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Noninvasive Tools to Assess Hepatic Fibrosis: Ready for Prime Time?

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Introduction

Often regarded as the gold standard for fibrosis assessment, liver biopsy does carry associated risks given its invasive nature. Moreover, liver biopsy is not a true gold standard due to inter/intra-observer variability and the small amount of tissue that is typically obtained with this procedure. Advances in the development of serologic tests and conventional imaging techniques have been shown to reduce the need for liver biopsy for diagnosing hepatic fibrosis. More commonly, it is a tool that is now reserved for evaluating indeterminate noninvasive tests or excluding features of particular diseases (e.g. autoimmune hepatitis, steatohepatitis).

The noninvasive assessment of hepatic fibrosis has been a popular topic of discussion over the past decade. Ideal properties of a noninvasive test include widespread availability, ease of use, cost-efficiency, reproducibility, and ability to detect changes in fibrosis over time. Furthermore, the ability of noninvasive testing to identify intermediate to advanced histological stages of fibrosis ($\geq$ stage F2) without liver biopsy is important as this is the usual threshold to start treatment in eligible subjects with chronic hepatitis C, for example. In addition, the identification of early-stage cirrhosis by noninvasive testing allows for the timely implementation of disease management strategies (i.e. HCC and variceal screening) to reduce the likelihood of complications.

This article will review salient aspects of hepatic fibrogenesis as well as the diagnostic performance of serum markers and imaging techniques that are currently available for

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detecting hepatic fibrosis. Finally, we will provide suggestions as to how these noninvasive methods can be incorporated into routine clinical practice.

Pathogenesis of Hepatic Fibrosis

The hepatic stellate cell is thought to play an integral role in hepatic fibrosis. When in a quiescent state, stellate cells serve as storage reservoirs for retinol (a precursor of vitamin A) and other lipid soluble substances. Moreover, they control extracellular matrix turnover and regulate sinusoidal blood flow.¹ Additional fibrogenic cells involved with hepatic fibrogenesis are derived from portal fibroblasts, circulating fibrocytes, bone marrow, and epithelial-mesenchymal cell transition. The proportion of fibrogenic cells from these various sources likely depends on the etiology of liver disease. For example, stellate cells are mainly involved when the damage is centered within the hepatic lobule. On the other hand, portal fibroblasts are observed to contribute more in cholestatic liver disease and ischemia.²

A variety of mediators have been shown to promote ongoing stellate cell activation and fibrogenesis, with platelet-derived growth factor and transforming growth factor beta described as two of the major cytokines involved with this process.³ Stellate cell activation occurs through several additional pathways as well. Oxidative stress in the form of reactive oxygen species can activate stellate cells. This pathway may be of particular relevance in alcoholic liver injury, non-alcoholic fatty liver disease, and iron overload syndromes. Parenchymal cell apoptotic bodies can induce an inflammatory response that activates stellate cells as well. Bacterial lipopolysaccharide can elicit a fibrogenic response by binding to stellate cells via Toll-like receptor 4. Lastly, paracrine stimuli from adjacent cell types (macrophages, sinusoidal endothelium, and hepatocytes) can also aid in the transformation of stellate cells from a quiescent to activated state.⁴

Once activated, stellate cells and fibroblasts transform into myofibroblasts which are cells that contain contractile filaments. Myofibroblasts have the capacity to alter the composition of the extracellular matrix (ECM). Progressive changes in the ECM include a change from type IV collagen, heparan sulfate proteoglycan, and laminin to types I and III collagen. Subsequently, ECM accumulates due to its increased synthesis and decreased degradation.⁵ Fibrogenesis is further propagated by positive feedback mechanisms that arise from changes in ECM composition. Changes in membrane receptors (e.g. integrins), activation of cellular matrix metalloproteases (MMPs), and increasing matrix stiffness all serve as stimuli to perpetuate stellate cell activation.⁶ It is this dynamic production and turnover of matrix, as well as increases in matrix stiffness, that form the basis for clinical techniques to noninvasively assess the extent of hepatic fibrosis.

Clinical Implication of Hepatic Fibrosis

The accurate detection of hepatic fibrosis stage has important clinical decision-making implications for the management of chronic liver disease. For example, the presence of clinically significant hepatic fibrosis (defined as histological stage 2 or greater) influences the timing for antiviral treatment for patients with chronic hepatitis B and C. In patients with non-alcoholic fatty liver disease (NAFLD), the presence of fibrosis overall is suggestive of non-alcoholic steatohepatitis (NASH) and thereby identifies a subset of patients who require closer monitoring and follow-up. Perhaps most importantly, the presence of early-stage or compensated cirrhosis necessitates interval screening for disease-related complications including esophageal varices and hepatocellular carcinoma.

