Assessment of Liver Fibrosis: Can Serum Become the Sample of Choice?

Chronic hepatitis is a relatively common problem; worldwide, an estimated 350 million individuals are chronically infected with hepatitis B (HBV), 170 million are chronically infected with hepatitis C (HCV), and nonalcoholic fatty liver disease is predicted to reach epidemic proportions with the rapidly increasing prevalence of obesity and type 2 diabetes mellitus. Chronic hepatitis causes relatively minor symptoms by itself, but it may progress to cirrhosis (the 10th leading cause of death in the United States) and hepatocellular carcinoma (the 3rd leading cause of cancer death worldwide). Although exact figures are lacking, it is estimated that ∼20%–30% of persons with chronic HBV or HCV will develop cirrhosis if untreated; however, successful treatment of HBV and HCV seems to prevent progression to cirrhosis and reduces the likelihood of or prevents development of hepatocellular carcinoma (1,2).

There are two major components to chronic hepatitis: necroinflammatory activity and fibrosis. The laboratory finding most correlated with activity is release of hepatocyte cytoplasmic enzymes such as aspartate and alanine aminotransferases. Unfortunately, the feature that best correlates with likelihood of developing cirrhosis is fibrosis; there is no correlation between aminotransferase activities and fibrosis.

Liver biopsy has been considered the gold standard for evaluation of extent of fibrosis, but it has considerable limitations. Biopsy is an expensive, invasive procedure with a considerable risk of complications (particularly bleeding) and a small chance (<1:1000) of death. Chronic hepatitis does not affect the liver uniformly; the extent of fibrosis may vary from one part of the liver to another, and liver biopsy samples only 1.5:000th of the mass of the liver. Even with adequate-sized biopsies, cirrhosis may be missed in 15%–30% of liver biopsies. Moreover, inter- and intraobserver agreement in liver biopsy interpretation are less than optimal, although agreement is generally high for the more important fibrosis component (3).

Because current treatments for both HBV and HCV have major limitations (e.g., high cost and frequent side effects when interferon is used) and are not universally effective (response rates typically 50% or less) (1,2), hepatologists generally recommend a liver biopsy, despite its drawbacks, to assess extent of disease before starting treatment. Individuals with minimal fibrosis (e.g., fibrosis stage <2 on the METAVIR scoring system) despite long duration of disease are generally felt to have little risk for progression to cirrhosis in the short term and are often assigned to watchful waiting, often with a repeat biopsy in 3–5 years. Because of the limitations of liver biopsy, there is strong interest in developing less invasive methods to assess liver fibrosis for both initial evaluation of patients and follow-up of those not treated or not responding to therapy.

Afjal and Nunes (3) suggest the following criteria for an ideal marker of liver fibrosis: it should be liver specific; should not be influenced by alterations in liver, renal, or reticuloendothelial function; should measure one or more of the processes related to fibrosis (stage of fibrosis, activity of matrix deposition, or activity of matrix removal); and should be easy to perform. Several approaches have been suggested. Evaluation of liver stiffness has recently been introduced. It is easy to perform, is related to stage of fibrosis, and is liver specific, but it has had limited evaluation (4). Markers of components of scar tissue, such as procollagen III aminopeptide, have been evaluated; although this marker is widely used in Europe for evaluating liver fibrosis in persons treated with methotrexate (5), it is probably not liver specific and may be affected by other disease processes. Although other markers of collagen formation have been evaluated, including hyaluronate (6), they are not liver specific and appear to be affected by activity at the time of the biopsy, rather than only cumulative fibrosis (7).

