

Clinical Utility of Biomarkers of Liver Fibrosis

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Abstract: Hepatic fibrogenesis is a dynamic process and reflects a balance between matrix synthesis and degradation. An accurate determination of hepatic fibrosis in chronic liver disease is important in determining prognosis, therapy outcomes, and disease progression. Needle liver biopsy is an invasive procedure that is associated with small sample size and inaccurate staging, and provides only a semi-quantitative assessment of fibrosis. A number of simple and specific extracellular matrix biochemical markers predictive of fibrosis have been developed and validated in patients with chronic liver disease. Further improvements in these noninvasive approaches, incorporating emerging technologies such as proteomics and transient elastography, will likely provide more accurate and reliable measures of disease severity in these patients.

Significant progress has been made in the last two decades in our understanding of the pathogenesis of the wound healing response of the liver to chronic injury. The advent of chronic hepatitis C as a major cause of end-stage liver disease has played a significant role in the drive to uncover pathogenic mechanisms involved in fibrogenesis. In hepatitis C disease, inflammation appears to be a key driving factor for the fibrogenic response, and the process in turn is likely to be influenced by an array of metabolic and genetic factors. We now appreciate that fibrogenesis is a dynamic process, reflecting a balance between matrix synthesis, deposition, and degradation. Extensive investigation currently indicates that the hepatic stellate cell is a key effector in the fibrogenic response.¹

Fibrosis is thought to lead to impaired synthetic function, portal hypertension, and, ultimately, reduced survival. Data are now available that indicate that the fibrogenic response is reversible; for example, antiviral therapy in chronic hepatitis B and C leads to histologic improvement of fibrosis.^{2,3} Thus, it is now appreciated that “therapeutic” modulation of common pathways involved in fibrogenesis has the potential to slow or reverse fibrosis progression in chronic liver disease. Given the apparent importance of fibrosis in predicting prognosis and, moreover, data indicating that it is important to stage fibrosis prior to therapy (and to judge the effect of therapy), histologic assessment of the liver has taken on a major role in the management of patients with liver disease.

While liver histology remains an important part of the clinical assessment of hepatic injury and indeed is considered the current

Keywords

Noninvasive, biopsy, extracellular matrix, fibrogenesis, serum markers.

Table 1. Typical Advantages and Limitations of Liver Biopsy in Comparison to Noninvasive Biomarkers

	Liver Biopsy	Biomarkers
Diagnosis	Fibrosis stage, necro-inflammation and activity, steatosis, iron content, hepatotoxicity, and other chronic liver diseases	Fibrosis stage, activity
Prognosis and guide to therapy	Established role	Exclusion of significant disease in chronic viral hepatitis. To be determined in other liver disease
Posttransplant	Established role	None at this time
False results	Subcapsular biopsy, nonliver sampling	Common causes include acute hepatitis or other inflammation, hemolysis, cholestasis
Assessment	Semiquantitative and qualitative	Semiquantitative or quantitative
Adverse events	Mortality 3/10,000 Morbidity 3/1,000 Localized pain 3/10	None
Sampling error	33% for fibrosis stage	None known
Observer error	20% for fibrosis stage 40% for activity (METAVIR)	None
Sample requirements	Interpretation limited by quality of biopsy size and staining	Standardized assays and analyzers
Hospital stay	Usually 4–6 hours	None
Contraindications	Inability to cooperate Severe comorbid disease Coagulopathy Obesity	None
Cost	Very expensive (uncomplicated biopsy)	Inexpensive to moderately expensive

gold standard, a number of questions have arisen as to the accuracy of liver biopsy in correctly staging disease. Thus, in recent years it has been recognized that noninvasive markers of hepatic fibrogenesis may be appropriate as alternatives to liver biopsy.^{4,5} Although the concept of using radiological, clinical, and laboratory parameters of inflammation and fibrosis as alternatives to liver biopsy has been around for some time, the importance of accurate staging of fibrosis for guiding antiviral therapy and following disease progression in patients with chronic hepatitis C (and other types of chronic liver disease) has resulted in the development of newer algorithms employing noninvasive markers. A number of biochemical marker panels have been developed by cross-sectional study design and validated mostly in patients with chronic hepatitis C. Given the common downstream pathways of hepatic fibrosis, some of these panels also appear useful for disease staging in non-virally mediated hepatic injury. Promising newer approaches being developed for assessment of fibrosis in chronic hepatitis C patients include the use of

transient elastography as well as emerging technologies such as proteomics and metabolomics.

