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Assessing Fibrosis Without a Liver Biopsy: Are We There Yet?

See article on page 1704.

A noninvasive test of hepatic fibrosis is needed. Although long sought, it has become a priority in the context of hepatitis C evaluation and treatment. The current antiviral regimen for hepatitis C is toxic, expensive, and effective in only half of those who take it. As a result, many physicians reserve it for patients with evidence of disease progression, defined as histologic fibrosis. The latter requires a liver biopsy with its attendant cost and risks. Among the possible alternatives, imaging is informative mainly for cirrhosis and not for lesser stages of fibrosis. In addition, it is nonquantitative and thus cannot track progression.¹

Fibrosis arises as a result of wound repair, a process that has been studied intensively for several decades. We now know much about the composition and genesis of histologic fibrosis, knowledge that we should be able to exploit in devising alternatives to biopsy. Many groups have taken up the challenge. Starting 20 years ago, assays for circulating fragments of collagen and other matrix proteins were developed. However, they proved to be relatively insensitive and in recent years have yielded to multicomponent tests (Table 1). The MULTIVIRC (Paris) group developed a test based on serum markers.² A group from Sydney published a test that includes 3 other biochemical markers and 2 clinical parameters.³ A test from Barcelona uses standard clinical information.⁴ A recent article examines the pattern of serum protein N-glycation in patients with liver disease. Glycan profiling adds sensitivity and specificity to the MULTIVIRC

test, according to this preliminary study.⁵ The newer multicomponent tests are touted principally for identifying 2 groups of patients: those with minimal or no fibrosis and those with advanced fibrosis or cirrhosis. Their accuracy for intermediate fibrosis is relatively poor.

The commercial potential of noninvasive testing has not gone unnoticed. Some of the single-component tests were marketed and continue to be available. The MULTIVIRC group has produced their test in Europe as FibroTest (Bio-Predictive, Paris, France) and in North America as HCV-Fibrosure (LabCorp, Burlington, NC). Another commercial test is FibroSpectII from Prometheus Laboratories (San Diego, CA); its panel consists of hyaluronic acid, tissue inhibitor of metalloproteinase (TIMP)-1, and α -2-macroglobulin. Formulas based on standard clinical data also have been developed.^{4,6} For all of these, prospective randomized studies are needed to compare an individual test with routine clinical evaluation (including imaging) as well as with other tests. Validation in a variety of practice settings also will be important. Approximately 20% of patients scored by these

Table 1. Multicomponent Fibrosis Tests

MULTIVIRC ²	Sydney ³	Barcelona ⁴	ELF ⁸
GGT	Age	Age	Age
α -2-Macroglobulin	AST	GGT	Hyaluronic acid
Haptoglobin	Cholesterol	Cholesterol	Procollagen III peptide
Apolipoprotein A1	Insulin resistance	Platelet count	TIMP-1
Total bilirubin	Past alcohol use	Prothrombin time	

AST, aspartate aminotransferase; GGT, γ -glutamyltransferase.

tests as having either no/minimal fibrosis or advanced fibrosis are misclassified when compared with biopsy staging.⁷ It remains to be seen if proprietary tests improve patient outcomes sufficiently to justify their added cost.

In this issue of *GASTROENTEROLOGY*, a European consortium, ELF, offers yet another multicomponent test.⁸ The study is large (921 patients), it surveys 9 potential markers, and the results are correlated with liver biopsy. Moreover, the markers selected for study are closely associated with metabolism of the extracellular matrix (ECM), including ECM constituents or fragments; ECM-degrading enzymes (matrix metalloproteinases); and TIMP-1, an inhibitor of matrix metalloproteinases. On statistical analysis of the data, 4 of these were most useful: the patient's age, serum hyaluronic acid, procollagen-III N-terminal propeptide, and TIMP-1. The approach is rational and, for this reason, attractive. Nonetheless, the diagnostic accuracy of the ELF test appears to be similar to that of the previously published tests, that is, best for the extreme ends of the fibrosis spectrum.

The significant failing of these panels relates to detection of intermediate fibrosis. As already noted in the case of hepatitis C, patients with stage 2 or 3 fibrosis are the prime candidates for treatment. Also, an ideal test should have a dynamic range suitable for noninvasively monitoring changes in fibrosis over time. With all that we have learned about the biology of fibrosis in the past 30 years, one could reasonably ask, "Where is the test that we need?" The answer is in the biology. What we have learned is that, in tissue injury, the ECM undergoes extensive remodeling, with both fibrogenesis (production of ECM) and fibrolysis (degradation of ECM). Fibrosis is the net of these 2 processes. The dynamics of ECM metabolism vary with the type and stage of injury and with the periodicity of the repetitive injury. In self-limited injury, increased fibrogenesis and TIMP expression dominate the early phase, whereas the inverse, along with increased matrix metalloproteinase activity, occurs during the resolution phase. Unfortunately, this simple paradigm likely does not apply when the injury is multifocal, repetitive, and asynchronous with overlapping fibrogenesis and fibrolysis. An additional variable, the time frame of which is many months, is the conversion of accumulated matrix to a form that is resistant to degradation and, thus, permanent (ie, scar).

