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Usefulness of Non-Invasive Tools in Liver Fibrosis Assessment

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Liver fibrosis occurs as the response of chronic liver injury. In the past, it was thought as irreversible but now is recognized as a dynamic process with possibility of significant resolution. Regardless of the cause, the response to liver injury includes collapse of hepatic lobules, formation of fibrous septae, and hepatocyte regeneration with nodular formation. Conventional biochemical and serological tests have no significant value for assessment of liver fibrosis and liver biopsy specimen is still the "gold standard" for staging of liver diseases. The incessant progress in production of new pharmaceuticals creates an enormous need for non-invasive diagnostics of liver fibrosis. The lack of appropriate non-invasive tools is currently the main limitation for monitoring of disease progression, predicting clinical outcomes and evaluating therapeutic effects. At this time, two groups of diagnostic tools are used for non-invasive assessment of liver fibrosis: imaging techniques and biologically-based markers. Some of them like transient elastogarphy are very promising and others like biologically based markers are helpful but cannot serve as independent markers of liver fibrosis. Therefore, experimental and clinical hepatology of the next decade have a great challenge to find a more accurate, reproducible and non-invasive technique for hepatic fibrosis assessment.

Keywords: **Liver Fibrosis, Transient Elastography, Direct Markers, Indirect Markers**

Introduction

Liver fibrosis results from chronic liver injury. Though previously considered irreversible, liver fibrosis is now recognized as a dynamic process with significant prospects for remission. While the exact "point of no return" is undetermined, increasing evidence suggests that even late stages of cirrhosis may be reversible. Regardless of the cause, liver injury leads to collapse of hepatic lobules, formation of fibrous septae, and hepatocyte regeneration with nodule formation. As a result of imbalances between production, deposition and degradation, the components of extracellular matrix (ECM) accumulate in the liver, often leading to progressive fibrosis, characterized by portal hypertension and impaired hepatic function.

While biomedical industries have great interest for treatment of hepatic diseases, the lack of reliable non-invasive tests evaluating hepatic fibrosis limits monitoring of disease progression and response to pharmacological treatment. Conventional biochemical and serological tests are of small value in assessing hepatic fibrosis and biopsy is still the "gold standard" for evaluating fibro-inflammatory activity in the injured liver. But even biopsy has its own limitations as this procedure is disliked by patients, and a small tissue sample can be source of a significant inter- and intra-observer error. Thus, experimental and clinical hepatologists would tremendously benefit from a non-invasive, accurate and reproducible technique for hepatic fibrosis assessment.

Despite increasing reliability of non-invasive tools for diagnosis of hepatic fibrosis, there is still no substitute for direct pathomorphological examination. We thus should ask how ideal the non-invasive fibrosis marker must be. In some ways, the answer is clear; in others it is only hypothetical. The

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most important features are high diagnostic sensitivity and specificity, providing near 1 value of area under the receiver-operating characteristics curve (ROC) (Table 1). This area ranges between 0.5 (no prediction) and 1.0 (perfect prediction). Desirable features are also an excellent reproducibility, close correlations to disease severity and clinical outcomes as well as resistance to effects of drugs or extrahepatic diseases. It should be acknowledged that at present no serum compound has emerged as the perfect measure of fibrosis, since all tests in current use have less or more serious limitations. Today, two groups of diagnostic tools are used for non-invasive assessment of liver fibrosis i.e., imaging techniques and biologically-based markers.

Imaging techniques

Classic cross-sectional imaging techniques like computerized tomography, magnetic resonance or ultrasound examination can demonstrate advanced liver fibrosis or cirrhosis (nodules formation) with aspects of portal hypertension. Such methods definitively fail to reveal early stages of fibrosis. A new and promising technique is transient hepatic elastography. This diagnostic tool measures the stiffness of hepatic tissue. By a probe attached to an ultrasonic transducer, a vibration of low frequency and low amplitude is transmitted into the liver. That wave induces an elastic shear wave that propagates through the liver tissue, with velocity correlating directly with tissue stiffness. Thus harder hepatic tissue permits faster propagation of the shear wave. The device measures stiffness of a cylinder of 1 centimeter diameter and 5 centimeter length (about 100 times the sample size of the standard liver biopsy).

