MEDICAL POLICY CRITERIA

Multianalyte assays with algorithmic analyses, including but not limited to the following tests are considered **investigational** for the evaluation and monitoring of patients with chronic liver disease:

A. HCV FibroSURE™ (FibroTest™)
B. Elasto-FibroTest®
C. FibroSpect II
D. ASH FibroSURE™ (ASH Test)
E. NASH FibroSURE™ (NASH Test)
F. Enhanced Liver Fibrosis™ (ELF) Test

G. LiverFASt™ Test

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Investigational Gene Expression, Biomarker, and Multianalyte Testing, Laboratory, Policy No. 77
2. Magnetic Resonance Spectroscopy, Radiology, Policy No. 27

BACKGROUND

CHRONIC LIVER DISEASES

Hepatitis C

Infection with the hepatitis C virus can lead to permanent liver damage. Liver biopsy is typically recommended prior to the initiation of antiviral therapy. Repeat biopsies may be performed to monitor fibrosis progression. Liver biopsies are analyzed according to a histologic scoring system; the most commonly used one for hepatitis C is the METAVIR scoring system, which scores the presence and degree of inflammatory activity and fibrosis. The fibrosis is graded from F0-F4, with a METAVIR score of F0 signifying no fibrosis and F4 signifying cirrhosis (which is defined as the presence throughout the liver of fibrous septa that subdivide the liver parenchyma into nodules and represents the final and irreversible form of disease). The stage of fibrosis is the most important single predictor of morbidity and mortality in patients with hepatitis C. Biopsies for hepatitis C are also evaluated according to the degree of inflammation present, referred to as the grade or activity level. For example, the METAVIR system includes scores for necroinflammatory activity ranging from A0 to A3 (A0=no activity, A1=minimal activity, A2=moderate activity, A3=severe activity.)

Hepatitis B

Most people who become infected with hepatitis B virus (HBV) recover fully, but a small portion will develop chronic HBV, which can lead to permanent liver damage. As with HCV, identification of liver fibrosis is needed to determine timing and management of treatment, and liver biopsy is the criterion standard for staging fibrosis. The grading of fibrosis in HBV also uses the Metavir system.

Alcoholic Liver Disease

Alcoholic liver disease (ALD) is the leading cause of liver disease in most Western countries. Histologic features of ALD usually include steatosis, alcoholic steatohepatitis (ASH), hepatocyte necrosis, Mallory bodies (tangled proteins seen in degenerating hepatocytes), a large polymorphonuclear inflammatory infiltrate, and, with continued alcohol abuse, fibrosis and possibly cirrhosis. The grading of fibrosis is similar to the scoring system used in hepatitis C. The commonly used Laënnec scoring system uses grades 0-4, with 4 being cirrhosis.

Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is defined as a condition that pathologically resembles ALD but occurs in patients who are not heavy users of alcohol. It may be associated with a variety of conditions, including obesity, diabetes, and dyslipidemia. The

characteristic feature of NAFLD is steatosis. At the benign end of the spectrum of the disease, there is usually no appreciable inflammation, hepatocyte death, or fibrosis. In contrast, non-alcoholic steatohepatitis (NASH), which shows overlapping histologic features with ALD, is an intermediate form of liver damage, and liver biopsy may show steatosis, Mallory bodies, focal inflammation, and degenerating hepatocytes. NASH can progress to fibrosis and cirrhosis. A variety of histological scoring systems have been used to evaluate NAFLD. The NAFLD activity score (NAS) system for NASH includes scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2). Cases with scores of 5 or greater are considered NASH, while cases with scores of 3 and 4 are considered borderline (probable or possible) NASH. The grading of fibrosis is similar to the scoring system used in hepatitis C. The commonly used Laënnec scoring system uses grades 0-4, with 4 being cirrhosis.

BIOPSY FOR CHRONIC LIVER DISEASE

The diagnosis of non-neoplastic liver disease is often made from needle biopsy samples. In addition to establishing a disease etiology, liver biopsy can determine the degree of inflammation present and stage the degree of fibrosis. The degree of inflammation and fibrosis may be assessed by different scoring schemes. Most of these scoring schemes grade inflammation from 0-4 (with 0 being no or minimal inflammation and 4 being severe) and fibrosis from 0-4 (with 0 being no fibrosis and 4 cirrhosis). There are several limitations to liver biopsy, including its invasive nature, small tissue sample size, and subjective grading system. Regarding small tissue sample size, liver fibrosis can be patchy and thus missed on a biopsy sample, which includes only 0.002% of the liver tissue. A noninvasive alternative to liver biopsy would be particularly helpful, both to initially assess patients and then as a monitoring tool to assess response to therapy.

MULTIANALYTE ASSAYS

A variety of noninvasive laboratory tests are being evaluated as alternatives to liver biopsy. Biochemical tests can be broadly categorized into indirect and direct markers of liver fibrosis. Indirect markers include liver function tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), the ALT/AST ratio (also referred to as the AAR), platelet count, and prothrombin index. In recent years, there has been growing understanding of the underlying pathophysiology of fibrosis, leading to direct measurement of the factors involved. For example, the central event in the pathophysiology of fibrosis is activation of the hepatic stellate cell. Normally, stellate cells are quiescent but are activated in the setting of liver injury, producing a variety of extracellular matrix (ECM) proteins. In normal livers, the rate of ECM production equals its degradation, but, in the setting of fibrosis, production exceeds degradation. Metalloproteinases are involved in intracellular degradation of ECM, and a profibrogenic state exists when there is either a down regulation of metalloproteinases or an increase in tissue inhibitors of metalloproteinases (TIMP). Both metalloproteinases and TIMP can be measured in the serum, which directly reflects fibrotic activity. Other direct measures of ECM deposition include hyaluronic acid or α2-macroglobulin.

While many studies have been done on these individual markers, or on groups of markers in different populations of patients with liver disease, there has been interest in analyzing multiple markers using mathematical algorithms to generate a score that categorizes patients according to the biopsy score. It is proposed that these algorithms can be used as an alternative to liver biopsy in patients with liver disease. The following proprietary, algorithm-based tests are commercially available in the United States.

FibroSURE and FibroTest

There are three different FibroSURE tests available depending on the indication for use: HCV FibroSURE, ASH FibroSURE, and NASH FibroSURE.

HCV FibroSURE

HCV FibroSURE (FibroTest) uses a combination of six serum biochemical indirect markers of liver function plus age and sex in a patented algorithm to generate a measure of fibrosis and necroinflammatory activity in the liver that correspond to the Metavir scoring system for stage (i.e., fibrosis) and grade (i.e., necroinflammatory activity). The measures are combined using a linear regression equation to produce a score between 0 and 1, with higher values corresponding to more severe disease. The biochemical markers include the readily available measurements of α2-macroglobulin, haptoglobin, bilirubin, γ-glutamyl transpeptidase (GGT), ALT, and apolipoprotein AI. Developed in France, the test has been clinically available in Europe under the name FibroTest since 2003 and is exclusively offered by LabCorp in the United States as HCV FibroSURE.

