Diagnosis and Quantitation of Fibrosis

Hepatic fibrosis is the final common pathway for many different liver insults. Originally considered to be irreversible, hepatic fibrosis is now known to be a dynamic process with a significant potential for resolution. The diagnosis and quantitation of fibrosis have traditionally relied on liver biopsy. However, there are a number of drawbacks including the invasive nature of the procedure, sampling error, and interobserver variability. This article reviews the current role of liver biopsy in the assessment of hepatic fibrosis and discusses the role of the newer noninvasive methods including serum markers and radiologic tests.

Liver Biopsy

Liver biopsy has long remained the gold standard for the assessment of hepatic fibrosis. However, because it is an invasive test with the potential for serious, albeit rare, complications, it is not undertaken lightly. The first percutaneous liver biopsy was performed in 1923, but only in the last 50 years has it become a standard test following Menghini’s description in 1958. Significant complications, defined as requiring hospital admission or prolonged hospital stay, occur in 1% to 5% of patients, and mortality has been reported in between 1 in 1000 patients and 1 in 10,000 patients.

Our deeper understanding of the mechanisms of fibrosis has led to the identification of many potential markers of fibrosis, which appear capable of identifying early and advanced hepatic fibrosis. Standard cross-sectional imaging studies will only identify or exclude advanced fibrosis. Novel technologies such as transient hepatic elastography and magnetic resonance imaging (MRI) elastography show promise as noninvasive methods of testing for hepatic fibrosis. In this article, we will review our current methods of diagnosing and quantifying hepatic fibrosis and discuss how the newer technologies may be integrated into clinical practice.

Abbreviations used in this paper: APRI, AST to platelet ratio index; ECM, extracellular matrix; HA, hyaluronic acid; MMPs, matrix metalloproteinases; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PIIINP, procollagen type III amino-terminal peptide; ROC, receiver operating curve; TIMPs, tissue inhibitors of metalloproteinases.
Serum Markers of Fibrosis

A large number of putative serum markers have been evaluated for the assessment of hepatic fibrosis. Despite the dynamic nature of hepatic fibrogenesis, most of the presumed tests are suitable for the cross-sectional diagnosis of fibrosis stage rather than determining the rate of fibrosis progression or regression. No true serum marker that would act as a surrogate marker of hepatic fibrosis has been validated to date. It is almost certain that combinations of biomarkers will probably have to be examined. A systematic review of 14 studies of fibrosis biomarkers in patients with chronic hepatitis C concluded that cut-off levels could rule out or rule in fibrosis in 35% of patients (Figure 1), but the panels of biomarkers could not differentiate stages of fibrosis accurately.33

Features that would apply to an ideal biomarker have been described and are shown in Table 1.34,35 Broadly speaking, serum markers of hepatic fibrosis can be considered in 1 of 2 categories: either indirect or direct. Indirect markers reflect alterations in hepatic function but do not directly reflect hepatic ECM metabolism, for example, platelet count, coagulation studies, and hepatic aminotransferases. Direct serum assays for markers of fibrosis reflect serum ECM turnover. The discovery of many of these direct biomarkers is directly attributable to advances in the understanding of the molecular mechanisms involved in hepatic fibrogenesis. Serum assays for enzymes and products of matrix synthesis or degradation have been evaluated as markers of fibrosis in many studies and show some promise as a simple alternative to liver biopsy.3,36–46

