Noninvasive diagnosis of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined by the presence of hepatic steatosis, either by imaging or by histology in the absence of other causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders. NAFLD has become the most common chronic liver disease in Western society[1] and its high prevalence is associated with the obesity epidemic worldwide. Currently NAFLD is considered the hepatic manifestation of the metabolic syndrome. The prevalence of NAFLD is 20–30% in the general population[2,3] and could be as high as 90% in patients with morbid obesity.[4] The spectrum of NAFLD starts from simple steatosis that can progress to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. Importantly, patients with NASH and fibrosis are considered a high-risk group for liver and cardiac-related morbidity and mortality. The diagnostic evaluation of patients with NAFLD usually starts with the assessment of an asymptomatic patient with abnormalities in liver injury tests (most commonly elevation of aminotransferases) or referred for evaluation of liver steatosis incidentally found on abdominal imaging performed for other clinical reasons. Most of these patients have components of the metabolic syndrome (obesity, hypertension, hyperlipidemia, diabetes). Liver sonogram and blood tests to rule out other etiologies of liver disease, as well as a detailed history of alcohol intake, are essential to establish the diagnosis. Because of their significantly different clinical outcomes, from a prognostic stand-point, it is very important to establish the distinction between simple steatosis and NASH with or without fibrosis. The three noninvasive diagnostic tests that have been independently validated compared with liver biopsy for diagnosis of NASH and fibrosis are serum levels of CK-18 for diagnosis of NASH and ultrasound elastography and NAFLD fibrosis score for the diagnosis of advanced fibrosis. Serum biomarkers that have been validated (NAFLD fibrosis score, FibroTest) could be used as screening tools in patients with metabolic syndrome. Imaging techniques have been developed and could have a more widespread use in the future, but only ultrasound elastography has been validated extensively. CK-18 is a direct serum biomarker that could be part of the routine diagnostic evaluation for NASH in the near future. Routine tests appear to be more accurate than nonroutine tests for prediction of NAFLD and advanced fibrosis.

Keywords: NAFLD, NASH, noninvasive diagnosis.
morbidity and mortality. The estimated prevalence of NASH among adults in the general population is 2–3%[3] and this increases significantly to approximately 35% in morbidly obese patients.[4]

The diagnostic evaluation of patients with NAFLD usually starts with the assessment of an asymptomatic patient with abnormalities in liver injury tests (most commonly elevation of aminotransferases) or referred for evaluation of liver steatosis incidentally found on abdominal imaging performed for other clinical reasons. Most of these patients have components of the metabolic syndrome (obesity, hypertension, hyperlipidemia, diabetes). Liver sonogram and blood tests to rule out other etiologies of liver disease, as well as a detailed history of alcohol intake, are essential to establish the diagnosis. If blood tests are negative and liver ultrasound reveals increased echogenicity (liver brighter than the cortex of the right kidney), NAFLD is suspected. Because of their significantly different clinical outcomes, from a prognostic standpoint, it is very important to establish the distinction between simple steatosis and NASH with or without fibrosis.[5]

Epidemiology and natural history of NAFLD

The prevalence of NAFLD in the U.S. population is approximately 30% and the prevalence of NASH between 3% and 5%. In several studies around the world the prevalence ranges from 6% to 35%. NASH is considered a progressive fibrotic disease with development of cirrhosis in 20% of cases over a 10-year period. Steatosis has a more benign clinical course with only a 3% lifetime risk of progression to cirrhosis. Risk factors associated with fibrosis are diabetes, obesity, and older age.[7]