ASH FibroSURE

ASH FibroSURE (ASH Test) uses a combination of 10 serum biochemical markers of liver function together with age, sex, height, and weight in a proprietary algorithm and is proposed to provide surrogate markers for liver fibrosis, hepatic steatosis, and ASH. The biochemical markers include α2-macroglobulin, haptoglobin, apolipoprotein AI, bilirubin, GGT, ALT, AST, total cholesterol, triglycerides, and fasting glucose. The test has been available in Europe under the name ASH Test and is exclusively offered by LabCorp in the United States as ASH FibroSURE.

NASH FibroSURE

NASH FibroSURE (NASH Test) uses a proprietary algorithm of the same 10 biochemical markers of liver function in combination with age, sex, height, and weight and is proposed to provide surrogate markers for liver fibrosis, hepatic steatosis, and NASH. The biochemical markers include α2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT, AST, total cholesterol, triglycerides, and fasting glucose. The test has been available in Europe under the name NASH Test and is exclusively offered by LabCorp in the United States as NASH FibroSURE.

FIBROSpect II

FIBROSpect II uses a combination of three markers that directly measure fibrogenesis of the liver, analyzed with a patented algorithm. The markers include hyaluronic acid, TIMP-1, and α2-macroglobulin. FIBROSpect II is offered exclusively by Prometheus Laboratories. The measures are combined using a logistic regression algorithm to generate a FIBROSpect II index score, ranging from 1 to 100 (or sometimes reported between 0 and 1), with higher scores indicating more severe disease.

Enhanced Liver Fibrosis Test

The Enhanced Liver Fibrosis (ELF) test uses a proprietary algorithm to produce a score based on three serum biomarkers involved in matrix biology: hyaluronic acid, Procollagen III amino terminal peptide and tissue inhibitor of metalloproteinase 1. The manufacturer recommends

the following cutoffs for interpretation for risk of development of cirrhosis or liver-related events in patients with NASH: <9.80 (lower risk) and ≥11.30 (higher risk).

LiverFASt

LiverFASt™ is a blood based diagnostic test marketed by Fibronostics that combines 10 biomarkers and algorithm technology to determine the fibrosis, activity and steatosis stages of the liver.[1] The markers include alpha-2-Macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, ALT (P5P), AST (P5P), fasting glucose, triglyceride, and total cholesterol. There is no FDA approval for the LiverFASt™ identified.

EVIDENCE SUMMARY

Validation of the clinical use of any diagnostic test focuses on three main principles:

1. Analytic validity of the test;
2. Clinical validity of the test (i.e., sensitivity, specificity, and positive and negative predictive values in relevant populations of patients and compared to the gold standard); and
3. Clinical utility of the test (i.e., how the results of the diagnostic test will be used to improve the management of the patient).

This evidence review focused on the clinical validity and utility of the tests.

LIVER BIOPSY IS AN IMPERFECT REFERENCE STANDARD

As mentioned in the Background, liver biopsy is an imperfect reference standard. There is a high rate of sampling error in biopsy, which can lead to underdiagnosis of liver disease.[2, 3] This will bias estimates of performance characteristics of the noninvasive tests to which it is compared and must be considered in apprising the body of evidence. Mehta estimated that, under the best scenario where sensitivity and specificity of liver biopsy are 90% and the prevalence of significant disease (Metavir ≥F2) is 40%, even a perfect alternative marker would have calculated area under the receiver operating characteristic (AUROC) curve of 0.90.[4] Therefore, effectiveness of alternative technologies may be underestimated. In fact, when the accuracy of biopsy is presumed to be 80%, a comparative technology with an AUROC curve of 0.76 may actually have an AUROC curve of 0.93 to 0.99 for diagnosing true disease.

FIBROSURE AND FIBROTEST

Hepatitis C Virus

Clinical Validity

In a systematic review published by Crossan (2015), FibroTest was the most widely validated commercial serum test.[5] Seventeen studies were included in the pooled estimate of the diagnostic accuracy of FibroTest for significant fibrosis (stage ≥F2) in HCV. With varying cutoffs for positivity between 0.32 and 0.53, the summary sensitivity in HCV was 68% (95% confidence interval [CI], 58% to 77%) and specificity was 72% (95% CI 70% to 77%). Eight studies were included for cirrhosis (stage F4) in HCV. The cutoffs for positivity ranged from 0.56 to 0.74 and the summary sensitivity and specificity were 60% (95% CI 43% to 76%) and

86% (95% CI 81% to 91%), respectively. Uninterpretable results were rare for tests based on serum markers.

Poynard (2012) assessed the relative accuracy of FibroTest and FibroScan using a method to estimate performance characteristics when no perfect reference standard exists.[6] The study included 1,893 subjects retrospectively extracted from four prospective cohorts: three cohorts with HCV (n=1,289) and one cohort of healthy volunteers (n=604). Four different tests (FibroTest, FibroScan, ALT, liver biopsy) were performed on all patients with HCV. Latent class models with random effects were used to combine the test results to construct a reference standard. When compared to biopsy as the reference standard, the sensitivity and specificity for the diagnosis of advanced fibrosis were 85% and 66% for FibroTest and 93% and 48% for FibroScan. However, when compared to the latent class reference standard, the specificity and sensitivity for the diagnosis of advanced fibrosis were 93% and 70% for FibroTest and 96% and 45% for FibroScan.

Following the initial research into FibroSURE (patients with liver fibrosis who had undergone biopsy),[7] the next step in the development of this test was further evaluation of the algorithm in a cross-section of patients, including patients with HCV participating in large clinical trials before and after the initiation of antiviral therapy. One study focused on patients with HCV who were participating in a randomized study of pegylated interferon and ribavirin.[8] From the 1530 participants, 352 patients with stored serum samples and liver biopsies at study entry and at 24-week follow-up were selected. The HCV FibroSURE score was calculated and then compared with the Metavir liver biopsy score. At a cutoff of 0.30, the HCV FibroSURE score had 90% sensitivity and 88% positive predictive value (PPV) for the diagnosis of Metavir F2-F4. The specificity was 36%, and the negative predictive value (NPV) was 40%.

Poynard (2004) also evaluated discordant results in 537 patients who underwent liver biopsy and the HCV FibroSURE and ActiTest on the same day; discordance was attributed to either the limitations in the biopsy or serum markers.[9] In this study, cutoff values were used for individual Metavir scores (i.e., F0-F4) and for combinations of Metavir scores (i.e., F0-F1, F1-F2). The definition of a significant discordance between FibroTest and ActiTest and biopsy scores was at least two stages or grades in the Metavir system. Discordance was observed in 29% of patients. Risk factors for failure of HCV FibroSURE scoring system were presence of hemolysis, inflammation, possible Gilbert syndrome, acute hepatitis, drugs inducing cholestasis, or an increase in transaminases. Discordance was attributable to markers in 2.4% of patients and to the biopsy in 18% and nonattributed in 8.2% of patients. As noted in two reviews, the bulk of the research on HCV FibroSURE was conducted by researchers with an interest in the commercialization of the algorithm.[10, 11]

One Australian study attempted to independently replicate the results of FibroSURE in 125 patients with hepatitis C.[12] Using the cutoff of less than 0.1 to identify lack of bridging fibrosis (i.e., Metavir stages F0-F1) and greater than 0.6 to identify fibrosis (i.e., Metavir stages F2-F4), the NPV for a score of less than 0.1 was 89%, and the PPV of a score greater than 0.6 was 78%.