Liver Biopsy

Liver biopsy provides useful information to the clinician for determining prognosis and the urgency of therapy, predicting response to treatment, and investigating the etiology of liver injury, as well as for providing a baseline to allow comparisons of future histologic outcomes (Table 1).⁶ However, percutaneous liver biopsy is an invasive procedure and may be associated with significant complications in 3% of recipients, with a mortality rate of 0.03%.⁷⁻⁹ A study evaluating complication rates from 2,084 liver biopsies performed in France noted severe complications in 0.57% of patients and no deaths.¹⁰ Risk factors such as age and cirrhosis increase the likelihood of adverse events from liver biopsy. Furthermore, liver biopsy is costly: the direct costs of an uncomplicated liver biopsy in the United States are estimated at \$1,500–\$2,000,

and do not account for lost productivity and time off work.¹¹ For these reasons many patients are reluctant to undergo liver biopsies, further limiting the ability to assess and follow disease progression or determine efficacy of treatment.

An additional concern with liver biopsy is that it samples only 1/50,000 of the liver and thus is subject to sampling error, particularly in non-homogeneously distributed chronic liver disease. Several studies have highlighted the inaccuracy of liver biopsy for staging of advanced liver disease. Single biopsies may misclassify cirrhosis in 10–30% of cases.^{12,13} The number of biopsies performed also appears important. One study that obtained liver biopsies immediately prior to autopsy demonstrated that the diagnostic accuracy of cirrhosis increased from 80% for a single biopsy to 100% with three samples.¹⁴ Additionally, in patients with cirrhosis, even when three consecutive biopsies obtained through a single entry site were performed, the rate of concurrent findings about the histologic presence of cirrhosis was only 50%.¹⁵ Varying the site of biopsy also appears to result in significant discordance. A recent study evaluated laparoscopic biopsies obtained from the right and left lobes in 124 patients with chronic hepatitis C. There was a discordance of at least one stage in one third of patients, and in 14% of cases cirrhosis was present in one lobe, and a diagnosis of bridging fibrosis only in the other lobe.¹⁶ Most liver biopsies in clinical practice, however, are not obtained using laparoscopy. Further, the type and size of needle used may also be important. Compared to the Menghini needle, cutting-type instruments may increase the likelihood of making a correct diagnosis of cirrhosis.^{17,18}

Another important limitation of liver biopsy is inter- and intraobserver variation among pathologists.^{19–21} In chronic hepatitis C, the use of standardized grading systems such as Knodell, METAVIR, or Ishak (among others) results in good concordance rates between pathologists about fibrosis (there is concordance in 70–80% of samples), but there is generally less agreement about inflammation scores.²² Specimen size appears to be very important for the pathologist, with smaller samples leading to an underestimation of disease severity.²³ A study from France that created digitized virtual image biopsy specimens of varying lengths from large picosirius-stained liver tissue sections noted that 75% of 25-mm biopsy specimens were correctly classified using the METAVIR system, compared to only 65% of biopsies 15 mm in length.²⁴ In clinical practice most needle biopsies are likely to be less than 25 mm; however, a recent study noted that the experience of the pathologist may have more influence than specimen length on interobserver agreement.²⁵

Assessment of Liver Fibrosis in Liver Biopsy Specimens

Given the limitations of liver biopsy, alternative methods for quantifying liver tissue in histologic specimens have been utilized with variable success. Computer-aided image analysis can provide an objective measurement of the proportion of liver with fibrous tissue.²⁶ However, the coefficient of variation for image analysis remains unacceptably high at approximately 45%, even for 25-mm biopsies, precluding its routine clinical use for assessing fibrosis.²⁴ Nonetheless, there may be a potential role for image analysis in evaluating paired biopsies, or as a research tool in the development of noninvasive markers when quantitation of total matrix deposition is required. By comparison, histologic staging takes into account additional, subjective factors such as architectural distortion or nodule formation, although such morphologic assessment is clearly dependent upon the inherent bias of the individual pathologist.

