CURRENT TREND

Noninvasive assessment of fibrosis and steatosis in NASH and ASH

Évaluation non-invasive de la fibrose et de la stéatose dans la NASH et la stéatopathie alcoolique

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Available online 1 October 2009

Summary  NAFLD and alcoholic liver disease affect a substantial proportion of the population worldwide. Although presence and amount of steatosis can be determined with a good level of accuracy using noninvasive imaging techniques, currently, there is no available noninvasive tests to distinguish between simple steatosis from steatohepatitis or to stage fibrosis that had demonstrated to be simple, reproducible, and valid in patients who have NAFLD or alcoholic liver disease. Liver biopsy remains a useful tool to confirm the diagnosis and exclude other liver disease and remains the only investigation able to provide prognostic information by staging and grading these diseases. Noninvasive serum markers offer considerable promise in their ability to stage liver fibrosis. Routine liver tests may detect occult cirrhosis but are insensitive at predicting lesser stages of fibrosis. Several serum markers of collagen synthesis and degradation are not validated sufficiently and are not available in most medical centers to replace liver biopsy. These markers currently may assist in stratifying patients who are more likely to have advanced fibrosis and, therefore, may benefit from proceeding with liver biopsy. It is likely the more complex models, which include multiple serum markers, will be more accurate at predicting fibrosis. These currently have limited ability at predicting the full range of liver fibrosis, generally having the greatest diagnostic accuracy at predicting advanced fibrosis or absent fibrosis but not in-between. Measuring liver stiffness with different imaging techniques holds promise, but further carefully designed studies are necessary before they can be recommended in clinical practice.

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Résumé La stéatopathie métabolique (NAFLD) et la maladie alcoolique du foie sont des affections fréquentes dans le monde. Bien que la présence et la quantité de stéatose puissent être diagnostiquées avec une bonne performance par les techniques non-invasives, il n’existe pas actuellement de méthode non-invasive permettant de différencier la stéatose de la stéato-hépatite ou permettant de diagnostiquer la fibrose hépatique de façon simple, reproductible et validée chez les patients avec NAFLD ou maladie alcoolique. La ponction-biopsie hépatique...
Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of fat (mainly triglycerides) in hepatocytes that results from insulin resistance. NAFLD is recognized as the most common chronic liver disease in the Western world. NAFLD encompasses a wide spectrum of disease from bland hepatic steatosis, which is generally benign, to nonalcoholic steatohepatitis (NASH), which when associated with increased liver fibrosis may progress to cirrhosis and liver failure. Hence, distinguishing between hepatic steatosis and NASH has important prognostic and management implications. NAFLD may be categorized as primary or secondary depending on the underlying pathogenesis. Primary NAFLD occurs most commonly, and is associated with insulin-resistant states, such as obesity, type II diabetes, and dyslipidemia. Other conditions associated with insulin resistance, such as polycystic ovarian syndrome and hypopituitarism, have also been described in association with NAFLD, although the exact prevalence and significance in these conditions remain unclear. Distinction from secondary types is important as these have differing treatment and prognosis. Primary NAFLD has reached epidemic proportions in many countries around the world as demonstrated in several population-based studies. In the United States, 34% of the population aged 30 to 65 years [1] and 9.6% of the population aged 2 to 19 years [2] have hepatic steatosis. If these figures are extrapolated to the 2008 US population, over 55 million Americans have NAFLD.

Alcohol-induced liver injury is the second most common liver disease in the United States and a common liver disease in many Western countries. In the United States, about 9% of the population aged 18 to 75 years [3], or over 20 million Americans, have some degree of alcohol-induced liver injury. Similar to NAFLD, alcohol-induced liver injury includes a wide spectrum of liver disease ranging from simple steatosis which is generally reversible with alcohol abstinence, to alcoholic steatohepatitis (ASH) which may progress to cirrhosis and liver failure. Just as a matter of comparison, hepatitis C virus (HCV) infection affects about 1.6%, or about four million people in the United States [4]. Thus, the prevalence of NAFLD in the general population in the US is almost 14-fold higher than HCV infection, and almost three-fold higher than alcohol-induced liver disease whereas alcohol-induced liver diseases is about five times higher than the prevalence of HCV infection.