Clinical Utility

The effect on patient outcomes of a test depends on a demonstration that the test can be used to improve patient management. The primary benefit of the FibroSURE (FibroTest) for HCV is the ability to avoid liver biopsy in patients without significant fibrosis. Thus, empiric data are needed that demonstrate that the FibroSURE test impacts clinician decision making on

whether a biopsy should be performed and that the net effect is to reduce the overall number of biopsies while achieving similar clinical outcomes. There are currently no such published studies to demonstrate effect on patient outcomes. However, FibroTest has been used as an alternative to biopsy to establish trial eligibility in terms of fibrosis or cirrhosis in several trials (ION-1,-3; VALENCE; ASTRAL-2, -3, -4) that established efficacy of HCV treatments.[13-18] For example, in the ASTRAL-2 and -3 trials, cirrhosis could be defined by liver biopsy, FibroScan, or FibroTest score of more than 0.75 and an APRI of more than 2.

These tests also need to be adequately compared with other noninvasive tests of fibrosis to determine their comparative efficacy. In particular, the proprietary, algorithmic tests should demonstrate superiority to other readily available, nonproprietary scoring systems to demonstrate that the tests improve health outcomes.

The test also has potential effect on patient outcomes as a means to follow response to therapy. In this case, evidence needs to demonstrate that use of the test for response to therapy impacts decision making and that these changes in management decisions lead to improved outcomes. It is not clear whether the HCV FibroSURE could be used as an interval test in patients receiving therapy to determine whether an additional liver biopsy was necessary.

Alcoholic Liver Disease and Alcoholic Steatohepatitis

Clinical Validity

FibroTest has been studied in patients with ALD.

A systematic review of studies of the prognostic performance of non-invasive tests in alcohol-related liver disease was published by Rhodes (2020).[19] Of the 11 articles included in the review, six were conference abstracts and one was an unpublished manuscript. One unpublished paper on ELF, four abstracts on FibroScan, four articles on FIB-4 (including one abstract), one abstract on FIB-4 + FibroScan, and one publication on FibroTest + FIB-4 were included. While low-moderate risk of bias for most domains was found, there was high risk in confounding/ statistical reporting domains, including six of the 11 studies having a high risk of bias in the confounding domain. Area under the receiver operating characteristic curves (AUROC) for outcome prediction ranged from 0.65 to 0.76 for FibroScan, 0.64 to 0.83 for FIB-4, 0.69 to 0.79 for FibroTest, and 0.72 to 0.85 for ELF. The low number of studies meeting inclusion criteria, as well as the heterogeneity in study design and/or data reporting precluded pooled data analysis. Additional adequately powered and appropriately controlled clinical trials are needed to evaluate the prognostic value of these tests in alcohol-related liver disease.

In the Crossan (2015) systematic review, one study was identified that described diagnostic accuracy of FibroTest for significant fibrosis (stage ≥ F2) or cirrhosis in ALD.[5] With a high cutoff for positivity (0.7) the sensitivity and specificity for advanced fibrosis were 55% (95% CI 47% to 63%) and 93% (95% CI 85% to 97%) and for cirrhosis were 91% (95% CI 82% to 96%) and 87% (95% CI 81% to 91%), respectively. With a low cutoff for positivity (0.3) the sensitivity and specificity for advanced fibrosis were 84% (95% CI 77% to 89%) and 65% (95% CI 55% to 75%) and for cirrhosis were 100% (95% CI 95% to 100%) and 50% (95% CI 42% to 58%), respectively.

The diagnostic value of FibroSURE (FibroTest) has also been evaluated for the prediction of liver fibrosis in patients with ALD and NAFLD.[20, 21] Thabut (2006) reported the development of

a panel of biomarkers (ASH FibroSURE [ASH Test]) for the diagnosis of alcoholic steatohepatitis (ASH) in patients with chronic ALD.[22] Biomarkers were initially assessed with a training group consisting of 70 patients, and a panel was constructed using a combination of the six biochemical components of the FibroTest-ActiTest plus AST. The algorithm was subsequently studied in two validation groups (one prospective study for severe ALD, one retrospective study for nonsevere ALD) that included 155 patients and 299 controls. The severity of ASH (none, mild, moderate, severe) was blindly assessed from biopsy samples. In the validation groups, there were 28 (18%) cases of discordance between the diagnosis of ASH predicted by the ASH Test and biopsy; 10 (36%) were considered to be false negatives of the ASH Test, and 11 were suspected to be failures of biopsy. Seven cases were indeterminate by biopsy. The AUROC curves were 0.88 and 0.89 in the validation groups. The median ASH Test value was 0.005 in controls, 0.05 in patients without or with mild ASH, 0.64 in the moderate ASH grade, and 0.84 in severe ASH grade 3. Using a cutoff value of 0.50, the ASH Test had sensitivity of 80% and specificity of 84%, with PPVs and NPVs of 72% and 89%, respectively.

Several authors have an interest in the commercialization of this test, and no independent studies on the diagnostic accuracy of ASH FibroSURE (ASH Test) were identified. In addition, it is not clear if the algorithm used in this study is the same as that used in the currently commercially available test, which includes 10 biochemicals.

Clinical Utility

No studies were identified that assessed clinical outcomes following use of ASH FibroSURE (ASH Test).

Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

Clinical Validity

In the Crossan (2015) systematic review described above, four studies were included in the pooled estimate of the diagnostic accuracy of FibroTest for advanced fibrosis (stage ≥ 3) in NAFLD.[5] The summary sensitivities and specificities were 40% (95% CI 24% to 58%) and 96% (95% CI 91% to 98%). Only one study included reported accuracy for cirrhosis, with sensitivity and specificity of 74% (95% CI 54% to 87%) and 92% (95% CI 88% to 95%), respectively. Munteanu (2018) published a cohort study evaluating the ten-year prognostic value of FibroTest for predicting liver-related death in patients with NAFLD.[23] A total of 7,082 patients in the FibroFrance cohort who underwent a FibroTest between 1997 and 2012 were prospectively enrolled. Of those, 1,079 had a diagnosis of NAFLD. In these patients, ten-year survival was 0.956 (95% CI 0.940 to 0.971, 38 events). The prognostic values for Fibrotest, AUROC (0.941, 95% CI 0.905 to 0.978) and Cox risk ratio (1,638, 95% CI 342 to 7,839), were statistically significant ($p<0.001$).

An independent study by Lassailly (2011) attempted to prospectively validate the NASH Test (along with the FibroTest, SteatoTest, and ActiTest) in a cohort of 288 patients treated with bariatric surgery.[24] Included were patients with severe or morbid obesity (body mass index >35 kg/m^2), at least one comorbidity for at least five years, and resistance to medical treatment. Excluded were patients with current excessive drinking, long-term consumption of hepatotoxic drugs, and positive screening for chronic liver diseases including hepatitis. Histology and biochemical measurements were centralized and blinded to other characteristics. The NASH test provided a three-category score for no NASH (0.25), possible

NASH (0.50), and NASH (0.75). The prevalence of NASH was 6.9%, while the prevalence of NASH or possible NASH was 27%. The concordance rate between histologic NAS and the NASH Test was 43.1%, with a weak κ reliability test (0.14). In 183 patients categorized as possible NASH by the NASH Test, 124 (68%) were classified as no NASH by biopsy. In 15 patients categorized as NASH by the NASH Test, seven (47%) were no NASH and four (27%) were possible NASH by biopsy. The NPV of the NASH Test for possible NASH or NASH was 47.5%. Authors suggested that the power of this study to validate agreement between the NASH Test and biopsy was low, due to the low prevalence of NASH. However, the results showed poor concordance between the NASH Test and biopsy, particularly for intermediate values.