Indirect Markers

A number of indirect markers of liver fibrosis have been used in clinical practice over the years, including serum aminotransferase levels, presence of coagulopathy, and platelet counts. A number of indices involving com-
The stage of fibrosis has not been found to be clinically
aspartate aminotransferase (AST) level alone to diagnose
sufficient numbers of portal tracts. Biopsy errors include small size, fragmentation, and in-
is a possibility for error of either biopsy or biomarker.
Results should always be undertaken to see whether there
in up to 20% of patients. An evaluation of discordant
cause there is the potential that liver biopsy is incorrect
ing the presence of mild disease or advanced fibrosis.
panel to determine whether they are best at discriminat-
ately. We also need to evaluate each fibrosis marker or
come from academic centers with high disease prevalence
the higher the sensitivity of a test. Many of the studies
and specificity of tests: the higher the disease prevalence,
disease prevalence has a major impact on the sensitivity
percentage of patients. We should also look at the prev-
ate 3 stages of disease is acceptable: mild with METAVIR
F0–F1; moderate to advanced, F2–F3; and cirrhosis, F4.
The number of indeterminate readings should be as low
as possible, but inaccuracy is still highly likely for a
percentage of patients. We should also look at the prev-
ience of disease in the populations studied because
disease prevalence has a major impact on the sensitivity
and specificity of tests: the higher the disease prevalence,
the higher the sensitivity of a test. Many of the studies
come from academic centers with high disease prevalence
for fibrosis and may overestimate the accuracy appropri-
ately. We also need to evaluate each fibrosis marker or
panel to determine whether they are best at discriminating
the presence of mild disease or advanced fibrosis.

There is also the question of “diagnostic truth” be-
cause there is the potential that liver biopsy is incorrect
in up to 20% of patients. An evaluation of discordant
results should always be undertaken to see whether there
is a possibility for error of either biopsy or biomarker.
Biopsy errors include small size, fragmentation, and in-
sufficient numbers of portal tracts.

Aspartate Aminotransferase/Alanine
Aminotransferase Ratio

The use of the alanine aminotransferase (ALT) or
aspartate aminotransferase (AST) level alone to diagnose
the stage of fibrosis has not been found to be clinically
useful. The ratio of AST to ALT tends to increase
with advancing stages of fibrosis from the level of ap-
proximately 0.8 in normal subjects. The value of this
ratio is greatest for the noninvasive diagnosis of cirrhosis
where a ratio of >1 suggests the diagnosis of cirrho-
isis. The use of the AST/ALT ratio is confounded by
the use of alcohol.

AST to Platelet Ratio Index

The AST to platelet ratio index (APRI) is calculated
as follows: APRI = (AST/upper limit of normal) ×
100/platelet count. This simple index is made up of easily
available, routine laboratory tests. With worsening fibro-
sis and increasing portal pressures, there is reduced
thrombopoetin production and increased platelet se-
questration in the spleen. Advancing liver fibrosis is also
associated with reduced clearance of AST. The
APRI test can thus potentially differentiate between those
with and without significant fibrosis or cirrhosis. The
studies that have focused on the APRI test have looked
mainly at patients with hepatitis C virus (HCV) or HCV/
human immunodeficiency virus (HIV) coinfection and
alcoholic liver disease. One of the first studies on
APRI was a retrospective analysis, which looked at a
number of clinical laboratory tests and examined the
relationship to significant hepatic fibrosis or cirrhosis.
The authors found that the APRI was the simplest and
most accurate test for the detection of significant fibrosis
or cirrhosis. The area under the receiver operating curve
(ROC) was 0.88 for significant fibrosis and 0.94 for cir-
rhosis. Subsequent studies have reported significant
variability in the performance of the index. A recent
meta-analysis of 22 studies, predominantly involving
chronic HCV patients, made a number of observations.
At an APRI threshold of 0.5, the sensitivity and specificity
for significant fibrosis were 81% and 50%, respectively. At
an APRI threshold of 1, the sensitivity and specificity for
predicting cirrhosis were 76% and 71%, respectively. The
authors concluded that APRI appears most useful for
excluding significant fibrosis in HCV.

PGA Index

The PGA index was the original indirect index of
hepatic fibrosis. Its original use was to detect alcoholic
liver disease in drinkers. It is a combination of the
prothrombin index, γ-glutamyl transferase (γGT), and
apolipoprotein A1. It has been validated in patients with
a variety of chronic liver diseases but particularly in
patients with alcoholic liver disease. Its accuracy for
detecting cirrhosis has been reported to range from 66%
to 72%.

