

Noninvasive Measures of Liver Fibrosis and Severity of Liver Disease

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Abstract: Determining the degree of fibrosis is an important step in the assessment of disease severity in patients with chronic liver disease. Liver biopsy has been the gold standard for estimating the extent of inflammation and fibrosis, although the procedure has limitations such as sampling error and variability. Noninvasive testing has been shown to be equally predictive in ruling out fibrosis or ruling in advanced fibrosis. Serum biomarkers and imaging-based tests have more limited predictive ability when classifying intermediate stages, but these tools can help identify which patients should receive antiviral treatment sooner and require ongoing cancer surveillance without the need for biopsy. Using a combination of serum markers and imaging tests may also be helpful in providing functional assessment of portal hypertension in patients with chronic liver disease.

Portal hypertension is the most serious of the consequences of chronic liver disease and the result of progressive liver fibrogenesis. It can lead to the development of esophageal varices, ascites, and encephalopathy, and carries a significant increase in mortality.¹ Advanced fibrosis is a risk factor for hepatocellular carcinoma (HCC) regardless of successful treatment of the underlying etiology.² Staging the severity of liver disease is important in stratifying which patients should be prioritized for treatment, as well as assessing the risk for HCC and subsequent need for long-term cancer screening.³

Fibrosis is a structural change that occurs in the liver secondary to chronic injury, notably progressive intrahepatic vascular remodeling with capillarization of sinusoids, fibrogenesis, neoangiogenesis, and development of intrahepatic shunts that lead to increased hepatic resistance. This eventually produces an increase in portal pressures and a decrease in effective hepatocyte perfusion.⁴ The resulting portal hypertension is also affected by the dynamic component of an increase in vasoconstriction and portal blood flow related to splanchnic arteriolar vasodilation.

The gold standard for the assessment of the degree of liver fibrosis is liver biopsy. There is a small risk for procedure-related

Keywords

Fibrosis, noninvasive, imaging, liver stiffness, diagnostic test, prognosis

Table 1. Noninvasive Markers of Fibrogenic Activity

Tests to Measure Liver Stiffness
<ul style="list-style-type: none"> • Transient elastography • Shear wave elastography • Acoustic radiation force impulse imaging • Magnetic resonance elastography
Magnetic Resonance– or Positron Emission Tomography–Based Technologies
<ul style="list-style-type: none"> • Liver inflammation score • Proton density fat fraction • Collagen or lysyl oxidase content • Receptor binding by positron emission tomography ligands
Serum Tests
<ul style="list-style-type: none"> • Fibrogenic panels that include extracellular matrix molecules • Other serum marker panels <ul style="list-style-type: none"> • FibroTest • FIBROSpect II • Fibrosis-4 test • Enhanced Liver Fibrosis test • Aspartate aminotransferase–to–platelet ratio index • HepaScore • FibroIndex • FibroMeter • Lipidomic profiles or markers
Functional Tests
<ul style="list-style-type: none"> • Cholate clearance test • ¹³C-methacetin breath test • Indocyanine green clearance test • Galactose elimination capacity test • Collagen synthesis quantification measuring ¹³C-labeled turnover
Clinical Scores
<ul style="list-style-type: none"> • Lok index • Forns index • Model For End-Stage Liver Disease score • Child-Pugh score • Maddrey discriminant function • Lille score

Adapted from Trautwein C et al.⁶⁷

complications, such as bleeding or pain.⁵ Inaccurate staging from sampling error can occur in up to 25% of cases with inter- and intraobserver variability in biopsy interpretation,⁶ and liver biopsy can be limited when distinguishing between fibrosis stages F1 and F2.^{7,8} There are various noninvasive modalities for assessing liver fibrosis, including serum tests, imaging-based modalities, and liver stiffness measurements (Table 1). The gold standard for assessing portal hypertension is the measurement of hepatic venous pressure gradients (HVPG), although this is an invasive procedure and is not routinely performed

at all medical centers. Although imaging can identify the presence of varices,⁹ upper endoscopy is the gold standard to assess patients for, and potentially treat, esophageal varices, which develop in the setting of clinically significant portal hypertension.¹⁰

Serum Testing

Platelet count, bilirubin, soluble CD163, aspartate aminotransferase–to–platelet ratio index (APRI), FibroTest (BioPredictive; known as FibroSure [LabCorp] in the United States), Forns index, Lok index, and FibroIndex are all laboratory-based measures that have been described in the assessment of fibrosis.