Serial measurements to evaluate for progression or regression of hepatic fibrosis also have clinical utility. The successful medical treatment of various chronic liver diseases including viral hepatitis, autoimmune hepatitis, and primary biliary cirrhosis is often associated with

histological regression of fibrosis in addition to clinical and biochemical improvement. Conversely, monitoring for disease progression will allow clinicians to implement treatment in eligible individuals to maximize the probability for a complete response.

Liver Biopsy

The introduction of a liver biopsy technique proposed by Menghini changed the field of hepatology in the 1960s.⁷ Subsequently, liver biopsy has been considered the gold standard for detecting hepatic fibrosis. In recent times, the indications for liver biopsy have shifted from diagnosis of chronic liver disease to staging and monitoring for disease progression due to improvements in serum laboratory testing,

Despite its widespread availability and use, there has been an increasing focus on the disadvantages of liver biopsy which call its value into question. As an invasive procedure, procedure-related complications such as pain are reported in an estimated 20% of patients while major complications including bleeding, hospitalization, and death are < 0.5%, respectively.⁸ The risk for complications increases further among patients with advanced liver disease manifested by thrombocytopenia, coagulopathy, or ascites. For these reasons, patients are becoming more reluctant to have repeated liver biopsies performed to assess disease progression as noninvasive test modalities become available.

Sampling error remains a major limitation for liver biopsy, and is difficult to overcome as only 1/50,000 of the liver is analyzed. Even when the specimen size is adequate (i.e. 25 mm in length), the probability for underestimating fibrosis stage remains as high as 25%.⁹ It is important to be aware of this concept when determining the accuracy of liver biopsy. Many studies that evaluate the diagnostic accuracy of noninvasive measurements of fibrosis utilize the area under the receiver operating characteristic curve (AUROC) to gauge performance. Unfortunately, even in the best case scenario, an AUROC > 0.90 cannot be achieved for the perfect marker because liver biopsy cannot achieve 100% accuracy based on inherent features of the technique.¹⁰

A variety of histologic scoring systems have been used to stage hepatic fibrosis. Some systems have 5 stages (0–4) while others (e.g. Ishak fibrosis score) have 7 stages (0–6). While systems that include additional stages convey more information, the likelihood of having excellent reproducibility declines. The fibrosis stages identified by classification systems are determined by both the quantity and location of the fibrosis. In other words, the numbered stages do not reflect equal units of severity but represent categories describing the histological changes. This semi-quantitative nature of histologic scoring systems is also an important consideration when comparing liver biopsy to noninvasive measures which provide a continuous quantitative assessment of liver fibrosis.¹¹

Serum Fibrosis Markers

Indirect Serum Fibrosis Markers—Significant attention has been paid to the development of serum fibrosis markers over the past two decades. (Table 1) Routine serum biochemical tests to assess hepatic fibrosis were first examined given their wide availability. One simple model proposed that an aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio of > 1 is indicative of cirrhosis. However, the sensitivity (53%) and negative predictive value (81%) for detecting cirrhosis is not robust enough for use in clinical practice.¹² The most commonly studied indirect serum marker test using widely available variables is the AST to platelet count ratio index (APRI). The APRI is calculated as $\text{AST (U/L)} / \text{upper limit of normal} \times 100 / \text{platelet count (10}^9\text{/L)}$.¹³ The performance characteristics of the APRI depend on the cutoff value used (i.e. < 0.5 to > 2.0).

Many studies have been conducted to externally validate initial results, but they have been conflicting, in part, due to differences in study methodology.

From a systematic review of 22 studies that examined the APRI in patients with chronic hepatitis C, the average prevalence of clinically significant fibrosis (stages F2-F4) was 47%. With an APRI cutoff set at 0.5¹³ for detecting clinically significant hepatic fibrosis (stages 2–4), the summary sensitivity and specificity values were 81% and 50%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) of the 0.5 cutoff were 59% and 75%, respectively. At a higher cutoff of 1.5, the sensitivity and specificity were 35% and 91%, respectively. The 1.5 threshold led to a tradeoff between PPV (77%) and NPV (61%). With regards to cirrhosis, twelve studies were examined using thresholds of 1.0 and 2.0. The NPVs were excellent at 91% and 94%, respectively, for each threshold value. However, the PPVs for these cutoffs were not high enough to allow one to “rule in” cirrhosis with high accuracy. Thus, the use of APRI in different populations appears to have variable performance in detecting clinically significant hepatic fibrosis in patients with chronic hepatitis C.¹⁴ Fewer studies have been performed using APRI in other chronic liver diseases, but are expected in the future.