Immunohistochemical analysis of extracellular matrix (ECM) components on liver biopsy may provide useful information regarding disease progression.²⁷ For example, matrix glycoproteins such as tenascin are deposited early in the fibrogenesis cascade into relatively immature matrix tissue that has the potential for reversibility.²⁸ Conversely, vitronectin is a marker of mature fibrous tissue that is unlikely to have significant potential for reversal.²⁹ Alternatively, assessment of stellate cell activation may be an attractive approach to evaluating fibrogenesis. Both assessment of ECM and stellate cell activation could potentially be correlated with serologic markers, thus providing a reflection of the dynamic state of fibrogenesis, as opposed to the standard static histologic measurements of disease stage. ECM and stellate cell activation could be useful in assessing treatment response and monitoring the disease process.

Limitations in liver biopsy have important implications for the development and validation of newer, noninvasive measures of fibrosis. Both the quality of the biopsy and the skill of the pathologist have to be taken into account. Furthermore, the semiquantitative grading systems developed for histopathologic analysis do not reflect linearity of fibrosis deposition or actual matrix content. For example, the Ishak system stages fibrosis on an ordinal scale where scores of 1 and 2 indicate portal fibrosis, 3 and 4 bridging fibrosis, and 5 and 6 incomplete and established cirrhosis, respectively.³⁰ Likewise, the METAVIR classification scores fibrosis on a 5-point scale from F0 to F4, with F1 and F2 indicating portal fibrosis with and without portal septae, respectively, F3 bridging fibrosis, and F4 cirrhosis.³¹ However, stage F2 does not imply twice as much fibrous tissue as F1. These

Table 2. Ideal Features for a Marker of Liver Fibrosis

• Liver-specific
• Inexpensive and easy to perform
• Measures: <ul style="list-style-type: none"> - Stage of fibrosis (or mass of extracellular matrix) - Activity of matrix deposition - Activity of matrix removal
• Levels not altered by changes in liver, renal, or reticuloendothelial function
• Reproducible performance characteristics
• Follows dynamic changes in fibrogenesis

Table 3. Biomarkers Evaluated in Relation to Fibrogenesis

Simple and Indirect Markers <ul style="list-style-type: none"> • Transaminases and GGT • Prothrombin time • Platelets • Apolipoproteins • Cholesterol • Total bilirubin • Gamma globulins
Cytokines Involved in Inflammation and Fibrogenesis <ul style="list-style-type: none"> • TGF-β • TGF-α • IL-10 • IL-2 receptor • IL-12 • Vascular endothelial growth factor • Leptin
ECM Remodeling and Other Markers <ul style="list-style-type: none"> • Tissue inhibitor of metalloproteinase • Procollagen peptides I and III • Type IV collagen • Matrix metalloproteinase • Tenascin • Laminin • Fibronectin • YKL-40 • Alpha-2 macroglobulin • Hyaluronic acid • Vitronectin • Cellular adhesion molecules • Urine hydroxylysylpyridinolines and (iso)desmosine

ECM = extracellular matrix; GGT = gamma glutamyltransferase; IL = interleukin; TGF = tumor growth factor; YKL-40 = human cartilage protein 39.

noncontinuous scales were developed to standardize and improve observer variability, and provide some assessment of the severity of chronic liver injury that could be used to determine thresholds for therapy in chronic hepatitis C in particular. Although certain ECM-specific serum markers are intuitively expected to reflect matrix deposition, their main limitation is that they are correlated with a semiquantitative morphologic assessment of fibrosis that is itself imperfect. Despite the obvious drawbacks of validating noninvasive markers against a liver biopsy for staging of fibrosis, alternative measures of fibrosis that achieve predictive area under receiver operating characteristics (AUROC) curve values above 0.85 should be acceptable as being equivalent to a liver biopsy.²⁷