Role of liver biopsy in nonalcoholic steatohepatitis and alcoholic steatohepatitis

The decision regarding whom and when to biopsy should take under consideration what information is to be obtained and how such information would affect patients. There are two general indications to perform a liver biopsy in patients who have suspected NASH or ASH: to confirm the diagnosis and stage of disease; and to determine prognosis based on severity of fibrosis. Liver biopsy is the only investigation that can differentiate ASH or NASH from simple steatosis, as well as stage the extent of fibrosis. Imaging studies such as ultrasound, computed tomography, and magnetic resonance imaging are not able to distinguish between steatosis and NASH, nor are they able to stage the degree of hepatic fibrosis. Recently, caspase-3-generated cytokeratin (CK)-18 fragments a marker of apoptosis measured in plasma has been evaluated in distinguishing simple steatosis from NASH [5]. Using plasma from 44 patients with NAFLD, the authors reported a specificity of 99.9%, a sensitivity of 85.7%, and positive predictive values (PPV) and negative predictive values (NPV) of 99.9 and 85.7% respectively of a value of CK-18 of 395 U/L for the diagnosis of NASH [5]. Although CK-18 levels looks promising to distinguish between simple steatosis and NASH, further validation is required before the test can be routinely used in clinical practice. In addition, the performance of CK-18 in differentiating simple steatosis from ASH in patients with alcohol abuse remains to be elucidated. Other investigations focused on whether noninvasive serum tests can differentiate NASH from simple steatosis among patients with NAFLD have proposed the NASH Test [6], and scores combining age, gender, aspartate aminotransferase (AST), body mass index, AST alanine aminotransferase (ALT) ratio and hyaluronic acid [7] or adiponectin, HOMA-IR, and...
serum type IV collagen [8]. These tests, however, require extensive further evaluation to determine their role in clinical practice.

Liver biopsy has several well-documented drawbacks, including sampling error, variability in pathologist interpretation, cost, and morbidity. Liver biopsy samples only a tiny portion of liver, estimated at 1/50,000. Sampling error thus may occur easily. Inter- and intraobserver variability of histopathologic interpretation of liver fibrosis is reasonable but not perfect and adds to the inaccuracy of liver biopsy for staging purposes [9]. Lastly, liver biopsy is associated with definite morbidity, with serious complications noted in 0.3% and mortality in 0.01% [10]. These shortcomings and drawbacks of liver biopsy have led investigators to examine noninvasive markers as potential substitutes for the assessment of liver fibrosis. The ideal noninvasive test is simple and reproducible, readily available, less expensive than biopsy, and able to predict the full spectrum of fibrosis and reflect changes occurring with therapy. Simple laboratory measures, serum markers of liver fibrosis, and potential pathogenic factors have been examined for their ability to predict liver fibrosis in patients who have NASH and ASH.

Serum markers of liver fibrosis in nonalcoholic steatohepatitis

Routine laboratory tests

Several routinely available laboratory tests may be abnormal in the presence of advanced liver fibrosis. Synthetic markers, such as albumin and prothrombin time, often are altered in the presence of cirrhosis, and bilirubin may be increased. A low platelet count in the setting of advanced liver disease generally is a sign of hypersplenism related to portal hypertension. Advanced liver disease often is clinically and radiologically apparent, however, when these laboratory markers are abnormal. Although these markers may assist in grading the severity of liver decompensation, they are insensitive at detecting milder degrees of fibrosis.

Aminotransferase levels are found to correlate with liver fibrosis in certain select populations of patients who have NALFD, such as those undergoing bariatric surgery [11–13]. Among nearly 1000 morbidly obese subjects undergoing gastointestinal bariatric surgery in Italy, an AST or ALT level greater than twice the upper limit of normal was found predictive of septal fibrosis. An AST greater than or equal to twice the upper limit of normal also was independently predictive of portal or bridging fibrosis in an Asian population of 60 patients who had NALFD [14]. Similarly, in 93 (nonmorbidly) obese subjects who had abnormal liver tests (78% of whom had NALFD) [14], an ALT greater than two times the upper limit of normal was found predictive of septal fibrosis. An AST greater than or equal to twice the upper limit of normal also was independently predictive of portal or bridging fibrosis in Asian patients who have NALFD [15]. Other studies, however, have failed to confirm an association between aminotransferase levels and degree of fibrosis in patients who have NALFD [16–18]. Furthermore, two studies comparing NALFD patients who had persistently raised ALT levels to those who had persistently normal ALT levels found the prevalence of advanced fibrosis and cirrhosis the same in both groups [19,20]. The association between aminotransferase levels and fibrosis, therefore, seems inconsistent and not sufficient to predict fibrosis stage in individual patients.