Poynard (2006) reported the development of a panel of biomarkers (NASH FibroSURE [NASH Test]) for the prediction of NASH in patients with NAFLD.[25] Biomarkers were initially assessed with a training group consisting of 160 patients, and a panel was constructed using a combination of 13 of 14 parameters of the currently available test. The algorithm was subsequently studied in a validation group of 97 patients and 383 controls. Patients in the validation group were from a prospective multicenter study with hepatic steatosis at biopsy and suspicion of NAFLD. Histologic diagnoses used Kleiner et al's scoring system, with three classes for NASH (NASH, borderline NASH, no NASH). The main end point was steatohepatitis, defined as a histologic NASH score (NAS) of 5 or greater. The AUROC curve for the validation group was 0.79 for the diagnosis of NASH, 0.69 for the diagnosis of borderline NASH, and 0.83 for the diagnosis of no NASH. Results showed sensitivity of 33% and specificity of 94% for NASH, with PPVs and NPVs of 66% and 81%, respectively. For borderline NASH or NASH, there was a sensitivity of 88%, specificity of 50%, PPV of 74%, and NPV of 72%. Clinically significant discordance (two class difference) was observed in eight (8%) patients. None of the 383 controls was considered to have NASH by NASH FibroSURE (NASH Test). Authors proposed that this test would be suitable for mass screening for NAFLD in patients with obesity and diabetes.

Clinical Utility

No studies were identified that assessed clinical outcomes following use of NASH FibroSURE (NASH Test).

Hepatitis B Virus

Analytic Validity

As above (see the Technical Performance: Hepatitis C Virus section).

Clinical Validity

While most multianalyte assay studies that have identified fibrosis have been in patients with HCV, studies are also being conducted in patients with chronic HBV.[26, 27] In the Crossan (2015) systematic review, six studies were included in the pooled estimate of the diagnostic accuracy of FibroTest for significant fibrosis (stage ≥ F2) in HBV.[5] The cutoffs for positivity ranged from 0.40 to 0.48, and the summary sensitivities and specificities were 66% (95% CI 57% to 75%) and 80% (95% CI 72% to 86%), respectively. The accuracy for cirrhosis in HBV was based on four studies with cutoffs for positivity ranging from 0.58 to 0.74. Sensitivities and specificities were 74% (95% CI 25% to 96%) and 90% (95% CI 83% to 94%).

Salkic (2014) conducted a meta-analysis of studies on the diagnostic accuracy of FibroTest in

chronic HBV.[28] Included in the meta-analysis were 16 studies (n=2,494) on liver fibrosis diagnosis and 13 studies (n=1,754) on cirrhosis diagnosis. There was strong evidence of heterogeneity in the 16 fibrosis studies and evidence of heterogeneity in the cirrhosis studies. For significant liver fibrosis (Metavir F2-F4) diagnosis using all of the fibrosis studies, the AUROC curve was 0.84 (95% CI 0.78 to 0.88). At the recommended FibroTest threshold of 0.48 for a significant liver fibrosis diagnosis, the sensitivity was 60.9%, specificity was 79.9%, and the diagnostic odds ratio (OR) was 6.2. For liver cirrhosis (Metavir F4) diagnosis using all of the cirrhosis studies, the AUROC curve was 0.87 (95% CI 0.85 to 0.9). At the recommended FibroTest threshold of 0.74 for cirrhosis diagnosis, the sensitivity was 61.5%, specificity was 90.8%, and the diagnostic OR was 15.7. While the results demonstrated FibroTest may be useful in excluding a diagnosis of cirrhosis in patients with chronic HBV, the ability to detect significant fibrosis and cirrhosis and exclude significant fibrosis is suboptimal.

Xu (2014) reported on a systematic review and meta-analysis of studies on biomarkers to detect fibrosis in HBV.[29] Included in the analysis on FibroTest were 11 studies (total n=1,640). In these 11 studies, AUROC curves ranged from 0.69 to 0.90. Heterogeneity in the studies was statistically significant.

Park (2013) compared liver biopsy and the FibroTest results obtained on the same day from 330 patients with chronic HBV.[30] Discordance was found in 30 (9.1%) patients for whom the FibroTest underestimated fibrosis in 25 patients and overestimated it in five patients. Those with Metavir liver fibrosis stages F3 or F4 (15.4%) had a significantly higher discordance rate than with stages F1 or F2 (3.0%, $p<0.001$). The only independent factor for discordance on multivariate analysis was a Metavir stages F3 or F4 on liver biopsy ($p<0.001$).

Clinical Utility

There are no studies of the effect on patient outcomes for patients with HBV. Of note, some researchers have noted that different markers (e.g., HBV FibroSURE) may be needed for this assessment in patients with hepatitis B.[31]

Section Summary: FibroSURE and FibroTest

FibroSURE (FibroTest) is the most widely validated of the noninvasive commercial serum tests. It has been studied in populations with viral hepatitis, NAFLD, and ALD. Although there are established cutoffs for positivity for FibroTest, they were not consistently used in validation studies. The methodologic quality of the validation studies was generally poor. There is no direct evidence that FibroSURE (FibroTest) improves health outcomes. However, FibroTest has been allowed as an alternative to biopsy to establish trial eligibility in terms of fibrosis or cirrhosis in several randomized controlled trials (RCTs) that established the efficacy of HCV treatments.

FIBROSPECT II

Clinical Validity

Patel (2004) investigated the use of these serum markers in an initial training set of 294 patients with HCV and further validated the resulting algorithm in a validation set of 402 patients.[32] The algorithm was designed to distinguish between no or mild fibrosis (F0-F1) and moderate-to-severe fibrosis (F2-F4). With the prevalence of F2-F4 disease of 52% and a cutoff value of 0.36, the PPVs and NPVs were 74.3% and 75.8%, respectively.

The published studies for this combination of markers continue to focus on test characteristics such as sensitivity, specificity, and accuracy.[33-35] In Crossan (2015), the summary diagnostic accuracy for detecting significant fibrosis (stage ≥ F2) in five studies of HCV with FIBROSpect II with cutoffs ranging from 42 to 72 was 78% (95% CI 49% to 93%) and the summary specificity was 71% (95% CI, 59% to 80%).[5] Using a FIBROSpect II cutoff score of 42, Christensen (2006) reported a sensitivity of 93%, specificity of 66%, overall accuracy of 76%, and a NPV of 94% for advanced fibrosis in 136 patients with HCV.[36]

Clinical Utility

The issues of effect on patient outcomes are similar to those discussed for the FibroSURE (FibroTest). No studies were identified in the published literature in which results of the FIBROSpect test were actively used in the management of the patient.