In a study of 775 patients with various forms and severity of chronic liver disease, the sensitivity and specificity of this technique, at an optimal cut-off value, ranged from 79 to 95 percent and from 78 to 95 percent, respectively. The elastography in this study was compared to a liver biopsy as the reference method [1]. Ziol *et al.* [2] prospectively enrolled 327 patients with chronic hepatitis C, in whom 5-degree Metavir fibrosis staging (F0-F4) was assessed by two independent pathologists and liver stiffness was measured by transient elastography (Fibroscan). Fibroscan measurements were correlated with histological assessment of fibrosis severity (Kendall correlation coefficient: 0.55; P<.0001) with following area under ROCs: 0.79 for F>2, 0.91 for F>3 and 0.97 for F=4. Fibrosis stages of F>2 and F=4 were most favorably detected by stiffness cutoff values of 8.7 and 14.5 kPa, respectively. In a study of Vizzutti *et al.* [3], a strong correlation between liver stiffness measurements and the hepatic venous pressure gradient was found. The authors suggested that this technique could serve as a non-invasive tool for identifying patients with severe portal hypertension. Moreover, it might be useful for recognition of patients needing a screening endoscopy to detect esophageal varices.

It seems that transient hepatic elastography possesses many desirable features of non-invasive tool for hepatic fibrosis assessment. This technique is rapid, painless, and inexpensive and what is very important- reproducible. Moreover, it allows examining a much bigger piece of liver tissue than liver biopsy does, which significantly reduces a sampling error. The limitations of Fibroscan use are ascites and extreme obesity because both the fluid and adipose tissue attenuate the elastic wave.

Table 1. Accuracy of laboratory panels for assessment of liver fibrosis.

Test	Sensitivity (%)	Specificity (%)	Area under ROC	Validation groups of patients	Remarks
APRI	37 - 80	30-100	0.61-0.94	HCV, ALD	Data largely scattered
PGA Index	97	73	0.89	HCV, ALD	Advanced fibrosis and cirrhosis
FibroIndex	78	74	0.83	HCV	Significant fibrosis (Metavir>2)
Fibrotest	75	85	0.73-0.87	HCV	Significant fibrosis (Metavir>2)
ActiTest	91	75	0.75-0.86	HCV	Significant fibrosis (Metavir>2)
NASH FibroSURE™	83	78	0.86	NASH	Significant fibrosis (Metavir>2)

ALD: alcoholic liver disease; NASH: non-alcoholic steatohepatitis

Biologically based markers

A variety of serological markers have been evaluated in the hope of accurately measuring the degree of liver fibrosis. It must be realized that hepatic fibrogenesis is a dynamic process. Majority of tests are more suitable for determining the rate of fibrosis development and/or response to therapy rather than for assessment of hepatic fibrosis stage at a particular point in time. Serological markers for hepatic fibrosis can be divided into two general categories i.e., indirect markers, which are not directly associated with ECM metabolism (Table 2), and direct markers, which reflect ECM turnover (Table 3).

Table 2. Indirect markers of hepatic fibrosis.

Test	Composition
AST/ALT ratio	Aspartate aminotransferase, alanine aminotransferase,
APRI	Aspartate aminotransferase to platelet ratio index
PGA Index	Prothrombin index, gamma-glutamyltransferase, apolipoprotein A1
FibroIndex	Platelet count, aspartate aminotransferase, gamma-globulin
Fibrotest	$Alpha_2$ macroglobulin, haptoglobin, gamma-globulin, apolipoprotein A1, gamma-glutamyltransferase, total bilirubin
ActiTest	$Alpha_2$ macroglobulin, haptoglobin, gamma-globulin, apolipoprotein A1, gamma-glutamyltransferase, total bilirubin, alanine aminotransferase
NASH FibroSURE™	Age, gender, $alpha_2$ macroglobulin, alanine aminotransferase, aspartate aminotransferase, apolipoprotein A1, total bilirubin, total cholesterol, gamma-glutamyltransferase ,glucose, haptoglobin, triglycerides
NashTest	Age, sex, height, weight, triglycerides, cholesterol, $alpha_2$ macroglobulin, apolipoprotein A1, haptoglobin, gamma-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, total bilirubin
Proteomics	Various protein patterns

Table 3. Direct markers of hepatic fibrosis.