FibroTest (Biopredictive, Paris, France) is a proprietary panel developed in 2001 that combines several indirect serum fibrosis markers including α 2- macroglobulin, haptoglobin, GGT, apolipoprotein A₁, and total bilirubin. Values of FibroTest range from zero to one with higher values indicating a greater probability of significant fibrosis. Serum α 2- macroglobulin is an acute-phase protein that is present at sites of inflammation. Haptoglobin is negatively associated with fibrosis because its synthesis is decreased by hepatocyte growth factor. GGT production could be caused by early cholestasis or an increase in epidermal growth factor.¹⁵ The initial work by Imbert-Bismut et al. identified these five variables as the most informative markers for staging fibrosis in patients with chronic hepatitis C. Subsequently, a meta-analysis of 16 publications demonstrated that a Fibrotest diagnostic cutoff value of 0.31 was associated with a NPV of 91% for excluding significant fibrosis.¹⁶ In the United States, a version of the Fibrotest assay called FibroSURE (Laboratory Corporation of America, Raritan, NJ, USA) is available for use. FibroSURE contains the same markers as Fibrotest in addition to ALT, patient age, and gender. The FibroSURE assay is mainly used for patients with chronic hepatitis C.

While FibroTest was initially validated in patients with chronic hepatitis C, further studies showed that it is an effective alternative to liver biopsy in populations with chronic hepatitis B, alcoholic liver disease, and nonalcoholic fatty liver disease. With regards to chronic hepatitis B, Sebastiani examined 110 consecutive patients who underwent assessment using multiple noninvasive methods including FibroTest and APRI. With a prevalence of 68% for significant fibrosis, FibroTest had a sensitivity of 81% and a specificity of 90%. However, the NPV was only 64% which severely limits its utility in clinical practice for these patients. The authors concluded that noninvasive serum markers alone may not be good enough to obviate the need for liver biopsy in staging chronic hepatitis B.¹⁷

Given the epidemic of obesity and the number of patients at risk for NAFLD in developed countries, one study evaluated FibroTest in 170 patients from a secondary care center and 97 from multiple centers who were at risk for NAFLD. The authors found that FibroTest reliably detected advanced fibrosis (F2-F4) in patients with NAFLD as the AUROC ranged from 0.75–0.86 in the two study groups.¹⁸

False positive results using FibroTest can occur when elevations in total bilirubin due to hemolysis, Gilbert’s syndrome, or other causes of cholestasis are present. Furthermore, test results can be impacted by acute hepatic and/or systemic inflammation, which leads to

spurious increases in serum α 2-macroglobulin and haptoglobin levels. These factors need to be accounted for when deciding to order this noninvasive test modality.

Other indirect serum fibrosis tests which have been studied include the Forns index which incorporates age, GGT, cholesterol, and platelets.¹⁹ This model has mainly been utilized in patients with chronic hepatitis C. Similarly, the Hepascore combines laboratory (bilirubin, GGT, hyaluronic acid, α -macroglobulin) and demographic data (age, sex) to predict fibrosis in chronic HCV.²⁰ The FIB-4 index, which uses age, ALT, AST, and platelets²¹, has also been evaluated in HIV/HCV coinfecting individuals and found to be accurate in detecting hepatic fibrosis. The above-mentioned scores are used less often than FibroTest in clinical practice mainly because of comparatively fewer supporting studies.

Direct Serum Fibrosis Markers—In contrast to indirect markers, the combined use of variables representing unique molecular aspects of hepatic fibrogenesis have been termed “direct” serum fibrosis marker panels. Two commonly available methods are the FIBROSpect II and European Liver Fibrosis (ELF) panels. The FIBROSpect II panel (Prometheus Laboratories Inc., San Diego, CA) is a proprietary technique comprised of hyaluronic acid, tissue inhibitor of metalloproteinase 1, and α 2- macroglobulin. Patel et al. evaluated the diagnostic accuracy of FIBROSpect II in 294 patients with chronic hepatitis C and validated the results in an external cohort of 402 patients. The sensitivity, specificity, and AUC in the external cohort was 77%, 73%, and 0.82, respectively.²² Their results showed relatively good diagnostic accuracy for the detection of moderate-to-severe fibrosis in patients with chronic hepatitis C. A subsequent investigation among 108 patients validated these initial findings with sensitivity, specificity, and AUROC values of 72%, 74%, and 0.826, respectively.²³