An association between an elevated AST/ALT ratio and fibrosis has been recognized in non-NALFD chronic liver disease and may reflect impaired AST clearance by sinusoidal cells in the liver [21]. Among patients who have NALFD, aminotransferase levels tend to fall over time [22] and the AST/ALT ratio tends to reverse as the degree of fibrosis progresses to bridging fibrosis or cirrhosis [16]. Consequently, several studies have found an association between advanced fibrosis on liver biopsy and an AST/ALT ratio greater than 1 [16,17,23–25]. Bugianesi et al. [23] found a higher AST/ALT ratio associated with advanced fibrosis in 167 patients who had NALFD, although this became nonsignificant after multivariable analysis. In contrast, a study examining 144 biopsy-proved patients who had NASH found an AST/ALT ratio greater than 1 remained significantly associated with advanced fibrosis when adjusted for multiple factors [16]. In that study [16], 81 out of 99 (82%) patients who had a ratio of 1 or less did not have fibrosis, whereas 21 out of 45 (47%) who had a ratio greater than 1 had severe fibrosis, indicating that the AST/ALT ratio may be a useful clinical adjunct for predicting or excluding advanced fibrosis in patients who have NASH.

Serum ferritin levels are elevated in 21 to 40% of patients who have NALFD and seem related to insulin resistance and liver damage rather than reflecting increased hepatic iron stores [18,23]. Ferritin was found a significant independent predictor of severe fibrosis in 167 subjects from Italy who had NALFD [23]. This has not been replicated in other studies, however, casting into doubt the usefulness of ferritin to discriminate fibrosis in patients who have NALFD [16–18,26].

Combination of serum and clinical markers of liver fibrosis

In an effort to increase the predictive value of simple laboratory parameters for liver fibrosis, several routine laboratory tests have been combined with simple clinical variables (Table 1). As insulin resistance is a driving force behind the pathogenesis of NALFD and is associated with stimulating fibrogenic hepatic growth factors [27,28], it is not surprising that the clinical correlates of insulin resistance (obesity, diabetes mellitus, and hypertriglyceridemia) are associated with advanced fibrosis and incorporated with laboratory tests to predict liver fibrosis.

Among 144 patients who had biopsy-proved NASH, 66% of those who had the combination of obesity, diabetes, age greater than or equal to 45 years, and AST/ALT greater than 1 had bridging fibrosis or cirrhosis [16]. In contrast, no patient had severe fibrosis in the absence of all of these factors. The French group [14] found age greater than or equal to 50 years, body mass index greater than or equal to 28 kg/m², elevated serum triglyceride, and ALT levels associated with septal fibrosis in 93 obese subjects who had abnormal liver tests. No patient in their cohort who had one or less of these factors had septal fibrosis, whereas all four patients who had all four factors had septal fibrosis. Lastly, an Australian algorithm revolving around systemic hypertension, elevated ALT, and insulin resistance (HAIR index) provided a sensitivity and specificity of 80 and 89%, respectively, for detecting NASH in
patients who were morbidly obese and undergoing bariatric surgery [26]. In the presence of at least two of these three predictive factors, 10 of 11 patients who had bridging fibrosis or cirrhosis also were identified; however, the specificity of the index was low, with at least 11 other patients who had a score of 2 or more not having advanced fibrosis.

In an international muticentric study [29], data from 733 patients with biopsy confirmed NAFLD were analyzed to create (480 patients) and validate (253 patients) a scoring system to distinguish between patients with (stage 3/4) and without (stage 0/2) advanced fibrosis using the Kleiner’s staging system. A score (named the NAFLD fibrosis score) was created using six variables that were significant by multivariate analysis: score = −1.675 + 0.037 × age (years) + 0.094 × body mass index (kg/m²) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet (× 10⁹/l) − 0.66 × albumin (g/dl). The area under the ROC of this score to distinguish between patients with and without advanced fibrosis was high, 0.88 in the estimation group and 0.82 in the validation group. A score less than −1.455 had high accuracy in excluding advanced fibrosis with a NPV of 93 and 88% in the training and validation groups, respectively; whereas a score greater than 0.676 had high accuracy in identifying advanced fibrosis with a PPV of 90 and 82% in the training and validation groups, respectively. If the NAFLD fibrosis score had been applied to the 733 patients, the liver biopsy for fibrosis staging could have been avoided in 75% of patients, that is those who were correctly identified, and performed in only 25% of patients, that is those that fell in the indeterminate range. Subsequently, two large studies of independent patient populations have reproduced the high accuracy of the NAFLD fibrosis score in distinguishing patients with and without advanced fibrosis in two independent patient population. In the study by Wong et al. [31], 162 Asian patients were enrolled; the lower cutoff score (less than −1.455) had a NPV of 91% in the exclusion of advanced fibrosis. In the study by Qureshi et al. [32], 331 morbidly obese patients with NAFLD who underwent bariatric surgery were enrolled; the lower cutoff score had a NPV of 98% in ruling out advanced fibrosis.