Radiology Imaging

Current imaging modalities are useful in the assessment of cirrhosis and related complications, but lack sensitivity for fibrosis staging. For example, while cross-sectional imaging techniques accurately detect evidence of portal hypertension such as varices and splenomegaly, they are not able to detect fibrosis at an early stage. Additionally, computed tomography and magnetic resonance imaging are expensive. Other radiographic tests, such as ultrasound, are heavily dependent upon the operator for accurate interpretation. One study that included a significant proportion of patients with decompensated cirrhosis indicated that a diagnosis of cirrhosis could be made in 82–88% of cases by using a few ultrasonographic parameters, such as liver surface nodularity and hepatosplenomegaly. The diagnostic accuracy of ultrasound for bridging fibrosis in this study was 84%, although technical issues and interobserver expertise and variability are likely to reduce the accuracy observed in clinical practice.³² Other quantitative imaging techniques such as liver-spleen scans using single photon emission computerized tomography of technetium-99 m-labeled colloid uptake are useful in the determination of cirrhotic liver disease and residual hepatic function, but little data are available for earlier stages of fibrosis.^{33,34}

Serum Markers of Fibrogenesis

There has been significant and renewed interest in recent years in serum markers as a tool for assessment of hepatic fibrosis, particularly in relation to guiding treatment decisions in chronic hepatitis C. The characteristics of an ideal marker of fibrosis are shown in Table 2. A large number of specific and nonspecific serum and urinary markers of fibrosis have been evaluated. These include simple or indirect biochemical measures that reflect hepatic function; cytokines involved in inflammation and fibrogenesis; and

markers related to the ECM and/or turnover (Table 3). The vast majority of studies have been performed using a cross-sectional or diagnostic test design and unfortunately have been retrospective. Further, these studies typically did not report on the quality of biopsy. A systematic review evaluated 66 studies in an attempt to determine the predictive utility of biochemical or serologic markers of fibrosis in chronic hepatitis C.³⁵ However, only three of these studies had used separate patient cohorts to develop and validate the proposed model.³⁶⁻³⁸ The results of this systematic review suggested that nonspecific functional markers such as serum aminotransferases had only a modest value in predicting fibrosis on biopsy. Cytokines were less predictive of fibrosis compared to more specific ECM markers such as hyaluronic acid (HA) and laminin. In general, panels of markers that combine a variety of types of tests appeared to have the greatest value in differentiating minimal from advanced liver disease stages in chronic hepatitis C. Studies evaluating marker panels to date have relied on using either simple or indirect biochemical measures that reflect hepatic function and/or more complex and direct markers that reflect the ECM turnover.²⁷ This approach has led to the establishment of marker panels that appear to have some utility in assessing fibrosis severity in chronic hepatitis C. Validation of many of the currently available marker panels in other forms of chronic liver disease is awaited.

Marker Algorithms Based on Common Laboratory Tests

A number of both simple and complex mathematical indices have been used to formulate predictive marker algorithms. Several studies have evaluated commonly available biochemical measures such as serum transaminases, prothrombin time, platelets, gamma glutamyltransferase (GGT), and apolipoproteins to derive relatively simple algorithms that can be used to predict the presence of advanced liver disease, which in turn reflects the degree of fibrosis. One early example was the "PGA index," which combined prothrombin time, GGT, and apolipoprotein A1 to predict the presence of alcoholic cirrhosis.³⁹ The diagnostic accuracy of this index was later improved by the addition of α -2-macroglobulin ("PGAA index").⁴⁰ Another study evaluated multiple clinical, biochemical, and radiological variables in patients with alcoholic and viral disease, and noted that prothrombin index and hyaluronate had an accuracy of over 85% for the diagnosis of cirrhosis, although the PGA and PGAA scores performed less well.⁴¹

An aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio of greater than 1 may also have some utility in the diagnosis of cirrhosis; it has a reported specificity of over 95% in chronic hepatitis C patients,⁴²

and improved predictive values when combined with a low platelet count.⁴³ The presence of alcoholic liver disease, however, may reduce the diagnostic value of this assessment.⁴¹