The European Liver Fibrosis (ELF) panel Group consists of age, hyaluronic acid, amino-terminal propeptide of type III collagen, and TIMP-1 for the detection of hepatic fibrosis. In a large cohort study examining patients with various chronic liver disease etiologies, the ELF panel also demonstrated good accuracy in detecting clinically significant hepatic fibrosis (stages F2-F4) in patients with alcoholic and nonalcoholic fatty liver disease as well as chronic hepatitis C. By adopting different test thresholds, sensitivities and specificities of over 90% could be obtained allowing the panel to reliably exclude or detect significant fibrosis.²⁴

The use of serum fibrosis markers to assess hepatic effects following antiviral treatment response has been a recent area of interest. Within the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial, a prospective study of maintenance pegylated interferon in chronic HCV patients with advanced fibrosis who failed prior treatment, a number of serum fibrosis markers were serially measured over the duration of the study. More specifically, serum samples of YKL-40, TIMP-1, amino-terminal peptide of type III procollagen, and hyaluronic acid were assessed at weeks 0, 24, 48, and 72. Among the markers examined, it was shown that a reduced YKL-40 level at baseline was an independent predictor of a virologic response at week 20. While the levels of some markers increased on antiviral treatment, there were statistically significant reductions in all 4 markers noted among patients achieving a sustained virological response (SVR) at week 72.²⁵ Patel et al. also studied changes in noninvasive serum markers (FibroSURE and FIBROSpect II) in treatment-naïve patients with chronic hepatitis C given albuterol and ribavirin. Similar to the HALT-C study, patients achieving SVR had lower baseline scores than non-responders. Moreover, there were significant declines in HCV FibroSURE and FIBROSpect II scores in those patients who achieved a SVR.²⁶ One drawback to the above-mentioned studies is that a second comparative liver biopsy was not

performed following treatment to document fibrosis regression. Future studies are expected to also define what minimum change in serum marker panel score is clinically significant.

Additional studies have evaluated the prognostic value of noninvasive serum biomarkers. A prospective cohort study compared the five year prognostic value of FibroTest to liver biopsy for predicting cirrhosis decompensation and survival among 537 HCV-infected patients. FibroTest values were used to classify those with minimal fibrosis (FibroTest < 0.32), moderate fibrosis (FibroTest 0.32–0.58), and severe fibrosis (FibroTest > 0.58). The authors found FibroTest to be a better predictor of both HCV-related complications (AUROC 0.96 vs. 0.91) and HCV-related deaths (AUROC 0.96 vs. 0.87) as compared to liver biopsy.²⁷ A similar study evaluated the ability of ELF to predict clinical outcomes (liver-related morbidity and mortality) in 457 patients with chronic liver disease of various etiologies. A unit change in ELF score was associated with a doubling of risk of liver-related outcome and ELF predicted outcome at least as well as liver biopsy.²⁸ Again, future studies are expected to confirm these initial findings and to see if treatment response influencing prognosis can also be measured by serum markers.

Imaging Techniques

Ultrasound-Based Transient Elastography—With the deposition of fibrotic tissue in organs such as the liver, the physical properties also change. Historically, this has been appreciated through physical examination where palpation of a hard or stiff liver often denoted the presence of significant disease. Recent advances in technology can now detect the extent of hepatic fibrosis by providing quantitative measurements of liver stiffness.

First described in 2003, ultrasound-based transient elastography (TE) using FibroScan (Echosens, Paris, France) is able to measure liver stiffness using a transducer probe mounted on a vibrating axis. Activation of the hand-held probe leads to vibrations of mild amplitude and low frequency (50 Hz) which generate one-dimensional mechanical waves that propagate through the liver. Pulse-echo acquisition then follows the propagating wave and measures its velocity, which is directly proportional to tissue stiffness. Therefore, subjects with higher degrees of fibrosis will have faster wave velocities detected by TE that result in higher liver stiffness measurements. Liver stiffness measurement is expressed in kiloPascals (kPa), with ranges between 2.5 to 75 kPa. Advantages of TE include 1) short acquisition time (100 ms) which allows measurements to be made even though the liver moves with respiration and 2) region of interest analysis within a cylindrical volume approximately 1 cm wide and 4 cm long, which results in a tissue volume over 100 times the size of a typical liver biopsy specimen. Criteria for valid liver stiffness measurement with TE include a success rate (percentage of successful measurements out of total acquisitions) of at least 60% and an interquartile range which should not exceed 30% of the median value.²⁹