FibroTest is a score utilizing total bilirubin, haptoglobin, gamma-glutamyl transferase, α 2-macroglobulin, and apolipoprotein A. It is widely available for use and is low in cost, although it is nonspecific for the liver and less accurate for intermediate stages.¹¹ False-positive results can occur from hemolysis, Gilbert syndrome, cholestasis, and inflammation related to increases in α 2-macroglobulin and haptoglobin. In a meta-analysis of 16 studies of fibrosis and 13 studies of cirrhosis in patients with chronic hepatitis B virus (HBV) infection, FibroTest was found to have high specificity for cirrhosis (91%), but its value was suboptimal for fibrosis.¹²

Soluble CD163 is a marker for macrophage activation and is associated with the severity of liver cirrhosis. In a review of 186 patients with chronic HBV infection, a soluble CD163 level greater than 1961 ng/L had high specificity in identifying greater than F2 fibrosis.¹³ A recent study from Korea utilized the enhanced liver fibrosis (ELF) test, which combines several biochemical parameters involved in the synthesis and breakdown of extracellular matrix: hyaluronic acid, N-terminal propeptide of collagen type III, and tissue inhibitor of metalloproteinase-1.¹⁴ When patients were stratified according to their ELF score, those with higher scores were predicted to have liver-related decompensation.

Combination testing has shown promise as a prognostic tool. In a single-center study, APRI and ultrasound were found to have a positive predictive value of 80% when identifying patients with cirrhosis.¹⁵ Newer tests, including FibroMeter (Echosens) and CirrhoMeter, have been used in combination to predict significant liver-related events better than fibrosis scoring from liver biopsy.¹⁶

Transient Elastography

Transient elastography (eg, FibroScan [Echosens]) utilizes a transducer probe, which emits low-frequency (50 Hz) vibrations into the liver to measure stiffness.¹⁷ A propagating shear wave induced by vibrations is detected by

pulse-echo acquisition, and the velocity of the wave is calculated. Liver stiffness, which is expressed in kilopascals (kPa), is proportional to shear wave velocity. The stiffer the liver, the faster the shear wave propagates. This form of imaging is more representative of the hepatic parenchyma, as it evaluates a larger area compared with a single liver biopsy. The reliability of the test is dependent on the interquartile range, which dictates the variability of the validated measures and should be less than 30% of the median value. The success rate is dictated by the ratio of successful measurements to the total number, and should be more than 60%.¹⁸

Transient elastography is painless and rapid, and can be performed in the outpatient setting. The sensitivity and specificity of the procedure are up to 90% for patients with cirrhosis.¹⁹ The cutoff values for patients with hepatitis C virus (HCV) infection and cirrhosis range from 11 to 17 kPa.⁶ The sensitivity and specificity are approximately 70% to 80% for F2 to F4 fibrosis.^{20,21} Diagnostic accuracy is similar in patients with advanced-stage nonalcoholic fatty liver disease (NAFLD), with an area under the receiver operating characteristic (AUROC) curve of 0.94, sensitivity of 94%, and specificity of 95%.²² In patients with autoimmune liver diseases, transient elastography is very sensitive and specific for predicting advanced fibrosis in patients with primary biliary cholangitis and primary sclerosing cholangitis,²³ although this tool is less reliable than in autoimmune hepatitis due to significant hepatic inflammation that can overestimate stiffness.²⁴

A recent Cochrane review examined transient elastography in 834 alcoholic liver disease patients from 5 retrospective studies and 9 prospective studies.²⁵ The authors concluded that transient elastography can be used when ruling out cirrhosis and may be helpful when ruling out severe fibrosis, although a liver biopsy can be obtained if there is uncertainty in staging.

There have been several meta-analyses of transient elastography testing, with a summary AUROC curve for diagnosing cirrhosis ranging from 0.90 to 0.95.^{20,21,26} A meta-analysis of 40 studies of patients with chronic liver disease found a sensitivity of 83% and specificity of 89% for cirrhosis; however, for stage 2 fibrosis, the sensitivity was only 79% and specificity was 78%.²⁷

Combination Testing

Transient elastography in combination with FibroTest was shown to obviate the need for liver biopsy in the evaluation of significant fibrosis in 72% vs 48% of patients who underwent solely serologic testing for fibrosis.²⁸ A multicenter study evaluated the combination of transient elastography with a different serum test, FibroMeter. In this retrospective review of 1785 patients with HCV infection, the combination eliminated the need for liver biopsy.²⁹

Transient elastography has been used with left lobe liver surface ultrasound in patients with suspected cirrhosis.³⁰ In a study of 90 patients, the combination of the 2 imaging modalities led to a positive likelihood ratio (LR) of 9.15 and a negative LR of 0.06 for predicting cirrhosis.