Harrison et al. analyzed data collected retrospectively from a group of 827 patients with NAFLD [30]. Based on logistic regression analysis, they reported the BAAR score which is a combination of three variables in a weighted sum (body mass index ≥ 28 = 1 point; AST/ALT ratio ≥ 0.8 = 2 points, and diabetes = 1 point). They reported a score of 2–4 associated with an odd ratio of 17 for advanced (stage 3–4) fibrosis. It remains uncertain however how many of those 827 patients evaluated retrospectively had the three variables measured and were in fact included in the evaluation of the accuracy of the BAAR score. In addition, the BAAR score needs to be cross-validated in other independent patient populations to determine its applicability in clinical practice.

### Serum markers of fibrogenesis

Hepatic fibrosis is a dynamic process involving complex interaction between enzymes involved in extracellular matrix synthesis and degradation. Extracellular matrix components, such as hyaluronic acid, collagen components, and

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**Table 1** Routine laboratory and clinical predictors of advanced fibrosis (stage 3—4) in patients who have non-alcoholic fatty liver disease.

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Patient population</th>
<th>Risk factors</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angulo et al., 1999</td>
<td>144</td>
<td>NASH</td>
<td>Age ≥ 45 years, Obesity (body mass index &gt; 30 kg/m²), Diabetes, AST/ALT &gt; 1</td>
<td>5.6 (1.5, 21.7)</td>
</tr>
<tr>
<td>Ratziu et al., 2000</td>
<td>93</td>
<td>Overweight, raised liver tests</td>
<td>Age ≥ 50 years, Body mass index ≥ 28 kg/m², Triglyceride ≥ 1.7 mmol/L, ALT ≥ 2 × ULN</td>
<td>14.1 (3.7, 54.0)</td>
</tr>
<tr>
<td>Dixon et al., 2001</td>
<td>105</td>
<td>Bariatric surgery patients</td>
<td>Hypertension, ALT &gt; 40 IU/L</td>
<td>NA</td>
</tr>
<tr>
<td>Angulo et al., 2007</td>
<td>733</td>
<td>Nonalcoholic fatty liver disease</td>
<td>Age (years), Body mass index (kg/m²), IFG/diabetes, AST/ALT ratio, Platelet count (× 10⁹/l), Albumin (g/dl)</td>
<td>1.04 (1.01, 1.07), 1.10 (1.04, 1.16)</td>
</tr>
<tr>
<td>Harrison et al.</td>
<td>827</td>
<td>Nonalcoholic fatty liver disease</td>
<td>Body mass index ≥ 28 kg/m², AST/ALT ratio ≥ 0.8, Diabetes</td>
<td>2.4 (1.2, 4.8), 9.3 (6.3, 13.6), 4.0 (2.8, 5.7)</td>
</tr>
</tbody>
</table>

NA: not available; ULN: upper limit of normal; AST: aspartate aminotransferase; ALT: alanine aminotransferase.
laminin, circulate in the serum at low levels and have been examined as potential predictors of liver fibrosis in NAFLD (Table 2).

Serum levels of hyaluronic acid are increased in liver fibrosis reflecting increased deposition of collagen and decreased clearance by sinusoidal endothelial cells [33]. Several studies have determined that hyaluronic acid predicts bridging fibrosis or cirrhosis in patients who have NAFLD, with accuracy between 80 and 89% [7,33,34]. Hyaluronic acid, however, is less accurate for detecting lesser degrees of fibrosis with an area under the receiver operator characteristic curve (AUC) for any degree of fibrosis varying between 0.67 and 0.73 [33,35]. In addition, hyaluronic acid increases in systemic inflammatory conditions, which may produce falsely positive predictive results.