In recent years, improved diagnostic indices have been developed in well-defined cohorts of chronic hepatitis C. Using the Ishak scoring system, a retrospective cohort study evaluated the AST-to-platelet ratio index (APRI) in a training and validation set of 270 patients.⁴⁴ The AUROC values for significant fibrosis (Ishak score >3) and cirrhosis (Ishak score 5 or 6) in the validation sets were 0.88 and 0.94, respectively. By using optimized cut-offs, significant fibrosis and cirrhosis could be accurately predicted in 51% and 81% of patients, respectively. The index could not accurately differentiate between the presence or absence of significant fibrosis in approximately 50% of patients. However, with simple parameters this index could potentially be used to avoid pretreatment liver biopsies in a significant proportion of chronic hepatitis C patients, if exclusion of significant fibrosis was the only aim of such a biopsy. External validation of this index in a French cohort using the METAVIR scoring system suggested a lower predictive AUROC for significant fibrosis of 0.74 and reduced discriminative ability compared to a validated 5-marker index.⁴⁵

Another predictive index, the Forns index, based on age, platelet count, GGT, and cholesterol, was developed in 476 patients with chronic hepatitis C to differentiate between METAVIR stages F2-F4 and F0-F1.⁴⁶ Using optimal cut-offs, the AUROC was 0.86 for the estimation group and 0.81 for the validation group. In the validation cohort of 125 patients the lower cut-off predicted that 39% of the cohort could be excluded as having stage F2-F4 disease with 96% certainty. The upper cut-off had a positive predictive value of 66% and applied to 12% of the validation set. As with the APRI model, although approximately half of patients would expect to have index values between the proposed cut-offs, where the predictive accuracy of the model is lower, there is the potential to reduce the need for a pretreatment liver biopsy in a significant number of patients. External validation of the Forns index indicated that the strengths of the model were in the exclusion of significant disease in approximately one third of patients.⁴⁷ Likewise, the Fibrotest was noted to have a slightly higher AUROC in a French cohort.⁴⁸ A potential limitation of this index may have been the use of cholesterol, which varies by genotype and is population dependent, as a variable. However, the fibrosis probability index that was developed in an Australian chronic hepatitis C cohort with a larger proportion of genotype-3 patients also included cholesterol as part of its regression model for prediction of stage F2-F4 disease. Other variables included age at biopsy, prior alcohol intake, serum

AST, and insulin resistance. The AUROC for the validation cohort was 0.77 in this study.⁴⁹

Marker Algorithms Using Combinations of Tests

In a single-center cohort of 339 chronic hepatitis C patients, the French MULTIVIRC group evaluated 11 potential markers of fibrosis that included transaminases, globulin fractions, and cytokines such as interleukin (IL)-10, and tumor growth factor β 1 (TGF- β 1). Neural connection and regression analysis selected a 5-marker predictive algorithm for stages F2–F4 that included α -2-macroglobulin, haptoglobin, GGT, apolipoprotein A1, and total bilirubin. Using distinct cut-off values, this index was able to accurately identify the biopsy category in 46% of patients in the validation cohort.³⁸ This marker panel has been validated in a number of separate hepatitis C study cohorts and has been found to have an AUROC of 0.73–0.87.⁵⁰ Addition of ALT to the marker panel allows for prediction of METAVIR necro-inflammatory activity with an AUROC 0.79–0.86.⁵⁰ Further modifications to the regression index allowed for a more linear assessment of fibrosis stage and activity grade, and the tests are now commercially available in several countries (FibroTest-ActiTest [FT-AT], BioPredictive), including in the United States (HCV-FibroSure, LabCorp). Limitations of this test include false-positive results due to increases in bilirubin or decreases in haptoglobin, for example from hemolysis secondary to ribavirin therapy. Likewise, false-positive results may also occur in Gilbert disease, cholestasis, and acute inflammation (increase in α -2-macroglobulin). False-negative results may be due to inflammatory states that lead to increases in haptoglobin. In a tertiary center evaluation of over 537 patients with chronic hepatitis C, only 14% of biopsies were over 25 mm in length and, in most cases, discordance between markers and biopsy related to inadequate sampling.⁵¹ Steatosis, inflammation, and biopsy size were also found to be associated with discordance between FT-AT and biopsy findings. The FT-AT has also been validated in HIV co-infection⁵² and other chronic liver diseases.^{53,54}