Test	Composition
PICP	Procollagen type I carboxy-terminal peptide
PIIINP	Procollagen type III amino-terminal peptide
MMPs	Matrix metalloproteinase
TIMPs	Tissue inhibitors of metalloproteinase
TGF-β	Transforming growth factor beta
TGF-α	Transforming growth factor alpha
PDGF	Platelet derived growth factor
HA	Hyaluronic acid
YKL-40	Chondrex

Among the indirect markers, there are some bedside tests like AST/ALT ratio, APRI, PGA or FibroIndex. It is well established that AST/ALT ratio in normal subjects is about 0.8; while in the presence of advanced fibrosis is frequently greater than 1. However, the pathogenesis of this finding still remains unclear and assessment of liver fibrosis exclusively with AST/ALT ratio is uncertain [4, 5]. The utility of APRI test as a non-invasive marker of hepatic fibrosis has been tested in many studies. Majority of them concerned patients with chronic hepatitis C and alcoholic liver disease. The diagnostic sensitivity ranged from 37 to 80 percent, specificity from 30 to 100 percent and positive predictive value from 57 to 100 percent, depending upon the selected cutoff value and whether the test was being used to predict precirrhotic or cirrhotic stage of fibrosis [6-9].

Oberti *et al.* [10] and Poynard *et al.* [11] tested PGA Index for detection of liver fibrosis and cirrhosis in patients with viral and alcoholic liver diseases. The authors found that accuracy of PGA Index for the detection of cirrhosis has ranged between 66 and 72 percent and proposed its general use to identify patients at high risk of severe liver damage. FibroIndex is one of the recently studied biomarker of significant fibrosis in patients with chronic HCV infection. It was also proposed to be a surrogate marker of response to antiviral therapy; however, its clinical significance needs to be validated in future trials [12].

Gawrieh *et al.* [13] tested the potential utility of serum levels of adipokines in differentiating between simple steatosis and nonalcoholic steatohepatitis (NASH). Serum levels of leptin, resistin and adiponectin were measured in 20 patients. In 10 patients fulfilling histological criteria of NASH, the serum levels of leptin and resistin were significantly higher, whereas serum levels of adiponectin were significantly lower than in 10 patients with simple steatosis. Moreover, the combination of leptin level equal to or above 40 ng/ml and/or resistin equal to or above 1.1 ng/ml had a sensitivity of 100% and specificity of 90% in differentiating between NASH and bland steatosis. Due to the small number of examined patients this observation needs to be confirmed in future trials.

Other tests like Fibrotest, ActiTest, NASH FibroSURE™ and NashTest are based on algorithms comprising several parameters [14, 15]. The sensitivity and specificity of Fibrotest for detection of significant fibrosis approximates 75 and 85 percent, respectively [16, 17]. Castera *et al.* [18] evaluated a combination of Fibrotest with Fibroscan in 183 patients with chronic hepatitis C. When the

figures of both tests agreed, liver biopsy examination was confirmative in 84 percent of cases for F>2, in 95 percent for F>3 and in 94 percent for F=4. According to authors' opinion, these two non-invasive tests complement each other and if used in combination increase the accuracy of fibrosis detection [18].

The data coming from a study of Halfon *et al.* [19] suggest that ActiTest reflects both liver fibrosis as well as necro-inflammatory activity. It seems that this tool improves identification of advanced fibrosis associated with coexistent inflammation. The results of meta-analysis of 1570 patients with chronic HCV infection made by Poynard *et al.* [20] confirmed that ActiTest and Fibrotest are useful in monitoring of the histological response to antiviral treatment, especially in patients who have achieved a sustained virologic response. Both tests were reliable alternatives to liver biopsy.

NASH FibroSURE™ is noninvasive test for assessment of liver fibrosis in patients with NASH. This test is based on algorithm of 12 quantitative variables containing laboratory and demographic data such as age and gender. In a study by Ratziu *et al.* [21] including 171 patients with nonalcoholic fatty liver disease (NAFLD), sensitivity and specificity for the detection of significant fibrosis (Metavir F2-F4) were 83% and 78%, respectively. At present, NASH FibroSURE™ is not recommended for patients with other liver diseases due to lack of validation studies. Poynard *et al.* developed and validated the NashTest, which is based on currently patented algorithm combining age, sex, height, weight and nine serum biochemical parameters. The clinical usefulness of NashTest was confirmed on a cohort of 160 patients with NAFLD [15]. The efficacy to identify NASH by this test was compared with Kleiner & Brunt histological scoring system with an area under ROC of 0.79. In authors' opinion, the NashTest reliably predicts the presence or absence of NASH in patients with NAFLD.