Assessment of the Severity of Cirrhosis/Portal Hypertension

Previous studies have demonstrated that transient elastography did not correlate well with HVPG measurements greater than 12 mm Hg.³¹ In contrast, a small study by Robic and colleagues revealed that the measurement of less than 21.1 kPa led to a 100% negative predictive value for the occurrence of portal hypertension–related complications.³² In this prospective study of 41 patients who underwent simultaneous HVPG and liver stiffness measurement, the AUROC curve was 0.830 for HVPG and 0.845 for liver stiffness in predicting the liver disease–related complications of bleeding, ascites, encephalopathy, HCC, sepsis, need for liver transplantation, or death.

A prospective study of 250 patients with chronic liver disease in an outpatient clinic in Barcelona, Spain utilized elastography, platelet count, and ultrasound to evaluate patients for nodularity and splenomegaly.¹⁰ Patients who had liver stiffness measurements greater than 13.6 kPa were divided into different risk groups based on platelet count and abdominal imaging. There were no varices in the low-risk group, and there was only 1 patient with varices in the intermediate-risk group; however, 90% of patients in the high-risk group had varices, suggesting that a combination approach may be helpful when assessing patients for severity of liver disease (Figure 1).

Lastly, a meta-analysis of 18 studies evaluating transient elastography in 3644 patients revealed a sensitivity of 90% and specificity of 79% for clinically significant portal hypertension, with an AUROC curve of 0.93.¹⁹ Specificity was below 60% for the identification of esophageal varices, although there was a 100% negative predictive value if the kPa value was less than 21.

Discordance

There are limitations to transient elastography, as this procedure is more difficult to perform in patients with obesity, ascites, and narrow intercostal spaces. A Canadian study prospectively evaluated patients undergoing liver biopsy and concomitantly performed liver stiffness measurements.³³ Of the 251 patients studied, 14% had discordance in biopsy and elastography results. Multivariate analysis revealed that mild fibrosis, higher body mass index (BMI), alanine aminotransferase elevation, and variability in liver stiffness measurement were independently associated with discordance. A transient elastography XL study performed to assess for discordance demonstrated that 10% of cases