The prevalence of NAFLD in the Dionysos Nutrition and Liver Study in Northern Italy was 25% in patients with suspected liver disease.[8] A study from the Brooke Army Medical Center in San Antonio, Texas of 400 patients found a prevalence of NAFLD of 46% on abdominal sonogram, while 12% had biopsy-proven NASH. In the same study, the prevalence of both NAFLD and NASH was higher among the Hispanic population, and highest among patients with type 2 diabetes mellitus (prevalence of NAFLD was 74% and NASH was 22%).[9] A study from an Asian population used transient elastography and proton-magnetic resonance spectroscopy as screening tools in persons randomly selected from the community; 28% of the individuals had evidence of NAFLD and 3.7% of persons had liver stiffness measurements suggestive of advanced fibrosis.[9] In a large population-based cohort of asymptomatic persons with diagnosis of NAFLD by ultrasound during routine check-ups, the prevalence of NAFLD increased 2.4-fold over a 12-year period (12.6% to 30.3%). Interestingly in this cohort, half of the persons were nonobese.[9] In Asian populations NAFLD has been described in nonobese individuals at a higher rate.[6,10,11] The prevalence of NAFLD in nonobese subjects has been considered as high as 25% in several studies from Asian countries; this group of “lean NAFLD” or “metabolic obese” patients has a higher incidence of metabolic risk factors including diabetes and cardiovascular disease.[11,12] A review of 12 observational and cross-sectional studies on the prevalence of NAFLD and NASH in more than 1600 morbidly obese patients undergoing bariatric surgery revealed a prevalence of simple steatosis of 91%, NASH of 37%, and cirrhosis of 1.7%. Diabetes mellitus and insulin resistance were associated with NASH and hypertension with advanced hepatic fibrosis.[9]

Studies of heritability of NAFLD have recently demonstrated that the I148M allele of the PNPLA3 gene that encodes adiponutrin has a genetic correlation with NAFLD and is most prevalent in Hispanics. I148M homozygotes have a higher frequency of NASH. A protective variant associated with lower hepatic fat, the PNPLA3-S4531 has been described in African Americans.[9] PNPLA3 polymorphism identifies patients with a higher risk of NASH but not fibrosis and is not useful yet as a noninvasive screening tool for significant liver disease.[13]

In a study that evaluated the risk factors and heritability in a Hispanic and African American population, NAFLD was twofold more prevalent in the Hispanic population compared with African Americans (24% vs. 10%). Serum triglycerides and levels of plasminogen activator inhibitor 1 (PAI-1) were associated with NAFLD in Hispanics.[14]

The prevalence of elevated aminotransferases as a surrogate of NAFLD was higher in Hispanics (39%) compared with Caucasians and African Americans in an obese urban population.[15]

Clinical relevance of NAFLD

As exemplified below, NAFLD, especially in the subgroup of patients with NASH, has a detrimental impact on morbidity and mortality in those affected by this disorder.

In a cohort of patients with NAFLD followed at the Cleveland Clinic for a median of 18.5 years, the mortality
Liver biopsy has been considered for decades as the “gold standard” for the diagnosis of chronic liver diseases including NAFLD/NASH; however it is far from being an ideal diagnostic method. One of the main drawbacks is sampling error since steatosis/steatohepatitis are distributed in a heterogeneous way in the liver parenchyma. For example a 2.5 cm long liver biopsy is able to evaluate only 1/50,000 of the total liver volume. There is also a significant inter- and intraobserver variability with up to 35% of false positives and false negatives in relation with fibrosis staging compared with larger size surgical biopsies. Another drawback of liver biopsy is its invasive nature, and although generally thought of as a safe procedure, there is associated morbidity and a reported mortality rate of 0.05%. The three main pathological features of NAFLD/NASH at the time of histologic evaluation of the liver biopsy include the presence of steatosis, the degree of hepatic inflammatory manifestations as “ballooning hepatocytes,” and the degree of inflammation. The presence of fibrosis is most relevant to establish prognosis, since patients with NASH and advanced fibrosis are considered at high risk for progression to cirrhosis. Epidemiologic studies show that simple steatosis in most cases does not progress to advance fibrosis or cirrhosis, in contrast, the presence of NASH is associated with risk of fibrosis and 20% of NASH patient’s will develop cirrhosis and associated complications. Most of the serum biological markers have been validated using the hepatic biopsy as the gold standard.

The diagnostic accuracy of a test can be measured as the area under the receiver operating curves (AUROC) that depends on the sensitivity and specificity of a test.
MRI techniques have been useful as a screening noninvasive diagnostic method. Advantages of this technique include wide availability, noninvasive nature, and low cost. The main drawbacks are the low sensitivity to detect liver steatosis, which has been estimated to be as low as 60% or as high as 94%, and that it is operator dependent and good visualization of liver parenchyma could be compromised in morbidly obese persons. Ultrasonography has a very poor diagnostic sensitivity when it comes to mild forms of liver steatosis (<30% steatosis).