Section Summary: FIBROSpect II

FIBROSpect II has been studied in populations with HCV. Cutoffs for positivity varied across studies and were not well validated. The methodologic quality of the validation studies was generally poor. There is no direct evidence that FIBROSpect II improves health outcomes.

OTHER MULTIANALYTE SCORING SYSTEMS

Clinical Validity

Other scoring systems have been developed, including FIB-4, NAFLD fibrosis score (NFS), APRI, AST/ALT ratio, and ELF. The ELF test combines measurements of biomarkers into a proprietary algorithm to produce a score. The other scoring systems use a simple nonproprietary formula that can be calculated at the bedside to produce a score for the prediction of fibrosis. Tables 1 and 2 summarize the characteristics and results of systematic reviews that have assessed the diagnostic accuracy of various noninvasive scoring systems. There are no established cutoffs for ruling in or ruling out advanced fibrosis for most tests. In the systematic reviews, two cutoffs were analyzed for each test (as selected by the authors); a lower threshold to rule out advanced fibrosis and a higher threshold to rule in advanced fibrosis. Patients that fall between the two thresholds are classified as "indeterminate" risk for whom a liver biopsy may be considered. Castellana (2021) conducted a meta-analytic comparison between FIB-4 and NFS and found no significant differences regarding relative diagnostic OR, positive likelihood ratio, and negative likelihood ratio.[37] FIB-4 was associated with fewer indeterminate findings compared to NFS. Mózes (2022) found that FibroScan, a transient elastography test, outperformed all of the serum-based tests.[38] Sharma (2021) qualitatively evaluated the diagnostic performance of ELF in patients with chronic liver disease.[39] Mózes (2023) found that all index tests evaluated (NFS, FIB-4, and FibroScan) performed as well as histologically assessed fibrosis in predicting clinical outcomes in patients with NAFLD.[40]

Table 1. Characteristics of Systematic Reviews Assessing Noninvasive Scoring Systems

Systematic Review	Studies	N (Range)	Population	Index Tests	Reference Standard
Mózes (2023)[40]	25	2,518 (NR)	NAFLD	FibroScan FIB-4 NFS	Histology

Systematic Review	Studies	N (Range)	Population	Index Tests	Reference Standard
Castellana (2021)[37]	18	12,604 (102 to 3,202)	NAFLD	FIB-4 NFS	Histology
Mózes (2022)[38]	37	5,735 (13 to 1,063)	NAFLD	FibroScan FIB-4 NFS APRI AST/ALT	Histology
Sharma (2021)[39]	36	NR (38 to 3,202)	Chronic liver disease (NAFLD, ALD, hepatitis, mixed etiologies)	ELF	Histology

ALD: alcoholic liver disease; APRI: AST-to-platelet ratio; AST: aspartate aminotransferase; ELF: Enhanced Live Fibrosis; NAFLD: nonalcoholic fatty liver disease; NFS: NAFLD fibrosis score; NR: not reported.

Table 2. Results of Systematic Reviews Assessing the Diagnostic Accuracy of Noninvasive Scoring Systems

Index Test	Studies/Sample Size	Index Test Threshold (low, high)	AUROC (95% CI) Sensitivity (95% CI) Specificity (95% CI)
Mózes (2023)[40]			Fibrosis (stages F0 to F4)
FibroScan	NR (2,518)	-	0.76 (0.70 to 0.83) at 5 years For ≥10.0 kPa (vs <10kPa): 70.6% (62% to 79%); 66.0% (64% to 69%) For ≥20.0 kPa (vs <20kPa): 29.4% (19% to 40%); 92.0% (90% to 93%)
FIB-4	NR (2,275)	-	0.74 (0.64 to 0.82) at 5 years For ≥1.30 (vs <1.3): 82.6% (77% to 88%); 54.5% (52% to 58%) For >2.67 (vs ≤2.67): 41.3% (32% to 51%); 87.7% (86% to 90%)
NFS	NR (2,040)	-	0.70 (0.63 to 0.80) at 5 years For ≥–1.455 (vs <–1.455): 78.9% (72% to 84%); 46.5% (44% to 51%) For >0.676 (vs ≤0.676): 31.6% (22% to 43%); 84.6% (82% to 87%)
Castellana (2021)[37]			Advanced fibrosis (stages F3 to F4)
FIB-4	14 (9,968)	1.3, 2.67	NR 65% (51% to 77%) 93% (89% to 96%)
NFS	14 (9,113)	-1.455, 0.676	NR 61% (45% to 76%) 93% (89% to 96%)
Mózes (2022)[38]			Advanced fibrosis (stages F3 to F4)
FibroScan	NR (5,489)	7.4, 12.1	0.85 (0.84 to 0.86) 84% (81% to 87%) 87% (85% to 88%)
FIB-4	NR (5,393)	0.88, 2.31	0.76 (0.74 to 0.77) 80% (76% to 83%) 79% (77% to 81%)

Index Test	Studies/Sample Size	Index Test Threshold (low, high)	AUROC (95% CI) Sensitivity (95% CI) Specificity (95% CI)
NFS	NR (3,248)	-2.55, 0.28	0.73 (0.71 to 0.75) 74% (70% to 79%) 78% (76% to 81%)
APRI	NR (5,477)	-	0.70 (0.69 to 0.72)[a] NE NE
AST/ALT	NR (5,434)	-	0.64 (0.62 to 0.65)[a] NE NE
Sharma (2021)[39]			Advanced fibrosis
ELF-HCV	11 (NR)	Varied among studies	AUROC range 0.773 (0.697 to 0.848) to 0.98 (0.93 to 1.00)
ELF-HBV	4 (NR)	Varied among studies	AUROC range 0.69 (0.63 to 0.75) to 0.86 (0.81 to 0.92)
ELF-NAFLD	7 (NR)	Varied among studies	AUROC range 0.78 (0.70 to 0.89) to 0.97 (no CI reported)
ELF-ALD	3 (NR)	Varied among studies	AUROC range 0.92 (0.89 to 0.96) to 0.944 (0.836 to 1.000)
ELF-mixed	7 (NR)	Varied among studies	AUROC range 0.63 (no CI reported) to 0.91 (0.88 to 0.95)

ALD: alcoholic liver disease; APRI: AST-to-platelet ratio; AST: aspartate aminotransferase; AUROC: area under the receiver operating characteristic; CI: confidence interval; ELF: Enhanced Liver Fibrosis; FIB-4: fibrosis-4; HBV: hepatitis B virus; HCV: hepatitis C virus; NAFLD: nonalcoholic fatty liver disease; NE: not evaluated; NFS: NAFLD fibrosis score; NR: not reported.
[a]Diagnostic performance not further evaluated after modest performance on AUROC

The APRI requires only the serum level of AST and the number of platelets as part of its calculation.[41] Using an optimized cutoff value derived from a training set and validation set of patients with HCV, authors have reported that the NPV for fibrosis was 86% and that the PPV was 88%. In Crossan (2015), APRI was frequently evaluated and has been tested in HCV, HBV, NAFLD, and ALD.[5] The summary diagnostic accuracies are in Table 3.