Type IV collagen is a product of collagen degradation and a marker of fibrolysis. Serum levels of the 7S domain are increased in the presence of severe fibrosis in patients who have NAFLD [25]. Among 112 Japanese patients who had NAFLD, a cutpoint of 5.0 ng/mL provided a PPV and NPV of 68 and 84%, respectively, for the presence of severe fibrosis [34]. Laminin is a component of extracellular matrix cleared by hepatic endothelial cells. A small recent study found levels greater than 282 ng/mL reasonably predictive for the presence of any fibrosis in 30 patients who had NAFLD [35]. Serum YKL-40 [39] levels also are proposed, based on small studies that lacked a validation group.

To increase the accuracy of noninvasive markers of liver fibrosis, multiple serum markers have been combined into mathematical models to produce predictive scores. The FibroTest is one algorithm, consisting of a combination of age, gender, bilirubin, γ-glutamyltransferase, apolipoprotein A1, haptoglobin, and α2-macroglobulin. It has been validated in a variety of chronic liver conditions and recently was examined in a cohort of 267 individuals, 85% of whom had NAFLD [36]. A score below 0.3 (range 0.0 to 1.0) provided a NPV of 98% for the presence of bridging fibrosis or cirrhosis, whereas a score above 0.7 provided a 60% PPV of bridging fibrosis or cirrhosis. However, 33% of individuals had a score between 0.3 and 0.7, indicating that the FibroTest cannot predict severity of liver fibrosis in a third of patients who have NAFLD. The European Liver Fibrosis Group assessed the combination of age and serum levels of hyaluronic acid, aminoterminal propeptide of type III collagen, and tissue inhibitor of matrix metalloproteinase 1 in predicting advanced fibrosis in patients who had a wide range of liver disease [40]. The proposed algorithm had an acceptable accuracy overall, but only 61 out of the 912 patients included in the original study [40] had NAFLD, a number too small to derive meaningful conclusions for the NAFLD population. Therefore, the group evaluated the same three serum markers hyaluronic acid, aminoterminal propeptide of type III collagen, and tissue inhibitor of matrix metalloproteinase 1 (named Enhanced Liver Fibrosis panel or ELF) in predicting advanced fibrosis in 192 patients with NAFLD. An ELF score of 0.3576 had area under the ROC of 0.93, and a sensitivity of 80% in detecting advanced (stage 3–4) fibrosis and a specificity of 90% in ruling out advanced fibrosis [37]. ELF was recently evaluated in 112 children with NAFLD [38]; the area under the ROC to distinguish among the several fibrosis stages varied from 0.90 to 0.99. In that study [38], values of ELF from 9.28 to 10.51 had a sensitivity of 88% and a specificity of 76 to 98% in predicting advanced fibrosis.

### Table 2: Serum markers of fibrogenesis and clinical predictors of advanced (stage 3–4) fibrosis in patients who have non-alcoholic fatty liver disease.

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Serum marker</th>
<th>Area under the ROC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wong et al. [33]</td>
<td>79</td>
<td>Hyaluronic acid &gt; 46.1 ng/mL</td>
<td>0.89</td>
<td>85.0</td>
<td>79.7</td>
</tr>
<tr>
<td>Sakugawa et al. [34]</td>
<td>112</td>
<td>Hyaluronic acid ≥ 50 ng/mL</td>
<td>0.80</td>
<td>68.8</td>
<td>82.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IV collagen 7S ≥ 5 ng/mL</td>
<td>0.82</td>
<td>81.3</td>
<td>71.4</td>
</tr>
<tr>
<td>Palekar et al. [7]</td>
<td>80</td>
<td>Hyaluronic acid &gt; 45.3 ng/mL</td>
<td>0.88</td>
<td>85.7</td>
<td>80.3</td>
</tr>
<tr>
<td>dos Santos et al. [35]</td>
<td>30</td>
<td>Hyaluronic acid &gt; 24.6 ng/mL</td>
<td>0.73</td>
<td>82.0</td>
<td>68.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IV collagen &gt; 145 ng/mL</td>
<td>0.80</td>
<td>64.0</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Laminin &gt; 282 ng/mL</td>
<td>0.87</td>
<td>82.0</td>
<td>89.0</td>
</tr>
<tr>
<td>Ratziu et al. [36]</td>
<td>267</td>
<td>Fibrotest 0.30</td>
<td>0.88</td>
<td>92.0</td>
<td>71.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibrotest 0.70</td>
<td>0.88</td>
<td>25.0</td>
<td>97.0</td>
</tr>
<tr>
<td>Guha et al. [37]</td>
<td>192</td>
<td>ELF score = −7.412 + (ln(HA)*0.681)</td>
<td>0.93</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ (ln(P3NP)*0.775) + (ln(TIMP1)*0.494)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ELF = 0.3576b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nobili et al. [38]</td>
<td></td>
<td>ELF (different cutoff values)</td>
<td>0.90–0.99</td>
<td>88–100</td>
<td>76–98</td>
</tr>
</tbody>
</table>