**Radiological evaluation of hepatic steatosis**

**Ultrasound**

Conventional ultrasound is considered the imaging technique of choice for screening for liver steatosis. Advantages of this technique include wide availability, noninvasive nature, and low cost. The main drawbacks are the low sensitivity to detect liver steatosis, which has been estimated to be as low as 60% or as high as 94%, and that it is operator dependent and good visualization of liver parenchyma could be compromised in morbidly obese persons. Ultrasonography has a very poor diagnostic sensitivity when it comes to mild forms of liver steatosis (<30% steatosis).

**Contrast ultrasonography**

It is considered the only imaging technique that is capable of discriminating among simple steatosis and NASH. It involves the use of an intravenous contrast agent (Leovist). The accumulation of micro-bubbles of Leovist in the hepatic parenchyma is decreased in NASH but not in NAFLD or chronic viral hepatitis. Currently it is in the experimental phase and needs further clinical validation. A Japanese study of 64 patients with NAFLD, NASH, and normal hepatic parenchyma revealed AUROC of 1 for diagnosis of NASH; however there was no histologic correlation with the degree of fibrosis or steatosis.

**Computed tomography (CT)**

CT allows for evaluation of the whole liver, and even non-enhanced CT can detect steatosis as a decrease in the attenuation of the liver parenchyma compared with that of the spleen; it has a high specificity but a low sensitivity. CT has a similar accuracy for diagnosis of NAFLD compared with ultrasound, with improved detection of focal steatosis. Cost and radiation are the main drawbacks for its use as a screening noninvasive diagnostic method.

**Magnetic resonance imaging (MRI)**

MRI can detect and quantify minimal amounts of steatosis. Magnetic resonance spectroscopy (MRS) can quantify the amount of triglycerides in liver parenchyma. It has been shown to be accurate for diagnosis and quantifying steatosis. MRI techniques have been useful as noninvasive screening methods for NAFLD. It is still considered an experimental test due to high cost, need for validation, and limited availability.

**Radiological modalities to evaluate liver stiffness (fibrosis)**

There are three imaging techniques to measure hepatic elasticity or compliance. In general, hepatic parenchymal elasticity is inversely proportional to the presence and severity of liver fibrosis. These methods include ultrasound elastography (UE), magnetic resonance elastography (MRE), and axial radiation force imaging (ARFI or shear wave elastography).

**Ultrasound elastography**

The rationale of the test is to measure hepatic stiffness; this has been considered equivalent to the degree of liver fibrosis on biopsy. The technique consists in the transmission of low amplitude shear waves through the liver parenchyma, and results are reported in kilo Pascals (kPa). A probe similar to an ultrasound probe is applied to the intercostal space. An ultrasound pulse-echo is used to measure the longitudinal shear wave velocity which has a direct correlation with hepatic stiffness (fibrosis). The greater the liver stiffness the faster the wave travels. Ultrasound elastography has many advantages: the test can be performed quickly (5–10 minutes), is painless, reproducible, and with less sampling error than liver biopsy since it is possible to evaluate a larger area of liver parenchyma (1/500 of the liver volume), and results are obtained immediately. The AUROC for fibrosis ≥ F2 has been estimated to be 0.84 and up to 0.94 for fibrosis ≥ F3. The principal limitation is in obese patients, in whom it may not be possible to obtain accurate results. However, to overcome this limitation, a special probe (XL) has been tested for obese patients, with good results.

**ARFI (axial radiation force imaging)**

ARFI evaluates the elastic properties of the area of interest and at the same time a conventional B-mode ultrasound in real time is performed. It measures the velocity of higher frequency shear waves that spread perpendicularly to the acoustic push pulse; displacement velocity is proportional to tissue stiffness and results are reported in meters/second. One of the advantages with this method is the possibility to select an optimal area of the liver to evaluate, unlike the limited intercostal approach in ultrasound elastography. Disadvantages are the smaller...
Magnetic resonance elastography (MRE)

MRE involves similar principles to those used in UE and ARFL. A 1.5 Tesla MR scanner is used to record the velocity of a radial shear wave. It has several advantages: MRE allows evaluation of all or at least the majority of the hepatic parenchyma, the quality of the imaging is not affected by the degree of obesity, it does not require an acoustic window, it is operator independent, and it does not require contrast. Recent studies have demonstrated an excellent diagnostic accuracy with sensitivity of 98% and specificity of 99% for detection of all grades of liver fibrosis. The other advantage of this technique is that it provides more successful measurements when compared with ultrasound elastography. Currently it is still considered as an experimental test and has not been extensively validated. The main drawbacks for its use are the high cost, the need for sedation in some patients and the limited accessibility.