Table 3. Diagnostic Accuracy for APRI from Crossan (2015)

Disease	Metavir Fibrosis Stage	Cutoff	Studies	Sensitivity (95% CI)	Specificity (95% CI)
HCV	≥ F2 (significant)	Low: 0.4 to 0.7	47	82% (77% to 86%)	57% (49% to 65%)
HCV	≥ F2 (significant)	High: 1.5	36	39% (32% to 47%)	92% (89% to 95%)
HCV	F4 (cirrhosis)	Low: 0.75 to 1	24	77% (73% to 81%)	78% (74% to 81%)
HCV	F4 (cirrhosis)	High: 2	19	48% (41% to 56%)	94% (91% to 95%)
HBV	≥ F2 (significant)	Low: 0.4 to 0.6	8	80% (68% to 88%)	65% (52% to 77%)
HBV	≥ F2 (significant)	High: 1.5	6	37% (22% to 55%)	93% (85% to 97%)
HBV	F4 (cirrhosis)	Low: 1	4	58% (49% to 66%)	76% (70% to 81%)

Disease	Metavir Fibrosis Stage	Cutoff	Studies	Sensitivity (95% CI)	Specificity (95% CI)
HBV	F4 (cirrhosis)	High: 2	3	24% (8% to 52%)	91% (83% to 96%)
NAFLD	≥ F3 (significant)	0.5 to 1.0	4	40% (7% to 86%)	82% (78% to 60%)
NAFLD	F4 (cirrhosis)	0.54 and NA	2	78% (71% to 99%)	71% (30% to 93%)
ALD	≥ F2 (significant)	Low: 0.5	2	72% (60% to 82%)	46% (33% to 60%)
ALD	≥ F2 (significant)	High: 1.5	2	54% (42% to 66%)	78% (64% to 88%)
ALD	F4 (cirrhosis)	High: 2.0	1	40% (22% to 61%)	62% (41% to 79%)

ALD: alcoholic liver disease; APRI: aspartate aminotransferase–platelet ratio index; CI: confidence interval; FIB-4: fibrosis-4; HBV: hepatitis B virus; HCV: hepatitis C virus; NA: not available; NAFLD: nonalcoholic fatty liver disease.

Giannini (2006) reported that use of the AST/ALT ratio and platelet counts in a diagnostic algorithm would have avoided liver biopsy in 69% of their patients and would have correctly identified the absence/presence of significant fibrosis in 80.5% of these cases.[42] In Crossan (2015), the cutoffs for positivity of AST/ALT ratio for diagnosis of significant fibrosis (stage ≥ F2) varied from 0.6 to 1 in seven studies.[5] Summary sensitivity and specificity were 44% (95% CI 27% to 63%) and 71% (95% CI 62% to 78%), respectively. Thirteen studies used a cutoff of 1 to estimate diagnostic accuracy of cirrhosis with AST/ALT ratio, and summary sensitivity and specificity were 49% (95% CI 39% to 59%) and 87% (95% CI, 75% to 94%), respectively.

Several studies have compared HCV FibroSURE (FibroTest) and other noninvasive tests of fibrosis with biopsy using ROC analysis. For example, Bourliere (2006) reported validation of FibroSURE (FibroTest) and found that, based on ROC analysis, FibroSURE (FibroTest) was superior to APRI for identifying significant fibrosis, with AUROC curves of 0.81 and 0.71, respectively.[43] A prospective multicenter study by Zarksi (2012) compared nine of the best-evaluated blood tests in 436 patients with HCV and found similar performance for HCV FibroSURE (FibroTest), FibroMeter, and HepaScore (ROC curve, 0.84, 0.86, 0.84, respectively).[44] These three tests were significantly superior to the six other tests, with 70% to 73% of patients considered well classified according to a dichotomized score (F0/F1 vs ≥F2). The number of “theoretically avoided liver biopsies” for the diagnosis of significant fibrosis was calculated to be 35.6% for HCV FibroSURE (FibroTest). To improve diagnostic accuracy, algorithms that combine HCV FibroSURE (FibroTest) with other tests (e.g., APRI) are also being evaluated.[44, 45] One of these, the sequential algorithm for fibrosis evaluation (SAFE), combines the APRI and FibroTest. Crossan (2015) reported that the algorithm has been assessed in four studies of HCV for diagnosing both significant fibrosis (stage ≥ F2) and cirrhosis.[5] Summary sensitivity and specificity for significant fibrosis were estimated to be 100% (95% CI 100% to 100%) and 81% (95% CI 80% to 83%), respectively. The summary sensitivity and specificity for cirrhosis were 74% (95% CI 42% to 92%) and 93% (95% CI 91% to 94%), respectively.

Rosenberg (2004) developed a scoring system based on an algorithm combining hyaluronic acid, amino terminal propeptide of type III collagen, and TIMP-1.[46] This test is manufactured by Siemens Healthcare as the Enhanced Liver Fibrosis (ELF) Test.[47] The ELF Test is available in the U.S., however, the test has not been cleared or approved for use by the

FDA.[48] The algorithm was developed in a test set of 400 patients with a wide variety of chronic liver diseases and then validated in another 521 patients. The algorithm was designed to discriminate between no or mild fibrosis and moderate-to-severe fibrosis. The NPV for fibrosis was 92%.

Younossi (2021) evaluated the diagnostic value of ELF to assess liver fibrosis in patients with NAFLD.[49] This was a retrospective, cross-sectional study including 829 patients; 462 had transient elastography data and 463 had liver biopsy data. A significant increase in ELF scores was correlated in patients with advanced fibrosis by biopsy or transient elastography. The AUROC for ELF for identifying fibrosis was 0.81 (95% CI 0.77 to 0.85) with biopsy as the reference standard and 0.79 (95% CI 0.75 to 0.82) with transient elastography as the reference standard. Predictive combinations of ELF and FIB-4 scores were additionally evaluated. For ELF score ≥7.2 with a FIB-4 score ≥0.74, the sensitivity and NPV were 92.5% (95% CI 87.4% to 97.5%) and 95.1% (95% CI 91.8% to 98.4%), respectively, for ruling out fibrosis. For ELF score ≥9.8 with a FIB-4 score ≥2.9, the specificity and PPV were 99.7% (95% CI 99.1% to 100%) and 95.0% (95% CI 85.5% to 100%), respectively, for ruling in fibrosis.

A prospective study by Kumar (2022) compared ELF to transient elastography to evaluate fibrosis severity in 49 patients with chronic hepatitis C.[50] Liver biopsy histopathology was used as the gold standard to determine the severity of liver fibrosis. In this group, the AUROC for significant fibrosis was significantly higher for transient elastography than for ELF (0.64, 95% CI 0.48 to 0.79 vs. 0.85, 95% CI 0.73 to 0.96, respectively, p=0.004). A similar difference was seen for the AUROC for the detection of advanced fibrosis (ELF: 0.77, 95% CI 0.57 to 0.97; transient elastography: 0.98, 95% CI 0.94 to 1.0, p=0.034).