FibroTest and FibroSure

FibroTest and FibroSure (Labcorp, Burlington, NC)
are identical groups of biochemical markers, which are
marketed under different names in Europe and the
United States. The test was first developed by Imbert-Bismut et al. and involves measurement of α2 macroglobulin, α1 globulin, γ globulin, apolipoprotein A1, γGT, and total bilirubin. The results from the test are formulated to determine 3 different categories of fibrosis: (1) mild (METAVIR F0–F1), (2) significant fibrosis (METAVIR F2–F4), (3) indeterminate—removed from the clinical reporting by making the fibrosis score linear.

The sensitivity and specificity for the detection of significant fibrosis, by their definition, were 75% and 85%, respectively (Table 2). The correct identification of disease as either mild or severe was correct in approximately 46% of cases. The score has been validated in other HCV cohorts, but not all studies achieved the same results. The paper by Rossi et al. found that the FibroTest could not reliably predict the presence or absence of fibrosis in 125 patients with HCV, using local assays and the original authors’ computer program to calculate the FibroTest score. However, a recent metaanalysis found that FibroTest is effective in the evaluation of hepatic fibrosis in chronic HCV and chronic hepatitis B virus (HBV), alcoholic liver disease, and nonalcoholic fatty liver disease (NAFLD); and the authors concluded that FibroTest is, in fact, an effective alternative to liver biopsy.

A paper by Sebastiani et al demonstrated simple stepwise algorithms to identify significant fibrosis and cirrhosis in a validation set of 100 patients with HCV following a training set of 190 patients. They evaluated APRI, followed by FibroTest and then liver biopsy if necessary. They found that a stepwise application of these methods of assessing fibrosis could reduce the need for liver biopsy by 50%–70%. The combined use of FibroTest and Fibroscan has also been evaluated. One study of 183 patients with HCV demonstrated an area under the ROC of 0.88 for F ≥ 2, 0.95 for F ≥ 3, and 0.95 for F ≥ 4. When the Fibroscan and FibroTest results were in agreement, liver biopsy confirmed the results in 84% of cases for F ≥ 2, 95% of cases for F ≥ 3, and 94% of cases for F ≥ 4.

**ActiTest**

ActiTest is a modification of the FibroTest, which includes ALT in addition to the other variables and reflects both hepatic fibrosis and necroinflammatory activity. This slight modification appears to improve the diagnostic value for the identification of more advanced fibrosis associated with more severe histologic inflammation. Patients with chronic HCV who have a sustained virologic response to antiviral treatment show a corresponding improvement in both FibroTest and ActiTest scores, supporting a role for these tests in monitoring response to treatment. A metanalysis that included 1570 patients concluded that these tests were a reliable alternative to liver biopsy in patients with chronic HCV. This finding was confirmed by a prospective multicenter study of 519 patients.

**Forns Index**

The Forns index is based on 4 routine clinical variables: age, platelet count, cholesterol levels, and γGT. The study was confined to patients with chronic HCV and included both a test cohort and a validation cohort. The test cohort of 125 patients was used to define the thresholds for individuals with a low or a high probability of significant fibrosis (METAVIR F2–F4). Fifty-one

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**Table 2. Serologic Tests for Liver Fibrosis**