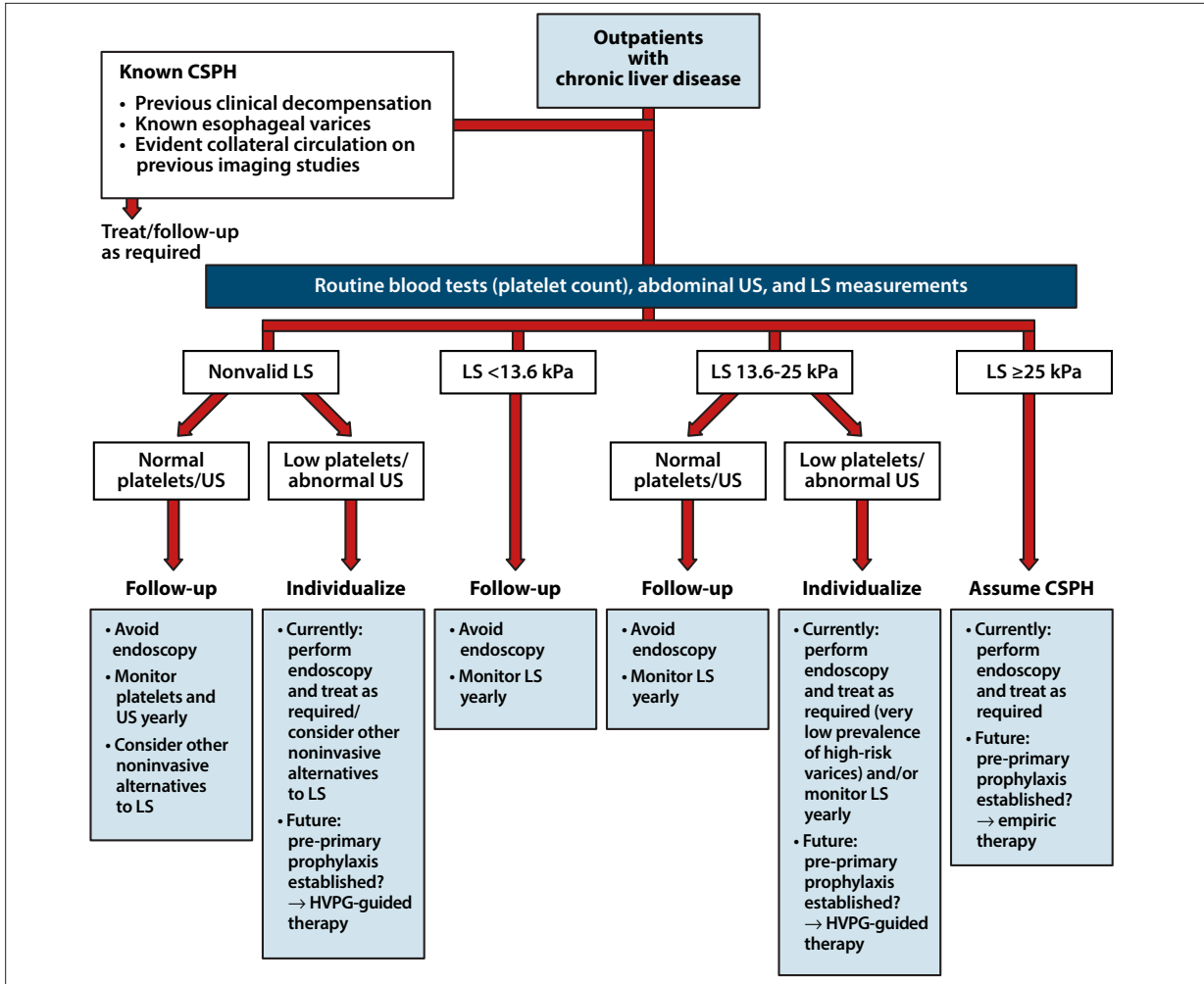


Figure 1. An approach to assessing patients for portal hypertension.

CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient; kPa, kilopascals; LS, liver stiffness; US, ultrasound.

Adapted from Augustin S et al.¹⁰

were discordant with liver biopsy and that a BMI greater than 40 led to a 4- to 5-fold increase in discordance.³⁴ In a review of 13,369 cases, liver stiffness measurements were unreliable in 1 of 5 cases due to obesity, increased waist circumference, and limited operator experience.³⁵ Incorrect probe size was also found to be a predictor of unsuccessful evaluation in a study of 2000 patients in Mexico.³⁶

Inflammation can also influence measurements in patients with viral hepatitis, as it can overestimate liver stiffness values.^{37,38} In a German study, 6 patients experienced complete reversal of abnormal stiffness scores after inflammation resolved.³⁹ However, a Belgian study of 135 patients who underwent transient elastography demonstrated that exclusion of patients with elevated transaminase levels lowered the cutoff for F4 disease marginally, from 18 to 14 kPa.⁴⁰ Liver stiffness values have also been shown to increase 25% after a light meal, and

it is recommended that patients fast for at least 4 hours prior to transient elastography.⁴¹

Ultrasound-Based Elastography

Ultrasound techniques for measuring stiffness are measured via strain displacement or shear wave imaging and quantification. In the first method, real-time tissue elastography (RTE) measures the relative stiffness of the tissue in the region of interest via the automatic displacement of the liver parenchyma induced by a heartbeat using a combined autocorrelation method.⁴² In a prospective study of 747 patients with HBV infection, the use of RTE resulted in a liver fibrosis score with a diagnostic efficiency similar to that of the liver fibrosis score for chronic HCV patients.⁴³ On the other hand, 2-dimensional shear wave elastography (Aixplorer, Supersonic Imagine) utilizes