Controlled attenuation parameter (CAP)

CAP is a new technique that evaluates the attenuation of ultrasound in liver parenchyma. It is based on the concept that fat decreases the propagation of ultrasound. It uses the same probe that is used for transient elastography and evaluates the same volume of liver parenchyma. The results are in decibels per meter (db/m) with a range from 100 to 400 db/m. Two studies compared CAP with serum steatosis scores (Steatotest, Fatty Liver Index and hepatic Steatosis Index) and demonstrated good accuracy for diagnosis of steatosis. However, this technique is not yet widely available.

Serum biomarkers for predicting steatosis

There are three main noninvasive predictive panels for steatosis that have been recently validated. The Fatty Liver Index (FLI) was designed using data from the Dionysus Nutrition and Liver Study in Northern Italy. An algorithm was designed based on BMI, waist circumference, triglycerides, and GGT with an AUROC of 0.84 for diagnosis of NAFLD. The index has a range from 0 to 100, a FLI < 30 excludes NAFLD and FLI ≥ 60 rules in NAFLD. The main use of FLI has been in epidemiologic studies. Kotronen et al., developed the NAFLD liver fat score (metabolic syndrome, diabetes, fasting serum insulin, AST, ALT) and liver fat equation. The study evaluated 470 patients with MRS. The AUROC was 0.87, the addition of genetic tests (PNPLA3 – rs738409) did not significantly increase accuracy. With the use of the same variables they developed a liver fat equation in which the individual percentage of liver fat can be estimated. A group from the Netherlands has recently validated these results in a cohort of their own.

Serum biomarkers for diagnosis of NASH and fibrosis [Tables 1 and 2]

Serum biomarkers for prediction of NASH and fibrosis can be classified as direct and indirect. Indirect serological markers of liver fibrosis include routine laboratory tests as well as routine clinical parameters, such as BMI, diabetes, and age, among others. These type of tests can detect 50–70% of patients with advanced fibrosis and have demonstrated excellent positive and negative predictive values; however they are not sensitive enough to identify early stages of fibrosis. On the other hand, direct serum biomarkers of liver fibrosis include specific serum tests that detect changes in extracellular matrix or components of the fibrogenesis pathway. Serum markers can be repeated several times and in the case of inconclusive results, a combination of methods can be used to increase diagnostic accuracy.

Serum biomarkers and scores for predicting NASH

There are several scores and panels that have been developed for detecting NASH. Most of them have been designed using cohorts of morbidly obese patients. The HAIR score (hypertension, increased ALT, insulin resistance) was designed in a cohort of 105 morbidly obese patients that underwent laparoscopic bariatric surgery; the presence of ≥ 2 of the score parameters had AUROC of 0.90 to predict NASH and fibrosis in these patients. NASH diagnostics (cleaved and total cyto-keratin-18, adiponectin, resistin) included 101 morbidly obese patients with liver biopsies and ELISA-based assays with a combined AUROC of 0.90 for the prediction of
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Table 1 Noninvasive serum biomarkers to evaluate NASH