<table>
<thead>
<tr>
<th>Patients, n</th>
<th>Name (serum markers)</th>
<th>AUROC (95% CI)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wai et al (2003)</td>
<td>APRI (AST, platelets)</td>
<td>0.88 (.80–.96)</td>
<td>41</td>
<td>95</td>
<td>88</td>
<td>64</td>
</tr>
<tr>
<td>Rosenberg et al (2004)</td>
<td>ELF (Propeptide III collagen, TIMP 1, HA)</td>
<td>0.80 (.76–.85)</td>
<td>90.5</td>
<td>41</td>
<td>99</td>
<td>92</td>
</tr>
<tr>
<td>Ziol et al (2005)</td>
<td>Fibroscan (hepatic elastography)</td>
<td>0.79 (.73–.84)</td>
<td>56</td>
<td>91</td>
<td>88</td>
<td>92</td>
</tr>
<tr>
<td>Imbert-Bismut et al (2001)</td>
<td>FibroTest (α2 macroglobulin, α2 and γ globulin, apolipoprotein A1, γGT, and total bilirubin)</td>
<td>0.87, SD 0.34</td>
<td>87</td>
<td>59</td>
<td>63</td>
<td>85</td>
</tr>
<tr>
<td>Castera et al (2005)</td>
<td>Combined FibroScan and Fibrotest</td>
<td>0.88 (.82–.92)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Patel et al (2004)</td>
<td>FibroSpect (Hyaluronic acid, tissue inhibitor of metalloproteinase 1, and α2 macroglobulin)</td>
<td>0.831</td>
<td>77</td>
<td>73</td>
<td>74</td>
<td>76</td>
</tr>
<tr>
<td>Adams et al (2005)</td>
<td>Hepascore (Bilirubin, γGT, hyaluronic acid, α2 macroglobulin, age, and sex)</td>
<td>0.82</td>
<td>63</td>
<td>89</td>
<td>88</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.90</td>
<td>88</td>
<td>74</td>
<td>88</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.89</td>
<td>71</td>
<td>89</td>
<td>89</td>
<td>98</td>
</tr>
</tbody>
</table>

ELF, European Liver Fibrosis (ELF) group; NPV, negative predictive value; PPV, positive predictive value.
percent of the population could be classified using these thresholds. The defined lower cut-off had a negative predictive value of 96%, whereas the upper cut-off value had a positive predictive value of only 66%. This test would appear useful at excluding patients with minimal or no fibrosis but was less useful for the identification of more advanced fibrosis. A comparison of the Forns index with the FibroTest demonstrated a slightly better performance by FibroTest. There are several criticisms of the Forns index including concerns about lipid abnormalities and the impact of medications and the variability of platelet measurements.

**FibroIndex**

The FibroIndex is calculated from the platelet count and γGT. Its accuracy is currently being determined. One study in chronic HCV patients included an estimation group of 240 patients and a validation group of 120 patients. Both these groups were treatment naïve. The authors also examined a longitudinal group of 30 patients who had undergone a liver biopsy before and after interferon treatment. By determining cut-off values, they found a significant decrease in the FibroIndex of 14 patients whose fibrosis stage improved and a significant increase in that of 5 patients whose fibrosis stage increased in the longitudinal group.

**Hepascore**

The Hepascore combines the following clinical and laboratory variables: age, gender, bilirubin, γGT, hyaluronic acid (HA), and α2-macroglobulin to create a score. One of the advantages of this score over the Forns index is the addition of a single direct biomarker of hepatic fibrosis—hyaluronic acid. One study compared this score to the APRI and Forns index and found in a group of 104 patients with chronic HCV that the area under the ROC was 0.82, 0.90, and 0.89 for significant fibrosis, advanced fibrosis, and cirrhosis, respectively, in their validation set.

**FIB-4**

The FIB-4 combines biochemical variables (platelet count, AST, and ALT) with age. It had reasonably good accuracy for predicting advanced fibrosis in patients with chronic HCV in 2 studies. Vallet-Pichard et al. found an area under the ROC of 0.85 for severe fibrosis and 0.91 for cirrhosis and found their results to be concordant with FibroTest results.

**NAFLD Fibrosis Score**

The NAFLD fibrosis score was recently described and examined in patients with NAFLD. This score consists of 6 readily available clinical and biochemical variables: age, hyperglycemia, BMI, platelet count, albumin, and AST/ALT. Seven hundred thirty-three patients with histologically proven NAFLD were divided into 2 groups: 480 to construct and 253 to validate the scoring system. The authors found that their scoring system of 6 variables had an area under the ROC of 0.88 and 0.82 in the estimation and validation groups, respectively. They validated a low cut-off score, which yielded a negative predictive value of 93% and 88% in the estimation and validation groups, respectively, for the presence of advanced fibrosis. Their high cut-off score yielded a positive predictive value of 90% and 82% in the estimation and validation groups, respectively, for the presence of advanced fibrosis. By applying this model, a liver biopsy could have been avoided in as many as 75% of the 733 patients. These findings were not validated, however, in a smaller study of 79 patients. A direct comparison of the NAFLD fibrosis score and FibroTest in 246 patients with NAFLD found that the FibroTest had better diagnostic value for the presence of advanced fibrosis.