Similar to the non-invasive serum fibrosis markers, the initial diagnostic evaluation studies using TE focused on patients with chronic hepatitis C. A preliminary study in 2003 showed excellent reproducibility and diagnostic accuracy for detecting clinically significant hepatic fibrosis and cirrhosis in 67 patients.³⁰ Subsequent work by Ziol et al. among 327 patients with chronic HCV infection revealed sensitivity, specificity, and AUROC values of 55%, 84%, and 0.79 for $\geq$ stage 2 and 84%, 94%, and 0.97 for stage 4 (cirrhosis) using cutoff values of 8.8 kPa and 14.6 kPa, respectively. In multivariate analysis, stage of hepatic fibrosis was highly correlated to liver stiffness as opposed to inflammation grade and extent of steatosis. It should be noted that seventy-six patients (23%) were excluded from the statistical analysis based on unsuitable liver biopsy specimens or TE examinations that failed to meet criteria for a valid result.³¹ Castera et al. in 2005 also studied 193 patients with chronic HCV infection who underwent liver biopsy as well as TE, APRI and FibroTest assessments. The diagnostic performance of FibroScan, FibroTest, and APRI was similar for

detecting stages F2-F4 hepatic fibrosis. For various combinations of noninvasive methods, the diagnostic use of FibroScan and FibroTest was most optimal for detecting stages F2-F4 and F4 alone. Agreement between FibroScan and FibroTest results for the presence of significant fibrosis was 84% when compared to histology from liver biopsy.³²

Several meta-analyses have confirmed the diagnostic accuracy of TE in various populations with chronic liver disease. Among nine studies that evaluated the diagnostic accuracy of TE compared to liver biopsy, a pooled sensitivity of 87% and specificity of 91% was observed for the detection of cirrhosis (F4). Chronic hepatitis C with or without viral co-infection was the main etiology of liver disease in eight of these studies. For patients with stages F2-F4 fibrosis, the accuracy of TE was lower and more variable (sensitivity ranged between 53%-93%, specificity ranged from 70%-84%) when compared to results for detecting cirrhosis.³³ A second meta-analysis examined the diagnostic performance of TE in 50 studies including work published in abstract form. Once again, TE was found to be excellent at distinguishing cirrhosis versus no cirrhosis while the diagnosis of clinically significant fibrosis (F2-F4) was less accurate. Meta-regression analysis identified underlying liver disease, the fibrosis staging system used, and the country in which the study was performed as major causes of heterogeneity in pooled results.³⁴ In summary, these meta-analyses show that TE is effective for identifying cirrhosis, but various factors leading to heterogeneity make it a less than ideal tool to detect stages F2-F4 fibrosis.

Fewer studies have assessed the use of TE in chronic liver diseases other than hepatitis C. Coco et al. studied 228 consecutive patients with chronic hepatitis B (n=79) and hepatitis C (n=149). At multivariate analysis, both ALT and fibrosis were the two factors independently associated with liver stiffness. Interestingly, 10 patients with ALT flares had up to 3-fold increases in liver stiffness that progressively declined to baseline following resolution of the flare in disease activity. This suggests that TE may not be as reliable for chronic hepatitis B.³⁵ A small study of 67 patients with NAFLD showed a stepwise increase in liver stiffness with increasing histologic fibrosis. The severity of fibrosis was not affected by grade of activity or amount of steatosis.³⁶ Patients with chronic cholestatic liver disease have also been examined by TE. Among 69 cases with primary biliary cirrhosis and 26 with primary sclerosing cholangitis, liver stiffness values were found to correlate with histological stages. The diagnostic performance of TE for detecting stages F2-F4 and F4 fibrosis were excellent with AUROCs values > 0.9 for both fibrosis categories.³⁷ Additional studies in patients with non-viral liver disease are expected to better define the diagnostic accuracy of TE in these populations.

Transient elastography is an attractive tool because it is painless, quick (can be performed in less than 5 minutes), easily reproducible, free of complications, and available at the bedside or on an outpatient basis. In contrast to serum markers as discussed above, TE is a more direct measure of fibrosis, is not affected by extrahepatic disorders, and is applicable to all chronic liver diseases. Limitations for using TE are seen in patients who are obese, have narrow intercostal spaces, have ascites (elastic waves do not propagate through liquids), or experience ALT flares (i.e. acute viral hepatitis or acute reactivation of chronic hepatitis B).³⁸ The impact of obesity on TE appears to be the most critical, as the frequency of incomplete and failed examinations exceeds 20% with body mass index values ≥ 28 kg/m² and continues to rise in frequency with further increases in BMI.³⁹ Furthermore, strict confinement of the right liver edge for TE measurement may limit the ability to identify the dominant stage of fibrosis if the geographic distribution of fibrosis within an individual patient's liver is heterogeneous (Figure 1).³⁸