Several predictive indices using more commonly performed laboratory tests have been proposed, including the Forns index (8), which uses age, γ-glutamyl transferase (GGT), cholesterol, platelet count, and prothrombin time; the APRI index, which uses aspartate aminotransferase activity and platelet count (9); and the Fibrotest/Actitest (10), which uses GGT, bilirubin, α2-macroglobulin, haptoglobin, and apolipoprotein A1 (along with alanine aminotransferase for the Actitest). All of these indices have been reported to have high negative predictive value for significant (stage 2 or higher) fibrosis; using ROC analysis, areas under the curve ≥0.8 or higher have been achieved, and the Fibrotest has high positive predictive value for significant fibrosis. In this issue of Clinical Chemistry, Adams et al. (11) report the development of Fibroscore, a similar multitest panel (using age, gender, bilirubin, GGT, hyaluronic acid, and α2-macroglobulin) to create an index (Hepascore) to predict likelihood of significant fibrosis. Some strengths of the current study are its comparison with other indices (including the APRI and Forns index) and its inclusion of a fibrosis-specific marker (hyaluronate); in the evaluable cases, Hepascore had a higher area under the curve than did the APRI or the Forns index. Like all of the reported indices, Hepascore has a “gray zone” in which the likelihood of significant fibrosis is uncertain; in most of the published studies, almost one-half of patients fall into the gray zone.

In light of the imperfect nature of liver biopsy, is it now appropriate to use such predictive indices as a replacement for liver biopsy? At least some have suggested that such an approach is reasonable (12,13). It is appropriate, however, to consider the limitations of the proposed indices in evaluation of liver fibrosis. First, none of these indices meet the criteria of Afdhal and Nunes (3) of being liver specific or measuring markers of fibrosis (although Hepascore does include one fibrosis marker). Most of the markers included in these indices are likely to reflect or be affected by necroinflammatory activity: α2-macroglobulin and haptoglobin are acute-phase response proteins, as well as hepatocyte products, and bilirubin, aspartate aminotransferase, and GGT increase with hepatocyte injury. In the most widely used Fibrotest, all 5 of the markers are also included.
in an index that evaluated necroinflammatory activity, highlighting the effect of this component on the markers. None of the studies controlled for degree of necroinflammatory activity, making it difficult to separate the effect of these two components on the indices. Few data have been published on the use of these markers to monitor response to treatment or their ability to monitor changes in stage of fibrosis over time. The developers of Fibrotest retrospectively found a correlation between changes in Fibrotest results and changes in fibrosis in those whose HCV virus concentrations became undetectable after treatment; however, there was no correction for changes in disease activity (14, 15). In addition, cutoff values may be affected by differences in the assays used to measure the individual markers used in the calculations (16, 17). Finally, most of the studies have been limited to patients with hepatitis C; it is not clear whether these indices will work as well in persons with other forms of liver disease. For the Fibrotest, correlation has also been shown with extent of fibrosis in persons with hepatitis B (18) or alcoholic hepatitis (19); however, the cutoff values for recognizing significant fibrosis were different from those used in persons with hepatitis C.

In light of these limitations, it does not seem appropriate to completely replace liver biopsy with predictive indices at the present time, despite the imperfections of biopsy as a gold standard. Because very low values in the indices have high negative predictive value, it may be appropriate to forego liver biopsy in those with low likelihood of significant fibrosis based on a prognostic index, as suggested by others (3).

In an editorial in this journal last year, Afadh (20) indicated that the important clinical question is how to best use the current established markers. What needs to be done before serum markers can be accepted as replacements for the imperfect gold standard, the liver biopsy? In addition to the suggestions of Afadh and Nunes (3), I offer the following considerations for future research into this promising field. First, as Adams et al. (11) imply in their report, algorithms or equations should be published to allow comparison of their predictive ability across institutions. Ideally, formulae should be validated by use of assays from different manufacturers, in different laboratories, or at least on different lots of reagents because standardization of assays is often less than ideal. Liver biopsies should be analyzed both for stage of fibrosis and degree of activity; comparisons among stages should be controlled for differences in necroinflammatory activity. Correlation of changes in marker concentrations to changes in degree of fibrosis over time is an ideal that will be difficult to accomplish quickly for nonroutine markers, but it may be possible with more routinely performed tests (such as the APRI) by selectively studying patients with multiple liver biopsies. Finally, additional tests or panels will be necessary to reduce the gray zone of patients who cannot be classified by current predictive indices. These approaches could provide answers to critical questions that must be clarified before widespread replacement of liver biopsy by serum tests.

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References


D. Robert Dufour
Pathology and Laboratory Medicine Service Veterans Affairs Medical Center Washington, DC and Department of Pathology George Washington University Medical Center Washington, DC Fax 202-745-8284 E-mail d.robert.dufour@med.va.gov

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