Specific Markers of Fibrogenesis

A potential limitation of markers and algorithms that utilize biochemical tests of hepatic inflammation, liver function, and/or the severity of portal hypertension, such as the APRI, FT-AT, or Forns index, is that they do not assess the underlying biology of the fibrogenic process. In recent years, significant progress in our understanding of the biology of the fibrogenesis cascade has led to the development of markers based on an understanding of ECM biology and metabolism. Markers that have been evaluated either alone or in combination include glycoproteins deposited in the basement membrane such

as HA, laminin, tenascin, and YKL-40 (human cartilage protein 39); procollagen peptides I and III and type IV collagen that reflect matrix deposition and remodeling; matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (eg, TIMP-1); and profibrogenic cytokines such as TGF- β and the antifibrotic IL-10. In theory, measurement of these markers should reflect the mass of ECM as well as fibrogenic activity in the liver. However, none of these markers are liver-specific, and in some cases serum levels are dependent upon hepatic and renal clearance. For example, HA levels can be elevated postprandially or in patients with inflammatory arthritis.^{55,56} Furthermore, these specific markers may reflect the dynamic state of fibrogenesis and their validation against static measures of histologic fibrosis has led to difficulties in determining their true utility. For example, altering the fibrogenic cascade through treatment may lead to changes in such markers before any significant changes in fibrosis stage. In addition, favorable biologic changes in fibrogenesis are difficult to collate with clinical outcomes.

Serum HA has been one of the most studied markers of fibrosis. Studies have shown that serum HA has a better ability to predict fibrosis than procollagen type III amino terminal peptide (PIIINP),⁵⁷ laminin, TGF- β , and a number of other clinical and biochemical variables.⁴¹ Although as a single marker a low serum HA can exclude the presence of significant fibrosis in patients with chronic hepatitis C,⁵⁸ it has limited utility in longitudinal assessment of fibrosis.⁵⁹

Two recent studies from the United States and Europe have developed diagnostic panels based on specific ECM markers.^{60,61} In one study, seven candidate markers were initially evaluated in 294 chronic hepatitis C patients. These markers included HA, TIMP-1, laminin, PIIINP, type IV collagen, YKL-40, and α -2-macroglobulin. Three markers, HA, TIMP-1, and α -2-macroglobulin, were selected as having the best diagnostic accuracy for F2–F4 fibrosis, with an accuracy of 75% in an external validation cohort of 402 patients. The derived algorithm and regression index provides a continuous scale for the determination of fibrosis stage, without indeterminate values, which was a limitation of earlier algorithms that relied on cut-off values. This test panel is commercially available in the United States (FIBROspect II, Prometheus Laboratories).⁶⁰ Prospective validation of this marker panel and determination of its utility in following changes in fibrosis are in progress.

Nine different markers were evaluated prospectively in a large multicenter study of 1,021 patients with chronic liver disease. These included collagen IV and VI, PIIINP, MMP-2, MMP-9, TIMP-1, tenascin, laminin, and HA. The derived algorithm included age, PIIINP, TIMP-1, and HA as being the most predictive for the detection

Table 4. Recent Serum Biomarker Algorithms

Study	N	Disease	Selected Markers and Variables	Fibrosis Stage Selected For	AUROC	Continuous Algorithm
Imbert-Bismut et al ³⁸	339	HCV	A2M Haptoglobin GGT APO A1 Total bilirubin	METAVIR F2–F4	0.84–0.87	No*
Forns et al ⁴⁶	476	HCV	GGT Cholesterol Platelets Age	METAVIR F2–F4	0.81–0.86	No
Wai et al ⁴⁴	270	HCV	AST Platelets	Ishak ≥ 3	0.80–0.88	No
Sud et al ⁴⁹	302	HCV	AST Cholesterol Insulin resistance Age Past alcohol	METAVIR F2–F4	0.77–0.84	No
Patel et al ⁶⁰	696	HCV	HA TIMP-1 A2M	METAVIR F2–F4	0.82–0.83	Yes
Rosenberg et al ⁶¹	496 64 61	HCV ALD NAFLD	HA TIMP-1 PIIINP	Scheuer stages 2–4	0.77 0.94 0.87	Yes