Magnetic Resonance Elastography—Liver stiffness is also measured using a technique called magnetic resonance elastography (MRE). The initial pioneering work in the

1990's has led to the use of MRE in human subjects for detecting hepatic fibrosis. Using conventional 1.5T MRI scanners, patients are placed in the supine position where a pneumatic driver is placed against the anterior abdominal wall. This driver vibrates at low frequencies (40–120 Hz) which then lead to mechanical wave formation and propagation into the liver. MRE measurements require the patient to hold his breath for 10 to 15 seconds on four occasions during the scanning period. A phase-contrast MRI sequence (which can be added to a conventional MRI system) then images the propagating waves. Data from the phase-contrast sequence is then analyzed by specialized computer-based algorithms (which can be added to post-processing workstations) to create elastograms – which are quantitative, color-coded images that depict tissue stiffness. Regions of interest are identified on each of the four cross-sectional images obtained, and averaged to obtain a mean liver stiffness value measured in kiloPascals. Of note, the numerical results obtained by MRE differ by a factor of 3 when compared to results obtained by TE.^{38,40}

Several investigations have been published demonstrating the excellent diagnostic accuracy for MRE in detecting hepatic fibrosis. A pilot study by Huwart et al. assessed the feasibility of MRE in a group of 25 consecutive patients with various etiologies of chronic liver disease. The mean live stiffness was 2.24 ± 0.23 kPa in the 11 patients with minimal fibrosis (F0-F1), 2.56 ± 0.24 kPa in the four patients with substantial fibrosis (F2-F3), and 4.68 ± 1.61 kPa in the 10 patients with cirrhosis. There were statistically significant increases in mean liver stiffness with increasing stage of fibrosis.⁴¹ Additional work by Yin and colleagues was performed by examining the performance of MRE in 50 patients with various forms of chronic liver disease and 35 healthy volunteers. In this study, mean liver stiffness also increased systematically with stage of histologic fibrosis. With a cut-off mean liver stiffness value of 2.93 kPa, MRE had a sensitivity of 98% and a specificity of 99% for differentiating any stage of fibrosis from normal liver tissue. For stages F2-F4, a sensitivity of 86% and specificity of 85% were associated with a mean cut-off value of 4.89 kPa. This study also demonstrated that there was no significant relationship between degree of steatosis and liver stiffness.⁴²

An important prospective comparative study was conducted by Huwart et al. comparing MRE to TE and APRI in 141 patients with chronic hepatitis C. The success rate of TE was 84% while the rate was 94% for MRE. Further analysis was performed in the 96 patients who had successful histologic and imaging studies. While MRE, TE, and APRI results increased according to the stage of liver fibrosis, the modality with the highest diagnostic accuracy for stages F2-F4 and F4 alone was MRE. It should be noted that both MRE and TE had excellent diagnostic performance for the detection of cirrhosis.⁴³

Advantages of MRE include 1) an acoustic window is not required, 2) it is operator independent, 3) it can be performed on obese patients, 4) large cross-sectional areas of hepatic parenchyma can be evaluated (Figure 1), and 5) a conventional MRI can be obtained at the same time as MRE.^{38,40} However, MRE does have its limitations. Standard contraindications for MRI (e.g pacemaker, defibrillator, aneurysm clip, etc.) still apply when performing MRE. While obesity is not as detrimental when compared to TE, obese patients still must be able to fit into the magnet bore. At the moment, MRE cannot be performed in patients with increased hepatic iron content due to signal-to-noise limitations that prevent wave visualization. As with TE, liver stiffness measurements can be affected by several other pathological process including hepatic inflammation, or congestion from cardiac disease^{38,40,42}

Emerging Imaging Modalities

Aside from TE and MRE, other non-invasive imaging modalities have been investigated. One such technique is acoustic radiation force imaging (ARFI). Basically, this is an ultrasound-based method that uses B-mode imaging to locate the region of interest. Longitudinal, short-duration acoustic pulses cause tissue displacement with generated shear waves moving laterally away from the region of excitation. The speed of these waves are then measured by ultrasound tracking beams.