There are several features of studies on indirect biomarkers of hepatic fibrosis that are applicable to clinical practice. In viral and nonalcoholic steatohepatitis (NASH), an AST/ALT ratio of >1 is often associated with advanced fibrosis or cirrhosis. γGT and prothrombin index are markers of progressive liver fibrosis and can be used to predict more advanced fibrosis. These indirect biomarkers simply reflect abnormalities of hepatic structure or function and do not reflect ECM turnover.

**Direct Markers**

Direct markers of liver fibrosis include a number of serum or urinary markers, which have been shown to be or are thought to be involved in the deposition of ECM. Liver fibrosis involves both quantitative and qualitative changes in ECM markers. Because some of the markers reflect fibrosis progression and others fibrosis regression, it is thought that a dynamic evaluation of ECM activity should be possible. Potential markers of fibrosis include products of collagen synthesis or degradation, enzymes involved in matrix biosynthesis or degradation, ECM glycoproteins, and proteoglycans/glycosaminoglycans. None of the currently available direct biomarkers completely fulfills the criteria for an ideal biomarker because none is liver specific and most are affected by changes in their metabolism, clearance, or excretion. Table 2 lists some of the currently available direct biomarkers.

**HA**

HA is a glycosaminoglycan synthesized by hepatic stellate cells and degraded by the liver sinusoidal cells and is a component of the ECM. High levels of HA in patients with liver disease, particularly cirrhosis, have been related to dysfunction of the sinusoidal endothelial cells and may reflect increased fibrogenesis. In evaluating single marker assays that reflect ECM concentration,
HA appears to be the best individual test. Studies have demonstrated that HA levels correlate with the degree of hepatic fibrosis in patients with chronic HCV. In alcoholic liver disease, the levels of HA correlate with both the degree of fibrosis and the severity of inflammation. There is a direct linear relationship between HA and procollagen type III amino-terminal peptide (PIIINP) with similar performance by both tests in discriminating between alcoholic patients with and without liver fibrosis. Moreover, abstinence from alcohol results in a reduction in HA levels. HA may, however, be increased in the postprandial state and is cleared by both liver and kidney. HA is included in the SHASTA index, Hepascore, and FibroSpect scores.

**PIIINP**

PIIINP is possibly the most widely studied marker of hepatic fibrosis. PIIINP levels are known to be elevated in acute hepatitis and correlate with aminotransferase levels. The levels reflect stage of fibrosis in chronic liver disease. Serum levels of PIIINP reflect the degree of fibrosis in alcoholic liver disease, viral hepatitis, and primary biliary cirrhosis. Reduction or normalization of PIIINP levels has been observed in those who abstain from alcohol. In chronic HCV, PIIINP levels have not been shown to correlate with degree of fibrosis but do correlate with the scores for necrosis. In patients receiving methotrexate, PIIINP appears to be a useful test for the evaluation of liver injury.

**Type I and Type IV Collagens**

Serum messenger RNA and protein levels of type I collagen are increased in all types of liver fibrosis and correlate with fibrosis but not necroinflammatory score. Type IV collagen has been immunolocalized to the periporal interstitium and large fibrotic bands in alcoholic liver disease. Serum type IV collagen is increased in hemochromatosis patients with advanced fibrosis compared with normal controls. In patients with alcoholic liver disease, there is a significant correlation between type IV collagen levels and fibrotic stage, particularly periportal fibrosis.

**Laminin**

Laminin is a noncollagenous glycoprotein synthesized by the hepatic stellate cells and deposited in the basement membrane of the liver. In chronic liver injury, basement components, particularly laminin, are deposited around the vessels, in the perisinusoidal spaces, and in the portal tracts. Serum levels of laminin and the laminin P1 fragment are elevated in patients with chronic liver disease due to alcohol and viral hepatitis. Laminin appears to be superior to PIIINP but inferior to type IV collagen in predicting fibrosis in chronic viral hepatitis.

**Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases**

The matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), are a group of proteins involved in the control of matrix degradation. The MMPs are produced intracellularly and secreted in proenzyme form that requires cleavage by cell surface mechanisms for functional activity. The actions of MMPs are, in turn, inhibited by TIMPs. These enzymes act to both degrade ECM and to permit new matrix deposition. The observation that MMPs are expressed in hepatic injury raises the hypothesis that degradation of normal ECM may contribute to hepatic fibrosis. The interaction between MMPs and TIMPs is complex, and, because these molecules act locally as well as having multiple activities including activation of growth factors, affecting cell proliferation, and inhibition of apoptosis, the relationship remains unclear. MMP-2 (gelatinase-A) is secreted by activated hepatic stellate cells and is increased in the presence of type I collagen. Studies examining the correlation of MMP-2 in chronic HCV have yielded conflicting results. Little is known about the role of MMP-3 (stromelysin) in liver injury. However, it would appear that levels are of little diagnostic value. MMP-9 (gelatinase-B) is mainly secreted by activated Kupffer cells. Plasma levels have been shown to be increased in patients with hepatocellular carcinoma but not those with chronic hepatitis or cirrhosis. The hypothesis that progression of chronic liver disease is associated with inhibition of matrix degradation is suggested by hepatic stellate cell cultures. MMP-13 is decreased in activated stellate cell cultures, but TIMP-1 and TIMP-2 are increased. This corresponds to findings in cirrhotic liver explant tissue showing an increase in TIMP-1 and TIMP-2 in patients with sclerosing cholangitis, primary biliary cirrhosis, autoimmune hepatitis, and biliary atresia. The study by Boeker et al found that TIMP-1 levels had a sensitivity of 100% for the prediction of cirrhosis, with a specificity of between 56% and 75%. A recent study showed reasonable sensitivity and specificity in predicting fibrosis in HIV/HCV coinfected patients.

**YKL-40**

YKL-40 or Chondrex is a novel marker of hepatic fibrosis. It is a 39-kilodalton glycoprotein that appears to function as a growth factor for fibroblasts, chondrocytes, and synovial cells and a migration factor for endothelial cells. Immunohistochemical staining has demonstrated positivity for YKL-40 in areas of hepatic fibrosis and fibrogenesis. A study of YKL-40 in alcoholic liver disease has suggested that it could function as a marker of clinical outcomes. A further study found it to be more accurate than HA in measuring hepatic fibrosis because of schistosomiasis.
Cytokines

A number of cytokines thought to mediate hepatic fibrogenesis have been studied as potential markers of fibrosis. Transforming growth factor (TGF)-β is a major stimulus for the production of the ECM by hepatic stellate cells. In one study of 88 patients with chronic HCV, there was a correlation between TGF-β and severity of fibrosis.\(^{115}\) A small study of 38 patients with chronic HCV found a close correlation between TGF-β levels and the rate of fibrosis progression.\(^{116}\) Tumor necrosis factor (TNF)-α has been associated with liver injury in patients with alcoholic liver disease.\(^{117}\) Platelet-derived growth factor is up-regulated following liver injury,\(^{118}\) and levels may correlate with the degree of liver injury.\(^{119}\)

Combinations of Indirect and Direct Biomarkers

**SHASTA Index**

The SHASTA index consists of measurements of serum HA, AST, and albumin and was developed in a cohort of 95 patients with HCV/HIV infection. It was capable of classifying mild fibrosis and advanced fibrosis and had similar accuracy to FibroTest and performed significantly better than APRI.\(^{57}\)

**FibroSpect**

The FibroSpect (Prometheus Laboratories, San Diego, CA) assay involves 3 parameters: HA, TIMP-1, and α2-macroglobulin. All patients were able to be evaluated by FibroSpect, a major advantage. In clinical practice, the assay gives a high likelihood of prediction as to the presence of mild and advanced fibrosis but performs less well for intermediate stages.\(^{120}\) The assay has been validated prospectively and appears to be particularly useful in excluding advanced fibrosis.\(^{121,122}\)