Advanced fibrosis defined as stage 3 or 4 [9]; ELF: enhanced liver fibrosis panel.

a Predicting presence of fibrosis versus absence of fibrosis.

b The area under the ROC is to distinguish between patients with and without advanced (stage 3/4) fibrosis. An ELF score of 0.3576 had a sensitivity of 80% in detecting advanced fibrosis and a specificity of 90% in ruling out advanced fibrosis.

**Serum markers of liver fibrosis in alcoholic steatohepatitis**

Similar to NAFLD, several routine laboratory tests and serum fibrosis markers have been evaluated to predict the
severity of liver disease in patients with alcohol-induced liver disease. They have been combined to create predicting formulas and scores as summarized in Table 3 [41–43].

### Assessment of steatosis in nonalcoholic steatohepatitis and alcoholic steatohepatitis

#### Imaging

Ultrasound, computed tomography, and magnetic resonance imaging can noninvasively diagnose fatty infiltration of the liver. Hepatic steatosis causes increased echogenicity on ultrasound, which can be contrasted against the lower echogenicity of the spleen or renal cortex. A similar pattern can be seen with diffuse fibrosis, giving rise to the term “fatty-fibrotic pattern,” although the echo shadows tend to be coarser in the presence of pure fibrosis. The sensitivity and specificity of ultrasound for detecting hepatic steatosis vary from 60 to 94% and 88 to 95%, respectively. However, the sensitivity of ultrasound decreases with lower degrees of fatty infiltration. In the presence of greater than or equal to 30% fatty infiltration, the sensitivity of ultrasound is 80% compared with a sensitivity of 55% when hepatic fat content is 10 to 19% [44]. Similarly, the sensitivity and specificity of ultrasound decrease in the presence of morbid obesity to 49 and 75%, respectively [45].

On noncontrast images by computed tomography scan, hepatic steatosis has a low attenuation and appears darker than the spleen. The sensitivity of computed tomography at detecting greater than 33% hepatic steatosis is up to 93%, with a PPV of 76% [44]. Both magnetic resonance imaging and magnetic resonance spectroscopy are reliable at detecting steatosis and offer good correlation with hepatic fat volume [46–48]. Magnetic resonance spectroscopy studies of the human liver have been based on the ubiquitous protons hydrogen (1H) and phosphorus (31P). More than 5% of hepatic fat content on magnetic resonance spectroscopy indicates presence of steatosis. However, the routine application of magnetic resonance images is limited by cost and lack of availability.

#### Serum markers

The only serum test that has been proposed to detect steatosis is the SteatoTest [49]. This test includes the six parameters of FibroTest-ActiTest plus body mass index, serum cholesterol, triglycerides and glucose adjusted for age and gender. It was constructed from a training group of 310 patients with various chronic liver diseases, using the presence of steatosis of greater than 5% on liver biopsy as the reference, and validated in 434 patients with hepatitis C and alcoholic liver disease. At cutoff of 0.3, the sensitivity of the test ranged from 85 to 100% whereas at cutoff of 0.7, the specificity ranged from 83 to 100%.