Friedrich-Rust et al. conducted a pilot study of 81 patients with chronic viral hepatitis (64 with HCV and 17 with HBV) that compared ARFI with TE and serologic fibrosis markers (APRI and FibroTest). Two percent of patients had to be excluded from the study due to failed TE (resulting from obesity). The median velocity in 20 healthy controls was 1.10 m/s (range 0.85–1.42 m/sec). The median velocities in the study patients were 1.13 m/s for patients with stage F0, 1.17 m/s for F1, 1.22 m/s for F2, 1.64 for F3, and 2.38 m/s (range 1.15–3.83 m/s) for F4. Of note, there were patients in the healthy control group who had velocities in the cirrhotic range. Despite this, all three modalities had similar diagnostic performances. Even though ARFI performed similarly to TE, it does have the advantage of being easily integrated into a standard ultrasound examination. Future studies with larger numbers of subjects are needed to define the role of ARFI in the future.⁴⁴

Diffusion-weighted MRI is another test that has been evaluated for detecting hepatic fibrosis. It allows for qualitative and quantitative assessment of tissue without using gadolinium chelates, thereby eliminating the risk of nephrotoxicity. This technique relies on differences in the mobility of protons (primarily associated with water) between tissues. Diffusion is restricted in highly cellular tissues and relatively free in cystic or necrotic tissues. The degree of diffusion is measured by the apparent diffusion coefficient (ADC) where lower values have been described in association with higher degrees of hepatic fibrosis. Other studies, however, have indicated that diminished hepatic perfusion in advanced fibrosis accounts for the change in ADC rather than the restriction of molecular diffusion. Further studies are needed to help delineate the exact mechanism behind diffusion changes seen in liver fibrosis. Other limitations of diffusion-weighted MRI include questionable ADC reproducibility and suboptimal image resolution due to motion artifact.⁴⁵

MR spectroscopy uses ³¹ phosphorus spectral profiles to provide direct biochemical information on hepatic metabolism. Theoretically, hepatic fibrosis leads to increased turnover of cell membrane synthetic and degradation products. The ratio of phosphomonoesters (PME) to phosphodiester (PDE) is thought to reflect this process. More specifically, studies have shown that the PME/PDE ratio increases with advanced liver disease. Lim et al. enrolled 15 healthy controls and 48 patients with biopsy-proven HCV-related liver disease. Fibrosis (F) and necroinflammatory (NI) scores were used to divide the subjects into three groups: mild hepatitis ($F \leq 2/6$, $NI \leq 3/18$), moderate-severe hepatitis ($3 \leq F < 6$ or $NI \geq 4/18$) and cirrhosis ($F = 6/6$). The PME/PDE ratio increased along with disease severity, providing statistically significant differences between the mild hepatitis, moderate hepatitis, and cirrhosis groups. Using a PME/PDE ratio of ≤ 0.2 , mild hepatitis could be detected with a sensitivity of 76%, and a specificity of 83% (PPV 72% and NPV 86%). Similarly, a PME/PDE ratio ≥ 0.3 could identify cirrhosis with a sensitivity of 82% and a specificity of 81% (PPV 56% and NPV 93%).⁴⁶ MR spectroscopy requires further refinement before it can be assessed more fully in patients with chronic liver disease.

Use of Noninvasive Tests in Clinical Practice

As previously described, the majority of studies examining the performance characteristics of serum fibrosis markers and noninvasive imaging were done on patients with chronic viral

hepatitis. Both serum tests (FibroTest) and imaging (TE, MRE) modalities can sufficiently identify stages F2-F4 and F4 alone. With the advent of direct-acting antivirals and IL-28 genotyping, the workup and management of hepatitis C will be rapidly changing and the role of noninvasive testing must be evaluated. The expected improvement in genotype 1 SVR rates, for example, may further obviate the need for an initial staging liver biopsy in these patients. Therefore, noninvasive testing could be used as a tool to exclude cirrhosis in those patients who do not have suggestive clinical, laboratory, or imaging findings. Furthermore, noninvasive testing could be performed serially to identify disease progression or regression in patients achieving SVR after therapy. .

Recurrent hepatitis C after liver transplantation is a difficult problem, and these patients are often subjected to multiple protocol liver biopsies to stage hepatic fibrosis. Stage 2 fibrosis is the usual threshold for starting HCV treatment post-transplant as with native liver disease. The use of serum fibrosis markers in this setting may be problematic because immunosuppressive agents can affect some of the variables. Carrion et al. evaluated the use of TE in 124 HCV-infected liver transplant recipients. With a liver stiffness cut-off value of 8.5 kPa, the sensitivity, specificity, NPV, and PPV for the diagnosis of $\geq$ F2 fibrosis were 90%, 81%, 79%, and 92%, respectively. Furthermore, there was a direct correlation between liver stiffness and hepatic venous pressure gradient for the diagnosis of portal hypertension. This is of potential importance as patients with greater than observed disease severity by liver biopsy may be detected with elastography.⁴⁷