The fibrosis-4 (FIB-4) index was developed in a cohort of patients with HCV and is similar to APRI in that it uses a simple nonproprietary formula to produce a score for the prediction of fibrosis, incorporating patient age, AST level, ALT level, and platelet count. In the original cohort studied by Sterling (2006), a low cut-off score of <1.45 had negative predictive value of 90% for advanced fibrosis whereas a high cut-off score >3.25 had a 97% specificity and positive predictive value of 65% for advanced fibrosis.[51] Overall, 70% of patients were stratified <1.45 or >3.25 and represented potential cases that could have avoided liver biopsy with a corresponding diagnostic accuracy of 86%. In a comparative study by Vallet-Pichard (2007) in patients with HCV utilizing the same cut-off values, a negative predictive value of 94.7% with a sensitivity of 74.3% and a specificity of 80.1% and a positive predictive value of 82.1% with a specificity of 98.2% and sensitivity of 37.6% were reported.[52] When the diagnostic performance of FIB-4 was compared against FibroTest (FibroSURE in the U.S.), the exclusion of severe fibrosis and the detection of severe fibrosis were found to agree between tests in 92.1% and 76.0% of cases, respectively.

Yan (2020) published a cross-sectional study in 667 patients evaluating the diagnostic value of total bile acid-to-cholesterol ratio (TBA/TC) as a serum marker for cirrhosis and fibrosis in chronic HBV-infected patients without cholestasis.[53] In a multivariate analysis, TBA/TC was independently correlated with cirrhosis in the study population (OR 1.102, 95% CI 1.085 to 1.166). Receiver operating characteristic (ROC) curve analyses yielded similar areas under the curve for TBA/TC, APRI, and FIB-4 at 0.87, 0.84, and 0.80, respectively. For diagnosing cirrhosis, the specificity and PPV of TBA/TC (83.33%, 91.10%) were higher than those of APRI (73.61%, 87.20%). The area under the curve of TBA/TC that distinguished significant liver cirrhosis was 2.70. In another multivariate analysis, TBA/TC was also independently correlated with significant fibrosis (OR 1.040, 95% CI 1.001 to 1.078). The area under the curve of

TBA/TC that distinguished significant liver fibrosis was 0.70. Among 32 patients who also had a liver biopsy performed, TBA/TC was significantly higher in both fibrosis and cirrhosis as well as significantly correlated with fibrosis stage (p<0.001 for all). This study was limited by its retrospective, cross-sectional design, as well as the lack of comparison to standard-of-care in determining the impact of the measurement on health outcomes.

Clinical Utility

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs. The primary benefit of the multivariate serum assays is the ability to avoid liver biopsy.

A systematic review and meta-analysis published by Cianci (2022) evaluated the use of noninvasive biomarkers for prediction of all-cause and cardiovascular mortality in patients with NAFLD.[54] Of 24 studies included in the review, noninvasive scoring systems were assessed in 16 studies, four of which had adequate data for meta-analysis based on review criteria that required two or more studies reporting the same outcome measure using equivalent cut-off values and statistical methods in a similar study population. All of of the studies included in the meta-analysis studies were retrospective (total n=9,725), and NAFLD diagnosis was based on liver biopsy or clinical diagnosis. Mean duration of follow-up ranged from 9 to 20 years in three of the studies and was not reported in the fourth study, but the total study duration was 17 years. A total of 1,697 deaths were reported in the four studies. Results of the meta-analyses appear in Table 4. Although high scores were associated with an increased risk of mortality relative to low scores across all scoring systems, the evidence is limited by the small number of included studies and high heterogeneity and imprecision for some estimates.

Table 4. Pooled Diagnostic Accuracy of Noninvasive Scoring Systems for Prediction of All-Cause and Cardiovascular Mortality in Patients with NAFLD

Scoring System	Number of Studies	Comparison (Score Cut-off)	Pooled HR (95% CI)
All-cause mortality			
NFS	4	High (>0.676) vs. Low (< -1.455)	3.07 (1.62 to 5.83; I^2=76%)
NFS	4	Intermediate (-1.455 to 0.676) vs. Low (< -1.455)	1.91 (1.18 to 3.09; I^2=82%)
FIB-4	3	High (>2.67) vs. Low (<1.30)	3.06 (1.54 to 6.07; I^2=73%)
FIB-4	3	Intermediate (1.30 to 2.67) vs. Low (<1.30)	1.60 (1.33 to 1.91; I^2=0%)
APRI	3	High (>1.5) vs. Low (<0.5)	1.90 (1.32 to 2.73; I^2=0%)
APRI	3	Intermediate (0.5 to 1.5) vs. Low (<0.5)	0.98 (0.76 to 1.26; I2=0%)
BARD	2	High (4) vs. Low (0 to 1)	2.87 (1.27 to 6.46; I^2=45%)
BARD	2	Intermediate (2 to 3) vs. Low (0 to 1)	
Cardiovascular mortality			
NFS	2	High (>0.676) vs. Low (< -1.455)	1.64 (1.21 to 2.23; I^2=0%)
NFS	2	Intermediate (-1.455 to 0.676) vs. Low (< -1.455)	2.12 (1.41 to 3.17; I^2=0%)

Adapted from Cianci (2022)[54]
ALT: alanine aminotransferase; APRI: aminotransferase-to-platelet ratio index; AST: aspartate aminotransferase; BARD: body mass index, AST/ALT ratio and diabetes status; CI: confidence interval; FIB-4: fibrosis-4 index; HR: hazard ratio; NAFLD: nonalcoholic fatty liver disease; NFS: NAFLD fibrosis score.

Sanyal (2019) reported on findings of two phase 2b, placebo-controlled trials of simtuzumab for NASH in patients with bridging fibrosis (F3, n=217) or compensated cirrhosis (F4, n=258) that assessed patients with liver biopsy and serum biomarker tests, including ELF, APRI, FibroSURE/FibroTest, and the FIB-4 index.[55] Laboratory screening was conducted at baseline

and every three months during the course of the trials. The trials were terminated after 96 weeks due to simtuzumab inefficacy, at which point data from treatment groups whereas combined for analysis. In patients with bridging fibrosis, increased risk of progression to cirrhosis was observed with higher baseline levels of all serum fibrosis tests ($p<0.001$). Change in the ELF score over time was also associated with progression to cirrhosis ($p<0.001$). For a cut-off score of 9.76, progression to cirrhosis had a reported hazard ratio HR of 4.12 (95% CI 2.14 to 7.93, $p<0.001$). For patients with compensated cirrhosis, higher levels of baseline biomarker tests were also associated with liver-related clinical events in 19% of patients, such as ascites, hepatic encephalopathy, newly diagnosed varices, esophageal variceal bleed, increase in Child-Pugh and/or MELD score, or death ($p<0.001$ to 0.006). Serum fibrosis test results were not directly used in patient management in the simtuzumab trials.

Section Summary: Multianalyte Serum Assays Other Than FibroSURE

For individuals who have chronic liver disease who receive multianalyte serum assays for liver function assessment other than FibroSURE, the evidence includes a number of observational studies and systematic reviews of those studies. Studies have frequently included varying cutoffs, some of which were standardized, and others not validated. Thresholds have often been modified over time, may be specific to certain patient populations, and in some cases, guideline recommendations differ from cutoffs designated by manufacturers and those utilized in studies. A comparison of transient elastography to various serum-based tests found that the former were superior in detecting fibrosis, and a meta-analysis of four studies found higher multianalyte scores associated with an increased risk of mortality relative to lower scores, but the evidence is limited by the small number of included studies and high heterogeneity and imprecision for some estimates. Given these limitations and the imperfect reference standard, it is difficult to interpret performance characteristics. There is no direct evidence that other multianalyte serum assays improve health outcomes; further, it is not possible to construct a chain of evidence for clinical utility due to the lack of sufficient evidence on clinical validity. FIBROSpect II has been studied in populations with HCV. Cutoffs for positivity varied across studies and were not well validated. The methodologic quality of the validation studies was generally poor. There is no direct evidence that FIBROSpect II improves health outcomes.