#### Imaging assessment of fibrosis in nonalcoholic steatohepatitis and alcoholic steatohepatitis

Conventional ultrasound, computed tomography, and magnetic resonance imaging have in general a good level of accuracy in the detection of cirrhosis, particularly portal hypertension in patients with chronic liver disease. Although the radiologic features of splenomegaly, reversal of hepatic blood flow, change in caudate to right lobe ratio, and hepatic vein narrowing aid the sensitivity of detecting severe disease, they are less useful in earlier disease. However, new imaging technologies, such as the ultrasonography-based transient elastography or FibroScan, and magnetic resonance imaging-based elastography offer promise in determining severity of liver fibrosis [50]. FibroScan transmits a transabdominal pulse wave through the liver and measures its reflectivity as a determinant of

<table>
<thead>
<tr>
<th>Author (Score)</th>
<th>n</th>
<th>End point fibrosis stage</th>
<th>Area under the ROC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lieber et al. [41] (APRI)</td>
<td>507</td>
<td>Septal fibrosis (Ishak score)</td>
<td>0.70</td>
<td>94</td>
<td>26</td>
</tr>
<tr>
<td>Rosenberg et al. [40] (ELF)</td>
<td>64</td>
<td>ELF = 0.087</td>
<td>0.94</td>
<td>100</td>
<td>16.7</td>
</tr>
<tr>
<td>Naveau et al. [42] (Fibrotest)</td>
<td>221</td>
<td>ELF = 0.431</td>
<td>0.84</td>
<td>84</td>
<td>66</td>
</tr>
</tbody>
</table>

APRI: AST to platelet ratio index; ELF: enhanced liver fibrosis panel, combines age, hyaluronic acid (HA), aminoterminal propeptide of type III collagen (PIIINP), tissue inhibitor of matrix metalloproteinase 1 (TIMP1) (score = −0.014 LN[age] + 0.616 LN[HA] + 0.586 LN[PIIINP] + 0.472 LN[TIMP1] − 6.38). FibroTest: combines age, gender, bilirubin, x-glutamyltransferase, apolipoprotein A1, haptoglobin, and α2-macroglobulin. Fibrometer: combines prothrombin index, α2-macroglobulin, hyaluronate, and age (the regression function was: −0.169 Prothrombin index [%] + 0.015 alpha 2-macroglobulin [mg/dL] + 0.032 hyaluronic acid [ug/L] − 0.140 age [years] + 16.541).

Author (Score): Cales et al. [43] (Fibrometer); n: 95; End point fibrosis stage: F 2/3/4; Area under the ROC: 0.96; Sensitivity (%): 91.8; Specificity (%): 92.6
liver stiffness. FibroScan has been evaluated in patients who have chronic hepatitis C infection [50,51], and in patients who have chronic cholestatic liver disease, such as primary biliary cirrhosis and primary sclerosing cholangitis [52], with a reasonable accuracy.

Ultrasound-based elastography (FibroScan)

Transient elastography is a technique whereby a shear wave, at a low frequency of 50 Hz, is created by a vibrating probe and transducer applied to the skin overlying the liver. The velocity of the propagated wave is correlated with the stiffness or elasticity of the underlying liver; simplistically, the propagated wave travels faster with increasing fibrosis. A pulse–echo ultrasound allows measurement of the wave velocity and the results are presented as kilopascals (kPa). The validity of measurement is assessed by the interquartile range and ratio of successful measurements to unsuccessful measurements (should be over 60%).

Transient elastography technique measures the liver stiffness within a cylinder of 1 cm in width and 4 cm in length, producing an estimated sampling area that is 100 times greater than biopsy. The reproducibility of the technique has been evaluated in a large study including 800 examinations in 200 patients who had heterogeneous liver disease; the intraclass correlation coefficient was 0.98 by two operators [53]. Finally, the test is inexpensive; the equipment has a capital cost, but the running cost thereafter is low compared with other noninvasive tests.

Only few studies have evaluated the performance of ultrasound-based transient elastography in detecting significant fibrosis in patients with alcoholic or NAFLD. The threshold for detecting significant fibrosis varied from 4 to 9 kPa in three selected studies [53–55] that have included over 400 patients with mixed causes of liver disease including alcohol-induced liver disease and NAFLD. Good diagnostic performance occurs above these critical thresholds. The area under the ROC varied from 0.74 to 0.86 with a sensitivity and specificity of the selected kPa threshold from 81 to 94% and 33 to 85%, respectively. Similar to blood tests, transient elastography shows better performance in detecting cirrhosis.