A recent study of 400 U.S. adults showed the prevalence of NAFLD to be 46% with NASH detected in 12.2% of the total cohort.^{48,49} These rates are higher than previously estimated, making NAFLD the most common liver disease where noninvasive tests of hepatic fibrosis can be used. The performance characteristics of certain serum markers (i.e. FibroTest, ELF) have been validated in patients with NAFLD and seem adequate for the detection of advanced fibrosis. The role of noninvasive imaging in these patients is somewhat more problematic for TE which can be technically difficult in obese patients as compared to MRE.

One interesting niche for noninvasive fibrosis assessment may be in screening patients prescribed methotrexate for chronic inflammatory diseases for clinically significant hepatic fibrosis. Past guidelines, which were based on expert opinion, recommended a liver biopsy after a cumulative methotrexate dose of 1500 mg and repeat biopsies after every additional 1000–1500 mg.⁵⁰ More recent work has suggested that advanced liver fibrosis is a rare event in patients treated with methotrexate for diseases including Crohn's disease and psoriasis.^{51,52} Furthermore, FibroTest and FibroScan have been shown to reliably detect advanced fibrosis in this group of patients. Given the rarity of methotrexate-induced fibrosis and the possible need for serial assessments, the use of these noninvasive tests is attractive in this setting.

In summary, the need for noninvasive assessment of hepatic fibrosis for disease staging, prognosis, progression, and treatment response is clear. The use of serum markers and elastography imaging with TE and MRE is promising though their role has mainly been studies in patients with chronic HCV infection. (Table 2) Additional areas of research include defining cut-off values for specific diseases, further head-to-head comparisons of various noninvasive modalities, and the examination of algorithms using both serum markers and imaging. Moreover, there is a paucity of data analyzing the cost-effectiveness of these various tests for diagnostic as well as screening purposes.

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Figure 1. Representative areas of hepatic parenchyma examined by transient elastography (*left panel*) and MRE (*right panel*) for detecting stage of hepatic fibrosis. A larger region of interest is available for calculating mean liver stiffness with MRE (From Talwalkar JA. Elastography for detecting hepatic fibrosis: options and considerations. *Gastroenterology* 2008;135:299–302 with permission)

Table 1

Diagnostic performance of serum fibrosis marker panels for detecting advanced hepatic fibrosis compared to the reference standard of liver biopsy (Adapted from Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology* 2006, 43: S113–S120 with permission).

Panel	Liver Disease	Se	Sp	PPV	NPV
AST/ALT ratio	AST/ALT	53%	100%	100%	81%
Forns test	Platelets, GGT, Cholesterol	94%	51%	40%	96%
APRI	AST, Platelets	41%	95%	88%	64%
Fibrotest	GGT, haptoglobin, bilirubin, apo A, alpha2-macroglobulin	87%	59%	63%	85%
Fibrospect	Hyaluronic acid, TIMP-1, alpha-2-macroglobulin	83%	66%	72%	78%
ELF	Numerous ECM proteins and proteinases	90%	41%	35%	92%

Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

Advantages and limitations of biopsy and noninvasive tests for detecting hepatic fibrosis Adapted from Castera L, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? Gut. 2010 Jul;59(7):861–6 with permission)

Table 2

	Liver Biopsy	Serum Markers	Transient Elastography	MR Elastography
Advantages	Direct observation of fibrosis	Non-invasive	Non-invasive	Non-invasive
	Examines 1/50,000 th of hepatic parenchyma	Examines indirect or direct markers of fibrosis	Examines 1 × 4 cm area over right liver edge	Examines multiple areas within right and left liver
	Accurate for detecting cirrhosis	Can be accurate for detecting cirrhosis	Accurate for detecting cirrhosis	Accurate for detecting cirrhosis
Disadvantages	Invasive with risk of complications	Non-invasive	Non-invasive	Non-invasive
	Contraindicated with ascites, coagulopathy	Delays in test result generation with send-out proprietary tests	Failure rate with obesity, narrow rib spaces	Limited by claustrophobia and typical MRI contraindications
	Sampling error and observer variation	False positive values with hemolysis, inflammation, Gilbert's syndrome	False positive values with inflammation, congestion	False positive values with inflammation, congestion
	Unsuitable for longitudinal monitoring	Indices may change with disease progression or response to therapy	Liver stiffness does change with disease progression or response to therapy	Liver stiffness does change with disease progression or response to therapy