Non-invasive approach for assessing the severity of fibrosis in NAFLD patients has been proposed by Angulo *et al.* [22]. NAFLD fibrosis score based on hyperglycemia, body mass index (BMI), platelet count, albumin and AST/ALT ratio was validated on a group of 253 patients with histologically proven NAFLD. The area under ROC for NAFLD Fibrosis score was 0.82 and positive predictive value for advanced fibrosis was 82%. Therefore, it seems that NAFLD fibrosis score can effectively select the subpopulation of patients in whom liver biopsy should be done. Further clinical trials which would combine the NAFLD Fibrosis score with other serum markers of fibrosis or imaging techniques are awaited. Next promising technique in the group of indirect markers is proteomics-based assessment of serum sample composition by mass spectroscopy. In a Belgian study a combined analysis of serum glycoproteins with Fibrotest provided impressive correlations, however, a clinical applicability of this technique requires further trials [23].

Direct markers of liver fibrosis include those associated with matrix deposition or degradation, and different cytokines and chemokines associated with the fibrogenesis. Markers linked to matrix deposition are generally based on detection of different carboxy and amino terminal procollagen peptides, which are cleaved off the procollagen molecule during the synthesis of collagen fibrils. Such tests involve procollagen type I carboxy-terminal peptide (PICP) and procollagen type III amino-terminal peptide (PIIINP). The usefulness of PICP and PIIINP in liver fibrosis assessment has been confirmed in various studies carried out in patients with alcoholic liver disease, viral hepatitis or primary biliary cirrhosis [24-28].

Another group of markers comprises hyaluronic acid (HA) and chondrex (YKL-40). Pares *et al.* [29] provided evidence that in patients with alcoholic liver disease serum concentration of HA reflects both the severity of inflammation and degree of fibrosis, however, abstinence from alcohol caused immediate reduction in serum HA levels. Similarly, Johansen *et al.* [30] showed that serum concentration of chondrex is increased in patients with alcoholic liver disease. Data coming from a study of Tran *et al.* [31] confirm the significant correlations between serum YKL-40 level, degree of liver fibrosis and HA serum concentration. Generally, markers associated with matrix deposition can be useful in assessment of liver fibrosis, but cannot serve as the only predictors.

Markers associated with matrix degradation measure the action of a family of enzymes called matrix metalloproteinases (MMPs). These enzymes are synthesized intracellularly and secreted as pro-enzymes requiring cleavage by cell surface mechanisms for functional activity. MMPs are inhibited by tissue metalloproteinases inhibitors (TIMPs). The most important MMPs are MMP-2, MMP-3, and MMP-9. The observation that MMPs are overexpressed in liver injury suggests that degradation of normal liver matrix may be an important event in pathogenesis of hepatic fibrosis. Research to date has not determined the usefulness of MMPs or TIMPs as markers of liver fibrosis. Among the large number of cytokines and chemokines, the most promising factors in hepatic fibrogenesis seem to have been transforming growth

factor beta (TGF-β), transforming growth factor alpha (TGF-α), and platelet derived growth factor (PDGF) [32-34]. In conjunction with other non-invasive tools, these substances are useful indicators of hepatic fibrogenesis, but cannot be used as solitary predictors.

Future perspectives

It is generally known that liver fibrosis is a dynamic process in which multiple genes interact with environmental factors. To date, human epidemiological studies have identified various polymorphisms in a number of genes influencing liver fibrosis [35]. It appears that polymorphism in genes encoding immunoregulatory proteins and proinflammatory cytokines, as well as fibrogenic factors like integrins, cadherins and selectins have a significant impact on progression of hepatic fibrosis. Finding the most important factor can be a challenge for future well-designed and large-scale studies.

Other sophisticated methods like proteomics and glycomics can help establish fibrosis-specific serum proteins and glycosylation patterns, and could play important roles in diagnosis and monitoring of fibrogenesis. Studies to date have been few, leaving their usefulness to be determined and research in this direction seems worth pursuing.

Conclusions

Published data suggest that elastography is promising technique, which shows correlation with stage of fibrosis and has a potential to reliably differentiate early from advanced fibrosis. The biologically-based markers of liver fibrosis used in different combinations may also become useful in the diagnosis and clinical management of various chronic liver diseases. Up to now, there is no evidence that any single test is able to discern a subtle progression or regression in liver fibrotic content in an individual patient over time. However, all these serum markers present in the market are valuable techniques in cohort studies in which median values reflect confidently general expansion of fibrosis in investigated population. Non-invasive techniques established an important alternative for patients who either have a high risk of complications from liver biopsy or are under the care of non-hepatologists like these co-infected with HIV.

It seems that we are very close to the point when non-invasive techniques for liver fibrosis assessment become an integral part of diagnostics in patients with chronic liver diseases. The general use of these tests is expected to considerably reduce, but not to completely eliminate the need for liver biopsy.

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