PRACTICE GUIDELINE SUMMARY

AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGY (AACE) AND AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES (AASLD)

A 2023 updated practice guidance issued by the AASLD included the following guidance statements on the use of noninvasive techniques for diagnosis and management of NAFLD and hepatic steatosis.[56]

- 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
- In patients with pre-DM [diabetes mellitus], T2DM, or 2 or more metabolic risk factors (or imaging evidence of hepatic steatosis), primary risk assessment with FIB-4 should be repeated every 1–2 years
- Although standard ultrasound can detect hepatic steatosis, it is not recommended as a tool to identify hepatic steatosis due to low sensitivity across the NAFLD spectrum

- CAP [controlled attenuation parameter] as a point-of-care technique may be used to identify steatosis. MRI-PDFF [proton density fat fraction] can additionally quantify steatosis

- If FIB-4 is ≥ 1.3, VCTE, MRE, or ELF [Enhanced Liver Fibrosis] may be used to exclude advanced fibrosis

- Improvement in ALT or reduction in liver fat content by imaging in response to an intervention can be used as a surrogate for histological improvement in disease activity

A 2022 joint guideline from the AASLD and the American Association of Clinical Endocrinology (AACE) on the Diagnosis and Management of Non-Alcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings included the following recommendations:[57]

- 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. (Grade B; Intermediate Strength of Evidence; Best evidence level [BEL] 2)
- Clinicians should consider persons belonging to the “high-risk” groups (as defined under R2.1.1) who have an indeterminate or high FIB-4 score for further workup with an LSM (transient elastography) or ELF test, as available. (Grade B; Intermediate Strength of Evidence; BEL 2)
- In persons with T1D [type 1 diabetes], clinicians may consider screening for NAFLD with clinically significant fibrosis (stages F2-F4) using the FIB-4, only if there are risk factors such as obesity, features of metabolic syndrome, elevated plasma aminotransferase levels (>30 U/L), or hepatic steatosis on imaging. (Grade C; Intermediate/Weak Strength of Evidence; BEL 2; downgraded based on the heterogeneity of studies and moderate to high probability of bias)
- Clinicians should further risk stratify persons with T2D [type 2 diabetes], or T1D with cardiometabolic risk factors and/or elevated plasma aminotransferase levels (>30 U/L) using the FIB-4, elastography, and/or ELF test. (Grade B; High/Intermediate Strength of Evidence; BEL 2)

AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES (AASLD), THE AMERICAN COLLEGE OF GASTROENTEROLOGY (ACG), AND THE AMERICAN GASTROENTEROLOGICAL ASSOCIATION (AGA)

The AASLD/ACG/AGA practice guideline on the Diagnosis and Management of Non-Alcoholic Fatty Liver Disease (NAFLD), updated in 2018, delineates when subsequent biopsy is recommended following unsuspected hepatic steatosis detected on imaging (strong and high to moderate recommendations).[58] Regarding non-invasive assessment of steatohepatitis and advanced fibrosis in NAFLD, the guideline stated that “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).” It also cited VCTE and MRE as “clinically useful tools for identifying advanced fibrosis in patients with NAFLD.”

A 2021 consensus-based clinical care pathway was published by the AGA on risk stratification and management of NAFLD, including some recommendations regarding the use of non-invasive testing for individuals with chronic liver disease.[59] Among individuals with increased risk of NAFLD or nonalcoholic steatohepatitis (NASH)-related fibrosis (i.e., individuals with type-2 diabetes, two or more metabolic risk factors, or an incidental finding of hepatic steatosis or elevated aminotransferases), assessment with a nonproprietary fibrosis scoring system

such as FIB-4 is recommended, although aspartate transaminase to platelet ratio index can be used in lieu of FIB-4 scoring. Depending on the fibrosis score, imaging-based testing for liver stiffness may be warranted with transient elastography (FibroScan), although bidimensional shear wave elastography or point shear wave elastography are also imaging options included in the clinical care pathway.

AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES (AASLD) AND THE INFECTIOUS DISEASES SOCIETY OF AMERICA (IDSA)

The AASLD/IDSA Guidance on Hepatitis (updated in 2020) recommends evaluation for advanced fibrosis in those with current (active) HCV infection.[60] They state, "Evaluation for advanced fibrosis using noninvasive tests (serum panels, elastography) 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 (e.g., hepatocellular carcinoma screening)"This recommendation has a rating of Class I: Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation, procedure, or treatment is beneficial, useful, and effective; and Level A: Data derived from multiple randomized clinical trials, meta-analyses, or equivalent.

The guidelines noted that there are several noninvasive tests to stage the degree of fibrosis in patients with HCV. Tests included indirect serum biomarkers, direct serum biomarkers, and VCTE. The guidelines asserted that no single method is recognized to have high accuracy alone and careful interpretation of these tests is required.

DEPARTMENT OF VETERANS AFFAIRS (VA) AND THE NATIONAL VIRAL HEPATITIS PROGRAM

The VA and National Viral Hepatitis Program Treatment Considerations for Chronic Hepatitis C Virus Infection (updated 2021) state, “routine blood tests can assist in identifying patients with advanced liver disease and, in some instances, predict the likelihood of decompensation or HCC. Serum markers of fibrosis (e.g., APRI, FIB-4, FibroSURE®) are quite good at diagnosing cirrhosis.”[61] This recommendation is based on observations that, “APRI and FIB-4 scores are easily calculated using standard clinical labs,” and that APRI and FIB-4 values have been associated with disease stage.

SUMMARY

There is not enough research to know if multianalyte assays with algorithmic analyses improve health outcomes for people with chronic liver disease. Therefore, the use of multianalyte assays to evaluate or monitor people with chronic liver disease is considered investigational.

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CODES

Codes	Number	Description
CPT	0002M	Liver disease, ten biochemical assays (ALT, A2-macroglobulin, apolipoprotein

Codes	Number	Description
		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)
	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 non-alcoholic steatohepatitis (NASH)
	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
	0166U	Liver disease, 10 biochemical assays (α2macroglobulin, 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
	0468U	Hepatology (nonalcoholic steatohepatitis [NASH]), miR-34a5p, alpha 2-macroglobulin, YKL40, HbA1c, serum and whole blood, algorithm reported as a single score for NASH activity and fibrosis
	81517	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
	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
	81599	Multianalyte assay with algorithmic analysis
	83520	Immunoassay, analyte, quantitative; not otherwise specified] tissue inhibitor of metalloproteinase (TIMP-1)
	83883	Alpha-2 macroglobulin– Nephelometry, each analyte not elsewhere specified
	84999	Unlisted chemistry procedure
HCPCS	None	

Date of Origin: *June 2013*