Only two studies, one in adults [56] and one in children [57], have evaluated the performance of FibroScan in detecting significant fibrosis in patients with NAFLD. The adult study [56] included 67 patients, with a prevalence of significant fibrosis of 49%, the area under the ROC was 0.87, and a threshold of 6.6 kPa had a sensitivity of 83% and a specificity of 81% in the detection of significant fibrosis. In the pediatric study [57], 52 patients were included. The area under the ROC for the prediction of any (≥1), significant (≥2), or advanced fibrosis (≥3) were 0.977, 0.992, and 1, respectively. Calculation of multilevel likelihood ratios showed that transient elastography values <5, <7, and <9 kPa, suggest the presence of any fibrosis, significant fibrosis, and advanced fibrosis, respectively. Transient elastography values between 5 and 7 kPa predicted a fibrosis stage of 1, but with some degree of uncertainty. Transient elastography values between 7 and 9 kPa predict fibrosis stages 1 or 2, but could not discriminate between these two stages. Transient elastography values of at least 9 kPa were associated with the presence of advanced fibrosis. The intraclass correlation coefficient for absolute agreement was 0.961. The study suggests that transient elastography is an accurate and reproducible methodology to identify pediatric subjects without fibrosis or significant fibrosis, or with advanced fibrosis, although this technique was not optimal to provide a reliable indication of the disease stage.

Several potential issues with ultrasound-based transient elastography are starting to be addressed. For instance, the intraclass correlation coefficient by two operators has been reported to be was lower in less-severe fibrosis, increased body mass index, and increased steatosis [53]. In addition, a recent prospective study of 2114 FibroScan examinations found presence of body mass index greater than 28 kg/m² the only independent factor associated with failure of FibroScan examination for the identification of liver fibrosis [58]. Body mass index of 28 kg/m² or greater is almost a universal finding in patients who have NAFLD; thus, the FibroScan’s usefulness in fibrosis quantification in patients who have NAFLD needs further evaluation. Further, steatosis may also have an independent effect on liver stiffness measurement [51].

Computed tomography- and magnetic resonance imaging-based elastography

The accuracy of imaging techniques other than ultrasoundography (ie, magnetic resonance imaging and computed tomography) to measure liver stiffness and predicting severity of liver fibrosis currently are under evaluation. Recently, the new Fibro-computed tomography tool was developed, which was shown to have an area under the ROC of 0.83 and 0.93 for detecting moderate and severe fibrosis, respectively, in patients who have chronic hepatitis C [59]. This technique does not require contrast and uses optical digital analysis of conventional images. Further studies are needed to determine the effect of steatosis, and necroinflammation on liver stiffness measurements, and to determine the reproducibility of the technique.

Magnetic resonance elastography is based on similar principles to those of ultrasound elastography. A shear wave is created by a driver (pneumatic or electromechanical) attached to the abdominal wall. A specialized magnetic resonance sequence is then used to measure the propagated waves and analysis is performed to quantify these sequences into elastograms. As the entire liver is sequenced, the area of sampling is greatly increased and the heterogeneous distribution of fibrosis is more commonly appreciated. Data on the performance of magnetic resonance elastography in fibrosis staging are scarce, but the data look encouraging. A recent study comparing magnetic resonance elastography with AST-to-platelet ratio index (APRI) in a cohort with viral and alcoholic liver disease found that the ROC curves were significantly greater for distinguishing moderate and severe fibrosis using elastography compared with the biochemical test [60]. The same group of investigators has recently reported the performance of magnetic resonance elastography as compared to ultrasound elastography and APRI for the staging of liver fibrosis [61]. A total of 141 patients were assessed. The technical success rate of magnetic resonance elastography was higher than that of ultrasound elastogra-
phy (133/141 [94%] vs 118/141 [84%]; p = 0.016). The areas under the ROC of magnetic resonance elasticity (0.994 for ≥ 2; 0.985 for F ≥ 3; 0.998 for F = 4) were larger (p < 0.05) than those of ultrasound elasticity, APRI, and the combination of ultrasound elasticity and APRI (0.837, 0.709, and 0.849 for F ≥ 2; 0.906, 0.816, and 0.936 for F ≥ 3; 0.930, 0.820, and 0.944 for F = 4, respectively). The study suggests that magnetic resonance elastography has a higher technical success rate than ultrasound elastography and a better diagnostic accuracy than ultrasound elastography and APRI for staging liver fibrosis.

Nevertheless, there are still some issues that are common to both fibro-computed tomography and magnetic resonance elastography techniques including the increased acquisition time of scanning, the costs of the equipment, the expertise in analysis, reproducibility, and standardized thresholds of measurement. Although these techniques are not yet ready to be used as first-line noninvasive assessment tools for liver fibrosis, they certainly hold much promise, particularly if large validation studies confirm their reported diagnostic accuracy.

References


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