The European Liver Fibrosis group reported an assay in a multicenter cohort of 1021 patients with chronic HCV, NAFLD, and alcoholic liver disease. An algorithm using age, HA, PIIINP, and TIMP-1 was developed. The algorithm accurately predicted the presence of fibrosis with a sensitivity of 90% and the absence of fibrosis with a negative predictive value of 92%.\(^{123}\)

**Proteomics and Glycomics**

Patterns of proteins or glycoproteins can be assessed using mass spectroscopy of samples of serum. A series of “peaks” are generated, the precise identity of which are generally not known. Callewaert et al developed a novel DNA sequencer-based serum glycomics test (GlycoCirrhoTest), which could be both cost-effective and rapidly determine a signature profile for fibrosis of n-glycans.\(^{124}\) Combining GlycoCirrhoTest with the FibroTest gave a sensitivity of 79% and specificity of 86% in distinguishing cirrhosis from noncirrhotic disease. Larger prospective studies are necessary to determine clinical application of these new technologies.

Radiologic Imaging

Radiologic assessment of hepatic fibrosis has really been limited to patients with cirrhosis and its associated complications. Resolution of the hepatic parenchyma with ultrasound, computed tomography, or MRI is inadequate to assess earlier stages of fibrosis. A reduction in the size of the right lobe of the liver with relative enlargement of the left and caudate lobes is a reliable indicator of cirrhosis.\(^{5,125,126}\) The specificities in these studies are high, but have lower sensitivities because these changes are relatively late signs of cirrhosis. In other reports, up to 11 sonographic and Doppler measurements reported accuracies in the detection of cirrhosis of between 82% and 88%.\(^{127,128}\) Other features seen on radiologic studies supporting the diagnosis of cirrhosis are those of portal hypertension including splenomegaly and varices. The main role of radiologic imaging is the confirmation of cirrhosis in patients in whom a high clinical suspicion exists and may be complementary in cases in which the biopsy results are indeterminate or at odds with the clinical suspicion.

Novel Imaging Modalities

**Transient Elastography**

Elastography is an evaluation of the liver based on the fact that, as the liver becomes progressively more fibrotic, it becomes harder and less elastic. This technique easily and noninvasively measures the mean liver stiffness. Using a probe (Fibroscan; Echosens, Paris, France), which includes an ultrasonic transducer, a vibration of low frequency (50 MHz) and amplitude is transmitted into the liver. This vibration wave induces an elastic shear wave, which propagates through the liver. The velocity of the ultrasonic wave correlates directly with tissue stiffness, ie, the harder or stiffer the tissue, the faster the wave propagates. Results are reported in kilopascals (kPa).\(^{129-131}\) Fibroscan measures liver stiffness in a volume of approximately a cylinder of 1-cm diameter and 5 cm long, which is roughly 100 times the volume of a percutaneous liver biopsy. The immediate advantage of less sampling error is clear.\(^{6}\) Fibroscan has been evaluated in a number of different liver diseases including hepatitis B and C, alcoholic liver disease, and NAFLD.

An initial ex vivo study in 19 hepatectomy specimens correlated with histologic analysis, although there were some discrepancies, particularly in the middle range of fibrosis scores.\(^{132}\) An in vivo study of 15 patients with HCV showed excellent interoperator and intraoperator reproducibility. Furthermore, in 91 patients with HCV, the areas under the ROC were 0.90, 0.88, 0.91, and 0.99 for hepatic fibrosis stage greater than or equal to F1, F2, F3, and F4, respectively. Of the patients with a Fibroscan <5.1 kPa, 93% were stage F0 or F1, whereas those with a score ≥7.6 kPa, 94% were stage F2 or higher.\(^{130}\) A large study of 327 patients with HCV concluded that transient
elastography was a reliable tool for the detection of significant fibrosis or cirrhosis. The areas under the ROC were 0.79 for >F2, 0.91 for >F3, and 0.97 for F4, with liver biopsy used as the reference standard. There was some overlap in scores for those with early stage fibrosis. However, using a cut-off value of 8.7 kPa allowed correct diagnosis of those with significant fibrosis (>F2) with an area under ROC of 0.79.