* In addition, a continuous index was developed from an integrated database of 1,570 HCV patients with AUROC METAVIR F2–F4 = 0.75–0.86.⁵⁰

A2M = α -2-macroglobulin; ALD = alcoholic liver disease; APO A1 = apolipoprotein A1; AST = aspartate aminotransferase; AUROC = area under receiver operating characteristic curve; GGT = gamma glutamyltransferase; HA = hyaluronic acid; HCV = hepatitis C virus; NAFLD = nonalcoholic fatty liver disease; PIIINP = procollagen type III amino terminal peptide; TIMP-1 = tissue inhibitor of metalloproteinase-1.

of significant fibrosis, with AUROC values of 0.77 for hepatitis C, 0.87 for nonalcoholic fatty liver disease, and 0.94 for alcoholic liver disease.⁶¹

The impact of biopsy size as a potential limiting factor in accurate staging was not assessed in these specific marker panel studies. Although direct comparisons have not been made between validated measures such as FT-AT and the specific marker panels incorporating ECM proteins, it seems unlikely that there would be any significant difference between these tests. Despite considerable progress, currently available serum marker panels do not quite meet all the criteria of an ideal fibrosis marker as outlined in Table 1. The ability of the marker panels to accurately differentiate between individual stages of fibrosis is still limited by the performance of the markers as much as the poor agreement between pathologists for staging moderate range fibrosis and sampling variability. Nevertheless, serum marker panels may be useful in patients hesitant to have a liver biopsy, those with relative contraindications such as coagulopathy, or when inadequate sampling limits histologic assessment. The high negative predictive

values of many of the marker panels suggest that their main utility at present could lie in excluding the presence of extensive fibrosis (Table 4).

Serum Markers and Change in Fibrosis

Accurate monitoring of changes in fibrosis would be helpful in following the natural history of disease, the need for intervention, and the response to treatment. Measurement of the dynamic process may be useful in determining the efficacy of potential antifibrotic agents.⁶² However, the ECM structure and content is certainly not linear and varies with advancing disease. Thus, longer treatment intervals may be required to observe histologic changes at the advanced stages compared to milder degrees of fibrosis. Serum correlates of ECM turnover may be particularly useful in this regard, and allow for the detection of early and favorable biologic changes in response to treatment. However, none of the currently available marker panels were designed to detect subtle changes in matrix turnover and, as of yet, none have been tested for

this purpose. Marker panels such as the FT-AT are currently being evaluated prospectively in ongoing long-term studies of maintenance interferon (IFN) therapy⁶³ and should provide useful information regarding their utility in following changes in fibrosis.

Some studies have noted that ECM markers decrease in proportion to virologic and biochemical responses induced by IFN therapy in patients with chronic hepatitis C,^{64,65} and do not change significantly in nonresponders.⁶⁶ Serum HA has been shown to reflect virologic response to therapy and parallel histologic changes,^{67,68} but is a poor predictor of changes in fibrosis stage.⁵⁹ In patients with hepatitis C virus treated with IFN monotherapy, the FT-AT fibrosis index was noted to decrease significantly following a sustained virologic response (SVR) compared to nonresponders or patients that relapsed. Similar differences were not observed for serum HA.⁶⁹ A retrospective study of 352 patients receiving combination therapy demonstrated that the FT-AT index decreased in patients who achieved an SVR, and that there was significant concordance between FT-AT and fibrosis stage. Importantly, the diagnostic value of FT-AT also improved when the biopsy size was larger than 15 mm.⁷⁰

Serum markers may also provide additional clinical or prognostic information in patients with advanced liver disease. Serum HA may be predictive of severe complications in patients with compensated cirrhosis due to hepatitis C.⁷¹ Markers such as PIIINP and YKL-40 may also predict clinical outcomes in other chronic liver diseases such as primary biliary cirrhosis⁷² or alcoholic liver disease.⁷³ It seems likely these markers reflect more advanced disease in general, and their ability to identify individuals at risk for disease progression and poor outcome remains to be determined.