Table 2. Comparison of Liver Fibrosis Measurements

	Advantages	Disadvantages
Serum Biomarkers	<ul style="list-style-type: none"> • Can be performed in an outpatient clinic • Reproducible • Well validated 	<ul style="list-style-type: none"> • Nonspecific to the liver • Poor at discriminating between intermediate stages of fibrosis • Affected by inflammation, hemolysis, Gilbert syndrome
Transient Elastography	<ul style="list-style-type: none"> • Can be performed in an outpatient clinic • Reproducible • Well validated 	<ul style="list-style-type: none"> • Not readily available unless the provider already has the equipment • Limited in patients with obesity, ascites, inflammation • Poor at discriminating between intermediate stages of fibrosis
Acoustic Radiation Force Impulse Imaging	<ul style="list-style-type: none"> • Can be implemented on a standard ultrasound machine • Examined area is smaller than that needed with transient elastography • Similar sensitivity/specificity to transient elastography 	<ul style="list-style-type: none"> • Stiffness values measured in m/s vs kPa • Poor at discriminating between intermediate stages of fibrosis
Magnetic Resonance Elastography	<ul style="list-style-type: none"> • Can be implemented on a standard magnetic resonance imaging machine • Evaluates entire liver • Similar sensitivity/specificity to transient elastography 	<ul style="list-style-type: none"> • Requires magnetic resonance imaging capabilities • Costly • Limited in cases of iron overload

the combination of a tissue-induced radiation force and focused ultrasonic beams to produce images of the transient propagation of shear waves in real time.^{44,45} This form of elastography can be implemented on a standard ultrasound machine and has been found to have accuracy similar to that of transient elastography.^{46,47}

Acoustic Radiation Force Impulse Imaging

Acoustic radiation force impulse (ARFI) imaging utilizes mechanical excitation of tissue with short-duration acoustic pulses that propagate shear waves and generate localized displacements in tissue.⁴⁸ Its sensitivity and specificity are greater than 90% for cirrhosis, but approximately 85% for stage F2 to F4 fibrosis, and its performance is likely similar to that of transient elastography. The benefits of this imaging modality are that it can be used with a standard ultrasound machine and overcomes the limitations of ascites and obesity seen with transient elastography. ARFI imaging's region of evaluation (10 mm × 6 mm) is smaller than that of transient elastography (1 cm × 4 cm), and a recent study showed superiority of ARFI imaging in comparison to transient elastography⁴⁹ (Table 2).

In a study of 172 patients with NAFLD, ARFI imaging was able to distinguish between low (F0-2) and high (F3-4) stages of fibrosis with a sensitivity and specificity of 90% and an AUROC curve of 0.90; in addition, a BMI greater than 40 was not a limiting factor.⁵⁰ A meta-analysis of 518 patients supported the diagnostic accuracy of ARFI

imaging, with AUROC curves of 0.87 for significant fibrosis, 0.91 for severe fibrosis, and 0.93 for cirrhosis.⁵¹ A single-center study from Taiwan demonstrated that ARFI imaging in combination with a spleen diameter to platelet ratio score can predict who would benefit from endoscopic screening vs those who do not need surveillance.⁵²

Magnetic Resonance Imaging

Magnetic resonance elastography utilizes a modified phase-contrast imaging sequence to detect propagating shear waves within the liver via a pneumatic driver placed on the upper abdomen. Liver stiffness measurements are obtained from wave displacement patterns via color-coded images (Figure 2). Calculation of elasticity is similar to that of transient elastography.⁵³ The sensitivity and specificity are greater than 90% for cirrhosis, but 85% for F2 to F4 fibrosis. Performance has been shown to be higher than with transient elastography for significant fibrosis. Benefits of this imaging modality include the ability to implement it on a standard magnetic resonance imaging machine to examine the entire liver, but the process can be time-consuming and costly. Further validation is warranted, and testing is not applicable in cases of iron overload. Initial studies in a swine model revealed a positive correlation between liver stiffness score and an increase in HVP measurements.⁵⁴

Diffusion-weighted imaging has been applied to assess for liver stiffness and evaluate patients with chronic