A recent metaanalysis of 9 studies involving Fibroscan showed excellent results for diagnosing cirrhosis, with a sensitivity of 87% and specificity of 91%. The ability to differentiate mild from advanced fibrosis was not as good, which may be explained, at least partially, by a lack of uniformity of stiffness cut-offs between the different studies. A further systematic review of test accuracy for Fibroscan found excellent accuracy for diagnosing earlier stages of fibrosis.

This technology fulfills many of the criteria required for noninvasive assessment of liver fibrosis. It is quick, inexpensive, has good reproducibility, is acceptable to the patient, and examines a relatively large proportion of the liver. However, there are a few limitations. The technique may be limited in those with narrow intercostal spaces and those patients with morbid obesity or significant ascites because fluid and fat will attenuate the ultrasonic wave. Although it has been shown to be useful in the evaluation of fibrosis in patients with NAFLD, the technique has not really been validated in patients with a BMI of over 40. A new elastography probe, which can penetrate chest wall fat, is under clinical evaluation in obese patients.

**Magnetic Resonance Elastography**

The technique of magnetic resonance elastography (MRE) involves applying a probe to the back of the patient, which sends low-frequency vibrations through the liver and measuring the MRI spin-echo sequence. The data are then processed to obtain shear elasticity and viscosity maps. The advantages of MRE include the ability to simply add the MRE protocol onto a standard MRI of liver, the potential to scan the whole liver, and the lack of need for an acoustic window. A feasibility study in 25 patients showed significant differences in elasticity among patients without substantial fibrosis (METAVIR F0–F1), with substantial fibrosis (F2–F3), and with cirrhosis (F4). A subsequent prospective comparison of MRE with APRI showed superiority for MRE in liver fibrosis staging. Another study showed that MRE could discriminate between advanced fibrosis (METAVIR F2–F4) and mild fibrosis with a sensitivity of 86% and specificity of 85%.

**Clinical Utilization of Biomarkers**

The number of choices of biomarkers for clinical practice is expanding, and commercialization of the FibroSure, FibroSpect, and Hepascore has occurred in the United States. The imaging modalities are not yet widely available but are expected to move rapidly into clinical practice. There are no definitive guidelines on the utilization of biomarkers, but we have suggested 2 general approaches to using them in practice. One is to combine them with liver biopsy at the initial evaluation, and the second is to use the biomarkers for initial screening and then limit the number of biopsies (Figure 2). These are personal recommendations, and there remains a need to develop some suggested guidelines for the practical use of biomarkers in the United States. In France, the health authorities have incorporated screening with biomarkers such as FibroTest as the first step in evaluation of liver disease, resulting in a marked reduction in liver biopsies.

**Conclusion**

Liver biopsy remains the gold standard for assessment of liver fibrosis. Although it remains an essentially safe procedure, complications do arise in a small number of cases. Bigger problems with liver biopsy are those of sampling error and interobserver variability. Understanding the limitations of liver biopsy has major clinical implications. Fibroscan and the currently available sero-
logic tests can make the differentiation between early and advanced disease, which means that they could be used to guide treatment decisions. Quantitation of fibrosis by liver biopsy remains an inexact science, even with technologies such as morphometric analysis. Unfortunately, there does not appear to be any way of improving on this. In the near future, we will have therapies specifically aimed at the reversal of hepatic fibrosis, and, currently, we are looking at maintenance interferon in patients with HCV and advanced fibrosis to reduce disease progression. Liver biopsy will not be suitable to monitor these treatments because of its limitations and invasive nature. It is clear that we need other methods of assessing and particularly quantifying fibrosis. We need to be able to monitor the dynamic nature of fibrosis progression and regression. This will really require serum markers that are sufficiently sensitive to measure small changes in the state of the ECM. Unfortunately, none of the current noninvasive methods has this degree of sensitivity.

References


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