Scar formation alters liver structure, to which the liver responds with regeneration. If the injury process is slow, as in typical hepatitis C, regeneration will keep pace such that, through early fibrosis, the liver remains functionally normal. Patients with midstage fibrosis (stage 2–3) likely represent a mix of fully compensated disease and mild-to-moderate functional compromise. Predictably, tests

designed to detect a functional deficit will have difficulty with this group of patients. As the scarring process progresses, it causes frank elevation of portal pressure and shunting. At this point, liver metabolism is broadly deranged, a state that will be detected by any number of tests. This is likely the reason that disparate test panels (Table 1) are similarly good at detecting advanced fibrosis or cirrhosis.

As this area advances with additional candidate tests, an issue that will need to be addressed concerns the use of liver biopsy as the gold standard. Sampling variation is a known problem but has come to the fore with attempts to quantify fibrosis progression by using liver biopsy. In one well-designed study, 124 patients with chronic hepatitis C underwent laparoscopic needle biopsy of both right and left hepatic lobes. Hepatitis C is believed to involve the liver uniformly. However, in one-third of the cohort, the paired samples differed by a full stage, a finding that could not be attributed to interobserver variation.⁹ This introduces a large amount of uncertainty when the disease under study (hepatitis C) progresses at a median rate of 0.15 stage per year,¹⁰ unless the study population is very large or the interval between biopsies is several years. No doubt sampling variation is caused by the fact that the 10–15 mg of tissue from a needle biopsy is but a tiny fraction of an organ weighing 1500 g, and even a disease that affects the liver relatively uniformly will vary from lobule to lobule. Recognition of this issue has led to efforts to define an adequate biopsy. The emerging standard is a core measuring at least 2.5 cm in length with 4 or more portal tracts.¹¹ A criticism of the ELF article is that these data were not recorded, although biopsies meeting these criteria would only lessen, not eliminate, the problem of sampling variation. It is entirely conceivable that a well-designed, noninvasive test would be not only safer but also more accurate than a biopsy.

For purposes of clinical decision-making, a test of fibrogenesis arguably would be more useful than a test of fibrosis. In general, the critical question is not how much scar is present but whether scar formation is ongoing. Quiescent scar, even if extensive, is compatible with normal health, provided the cause of injury has been eliminated and the liver has been able to compensate. This is apparent clinically in patients with hepatitis C cirrhosis who have undergone successful treatment, and also in those with alcoholic cirrhosis who abstain. A test for fibrogenesis would confirm that these patients are not progressing despite their extensive fibrosis. It would also identify patients with active hepatitis C, who are fully compensated but have progressive fibrosis. Finally, such a test would be useful for tracking the response to

therapy. In some patients with chronic hepatitis C, the current treatment may slow fibrosis progression even if the viral response is unsustainable. At present, identifying those patients requires initial and follow-up biopsies. The alternative, which some hepatologists are adopting, is to forego serial biopsy and simply place all patients with significant fibrosis on a long-term antiviral regimen. Neither approach is satisfactory for reasons of safety and cost.

In most liver diseases, inflammation appears to drive fibrogenesis, suggesting that clinical tests of inflammation, such as ALT and AST, may serve as surrogates. However, for hepatitis C it is clear that fibrosis progression correlates imperfectly with ALT and AST.¹⁰ A more accurate test, in principle, would involve markers directly related to fibrogenesis, such as ECM protein fragments. The collagen polypeptide is secreted with propeptides, which are cleaved before assembly of the triple-helical molecule and therefore reflect production of collagen, much as C-peptide is a measure of insulin production. However, the same peptides are released during normal turnover and replacement of collagen in skin, bone, and the vasculature. Because these tissues are rich in collagen, the background level of circulating peptide is substantial, making it difficult to detect a contribution from the injured liver. More promising as a test of hepatic fibrogenesis would be proteins expressed selectively during injury and related to ECM formation. Markers of the fibrogenic response to injury are known but have not been examined comprehensively. Expression arrays now represent most of the genome, are able to detect low-abundance message, and could be used to compare normal and acutely injured liver including stellate cells isolated from these tissues. Also worth exploring is the ability of the existing tests to track changes in fibrogenesis, independent of fibrosis. This would involve correlating test improvement with elimination of the injury factor (for example, hepatitis B virus, hepatitis C virus, or alcohol) rather than with a change in fibrosis on biopsy (although these will correlate in some patients). The MULTIVIRC panel includes 2 acute phase reactants, α -2-macroglobulin and haptoglobin, which will reflect on-going inflammation and thus may track fibrogenesis. The ELF panel has 2 constituents, procollagen-III peptide and TIMP-1, which increase with acute injury and are directly involved in fibrogenesis.

The statistical methods used in developing these tests are powerful even if, for some readers, they represent the fog of statistics. They have the virtue of being unbiased

and potentially are able to reveal new facets of fibrosis. Test performance will improve with introduction of markers that are closely related to wound repair, particularly when the focus is fibrogenesis rather than fibrosis. We are not there yet, but we are getting closer.

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