Other Diagnostic Approaches

Newer diagnostic approaches for fibrosis that have shown promise include serum protein profiling and transient elastography. A number of protein profiling arrays are now available and can be configured to measure protein expression, protein-protein interaction, or enzymatic activity. The availability of such high-throughput techniques continues to provide exciting and new diagnostic approaches to the biomarkers discovery arena in general. A recent study in 46 patients with chronic hepatitis B used the SELDI ProteinChip system (Ciphergen Biosystems) to identify 30 proteomic features predictive of significant fibrosis (Ishak stage ≥ 3) and cirrhosis. The AUROC for this analysis was 0.906 and 0.921 for advanced fibrosis and cirrhosis, respectively, although close to 60% of the study cohort had moderate- to severe-stage fibrosis. Inclusion of ALT, bilirubin, and international normalized

ratio improved the performance of the model.⁷⁴ Another study in 193 patients with chronic hepatitis C identified eight peaks that differentiated METAVIR fibrosis stages with an AUROC of 0.88 compared to 0.81 for the FibroTest.⁷⁵ Other novel serodiagnostic approaches have included the measurement of serum protein N-glycan profiles (GlycoCirrho Test) with 100% specificity and 75% sensitivity for diagnosis of compensated cirrhosis when combined with the FibroTest.⁷⁶ These early studies will need to be validated in separate cohorts, and although encouraging, potential issues including the marginal benefit in fibrosis staging, cost, and reproducibility of results remain.

Transient elastography (FibroScan, Echosens) is a novel noninvasive method that uses pulse-echo ultrasound acquisitions to measure liver stiffness and predict fibrosis stage. In a prospective multicenter study in France of 327 chronic hepatitis C patients, the AUROC for METAVIR stage F2–F4 and cirrhosis were 0.79 and 0.97, respectively.⁷⁷ Steatosis and necroinflammatory activity did not impact significantly the correlation between liver stiffness and fibrosis in this study. In a separate study of 183 chronic hepatitis C patients, transient elastography compared favorably with the FibroTest and APRI panel (AUROC for F2–F4=0.83, 0.85, and 0.78, respectively). When transient elastography was combined with the FibroTest, the predictive value for fibrosis stages F2–F4 was improved, with an AUROC of 0.88.⁷⁸ The technique is reported to have good reproducibility with low inter- and intraobserver variability. Ten validated measurements are acquired in each patient, further reducing the potential for sampling-type errors. The depth of measurement from the skin surface is 25–65 mm, raising the possibility that this technique may be difficult to use in obese patients or ascites. However, newer probes are being developed for obese patients, and further study is expected.

Conclusions

Recent advances in our understanding of the biology and dynamic nature of fibrogenesis have led to an increasing challenge to accurately measure fibrosis and, moreover, to question the role of the liver biopsy. Although the qualitative information obtained from a biopsy remains of central importance to the clinician, liver biopsy is limited by the ability to reliably obtain an adequate sample, accurate representation of the usually non-homogeneously distributed disease process, and, importantly, the invasiveness of the procedure, which poses a small but definite risk to the patient. Serodiagnostic algorithms and other noninvasive diagnostic tests may provide a more accurate reflection of disease severity. However, overcoming the inherent issue of validation against a static and imperfect test such as

liver biopsy has been a significant limitation. Refinements in our understanding of the quality of biopsy have certainly helped in this regard. Our noninvasive armamentarium for diagnostic testing has increased in recent years. As with liver biopsy, noninvasive markers are imperfect. Limitations include the fact that serum fibrosis markers have been developed mostly in chronic hepatitis C cohorts in tertiary centers and the widespread clinical application of these noninvasive serodiagnostic panels to community-based populations and other chronic liver diseases requires further validation. Ongoing longitudinal assessment of various noninvasive measures is required and indeed could provide clinicians with an opportunity to reassure and follow many of their patients without the need for repeat biopsies. The integration of novel imaging and emerging technologies such as metabolomics into the realm of noninvasive tests is still required; movement of this technology from the bench to the bedside is expected to further improve the ability to noninvasively monitor liver fibrosis. Although no definite single test or algorithm can be currently recommended as an optimal tool to stage or quantitate fibrosis, noninvasive markers will improve and are likely to play an integral role in the management of patients with liver disease.

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