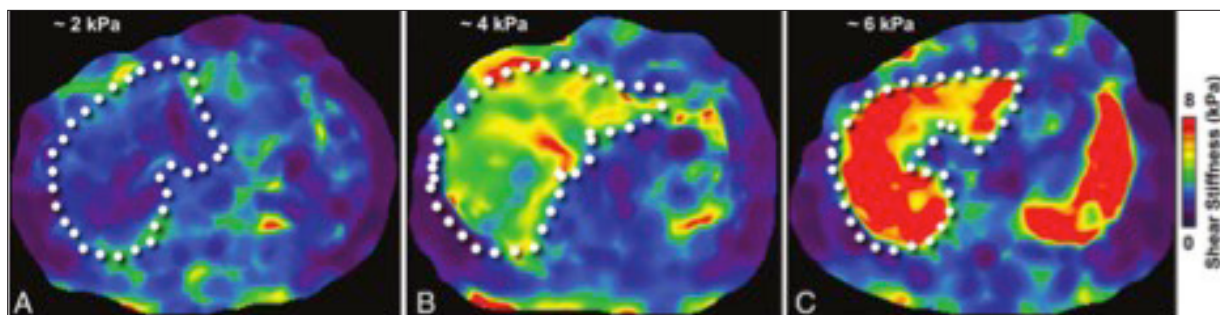


Figure 2. Magnetic resonance elastography from 3 patients. **A** is from a 63-year-old woman with a history of autoimmune hepatitis but no evidence of fibrosis, with a normal stiffness value of 2 kPa. **B** is from a 52-year-old woman with chronic cholestatic hepatitis and significant hepatic fibrosis, with a stiffness value of 4 kPa. **C** is from a 46-year-old man with chronic hepatitis C virus and advanced hepatic fibrosis, with a stiffness value of 6 kPa.

kPa, kilopascals.

Adapted from Barr RG et al.⁶⁶

viral hepatitis.⁵⁵ Magnetic resonance imaging can also integrate inherent cardiac motion,⁵⁶ where heart-induced shear wave velocity and subsequent stiffness can be measured inside the liver. Texture-based classification of liver fibrosis using magnetic resonance imaging is another option.⁵⁷ In this study, 49 patients with biopsy-confirmed fibrosis were scanned with a T2-weighted, high-resolution, spin-echo sequence with Haralick texture features. The AUROC curve was 0.81 for separating mild from severe fibrosis, and the AUROC curve was 0.91 when adding age and liver fat into the model.

Spleen Stiffness and Size

Splenomegaly is seen in approximately 65% of patients with cirrhosis, mainly related to congestion from portal hypertension, but also from an increase in splanchnic inflow.⁵⁸ Spleen stiffness can be measured through transient elastography and may correlate with the presence and severity of varices, as first described by Colecchia and colleagues in a study of 100 patients with chronic HCV cirrhosis.¹⁷ In a meta-analysis of 9 studies that evaluated spleen stiffness measurement and diagnostic upper endoscopy, the pooled sensitivity was 81%, specificity was 66%, positive LR was 2.5, and negative LR was 0.2.⁵⁹ There was heterogeneity among the studies due to differences in technique, and there was risk for spectrum, review, and disease progression bias.

ARFI can also evaluate patients for spleen stiffness by identifying shear wave velocity through ultrasound. In one study, a spleen stiffness cutoff value of 3.18 m/s identified patients with varices with a 98.4% negative predictive value, 98.5% sensitivity, 75% accuracy, and 0.025 negative LR, although testing could not be measured in 16 patients (4.5%) due to poor visualization of the spleen related to obesity and interference with bowel gas.⁶⁰

Spleen diameter has been assessed with platelet count for the detection of portal hypertension. In a meta-analysis of 3063 patients from 20 studies,⁶¹ the hierarchical summary receiver operating characteristic curve was 0.95 for a platelet count/spleen diameter ratio (PSR) cutoff of 909. If the PSR was less than 909, the posttest probability was 87% for the presence of esophageal varices. If the PSR was greater than 909, the posttest probability for varices was only 9%.

The combination of liver and spleen stiffness has also demonstrated a correlation with significant portal hypertension.^{62,63} In an Italian study that evaluated both spleen and liver stiffness through transient elastography, only 110 of 132 patients had reliable liver and spleen test results, but the combination of both parameters led to 93% sensitivity for cirrhosis and 91% sensitivity for esophageal varices.⁶⁴

Transient elastography has also been combined with platelet count and spleen size.⁶⁵ In 117 patients with cirrhosis, the AUROC curve of transient elastography was 0.883 for clinically significant portal hypertension. When combined with platelet count and spleen size, the AUROC curve was 0.918.

Conclusion

Liver biopsy remains the gold standard for the detection of fibrosis and cirrhosis. It is an imperfect measure of fibrosis and cannot quantify the degree of portal hypertension without concomitant hepatic venous pressure measurements. Noninvasive measures such as transient elastography have similar sensitivity and specificity in predicting mild vs advanced liver disease. These measures are poor at distinguishing between intermediate stages, which may be less important in the era of direct-acting antiviral agents for HCV infection.

The current focus should be on the safest and most accurate method for distinguishing between minimal or no fibrosis and cirrhosis to aid in prioritizing treatments and identifying those who require long-term screening for HCC. Once cirrhosis is established, there are several promising imaging modalities that can accurately predict portal hypertension in combination with platelet count.

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