<table>
<thead>
<tr>
<th>Test</th>
<th>Variables</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-18 Musso 2011[41]</td>
<td>ELISA plasma cytokeratin 18 fragments</td>
<td>0.82</td>
</tr>
<tr>
<td>NASH test (patented algorithms)</td>
<td>Age, sex, height, weight, triglycerides, cholesterol, alpha2-macroglobulin, AST, ALT, GGT, Bilirubin</td>
<td>0.79</td>
</tr>
<tr>
<td>Poynard 2006[44]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NASH Predictive Index (NPI)</td>
<td>Age, BMI, HOMA-IR, log(AST × ALT), female sex</td>
<td>0.86</td>
</tr>
<tr>
<td>Zein 2007[63]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palekar 2006[60]</td>
<td>Age, Sex, AST, BMI, AST/ALT ratio, serum hialuronic acid</td>
<td>0.76</td>
</tr>
<tr>
<td>Obesity-related NASH Diagnostics</td>
<td>Serum cleaved and Intact CK-18, serum adiponectin, serum resistin</td>
<td>0.85</td>
</tr>
<tr>
<td>Younossi 2008[67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NASH Clinical score for morbid obesity</td>
<td>Hypertension, diabetes, AST ≥ 27 IU/L, ALT ≥ 27 IU/L, sleep apnea, non-black race</td>
<td>0.80</td>
</tr>
<tr>
<td>Campos 2008[69]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAFIC score</td>
<td>Serum ferritin, serum fasting insulin</td>
<td>0.79</td>
</tr>
<tr>
<td>Sumida 2011[61]</td>
<td>Serum type IV collagen 7s</td>
<td></td>
</tr>
<tr>
<td>HAIR</td>
<td>Hypertension, ALT, insulin resistance</td>
<td>0.90</td>
</tr>
<tr>
<td>Dixon 2001[70]</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2 Noninvasive serum biomarkers to evaluate fibrosis in NAFLD

<table>
<thead>
<tr>
<th>Test</th>
<th>Variables</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrotest</td>
<td>Alpha 2-macroglobulin, apolipoprotein A1, haptoglobin, GGT, total bilirubin</td>
<td>0.78</td>
</tr>
<tr>
<td>Ratziu 2006[71]</td>
<td></td>
<td>2 studies[43]</td>
</tr>
<tr>
<td>Adams 2008[72]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAFLD Fibrosis Score</td>
<td>Age, BMI, hyperglycemia, platelet count, albumin, AST/ALT ratio</td>
<td>0.85</td>
</tr>
<tr>
<td>Angulo 2007[73]</td>
<td></td>
<td>13 studies[43]</td>
</tr>
<tr>
<td>BARD Score</td>
<td>BMI ≥ 28 (1 point), AST/ALT ratio ≥ 0.8 (2 points), diabetes (1 point)</td>
<td>0.60</td>
</tr>
<tr>
<td>Harrison 2008[74]</td>
<td></td>
<td>3 studies.</td>
</tr>
<tr>
<td>European Liver Fibrosis (ELF) panel</td>
<td>HA, PIIINP, TIMP-1</td>
<td>0.76</td>
</tr>
<tr>
<td>Guha 2008[75]</td>
<td></td>
<td>0.82</td>
</tr>
<tr>
<td>Combined panel</td>
<td>NAFLD fibrosis score + ELF</td>
<td>0.93</td>
</tr>
<tr>
<td>Guha 2008[76]</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Fibrometer</td>
<td>Age, weight, glucose, AST, ALT, ferritin, platelet count</td>
<td>0.94</td>
</tr>
<tr>
<td>Cales 2009[76]</td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td>BAAT</td>
<td>BMI, age, ALT, TG</td>
<td>0.84</td>
</tr>
<tr>
<td>Ratziu 2000[77,78]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIB-4</td>
<td>Age, AST, ALT, platelet count</td>
<td>0.86</td>
</tr>
<tr>
<td>McPherson 2010[79]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APRI</td>
<td>AST/platelet × 100</td>
<td>0.86</td>
</tr>
<tr>
<td>Wai 2003[79,80]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST/ALT ratio</td>
<td>AST/ALT</td>
<td>0.83</td>
</tr>
<tr>
<td>McPherson 2010[80]</td>
<td></td>
<td></td>
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</tbody>
</table>


biopsy-proven NASH.[83] The same group designed the NASH Diagnostic panel (diabetes, gender, BMI, triglycerides, cleaved and total cytokeratin-18, TIMP-1, AST) developed in a group of 79 morbidly obese patients. The panel included three models: NASH (AUROC 0.81), NASH-related fibrosis (AUROC 0.80), and NASH-related advanced fibrosis (AUROC 0.81).[84] A group from Nice, France created the Nice Model (cytokeratin-18 fragments, metabolic syndrome, ALT). The study included 464 morbidly obese patients who had bariatric surgery. The model was able to predict a nonalcoholic fatty liver disease activity score (NAS) ≥ 5 with AUROC 0.83–0.88.[83] A study from Belgium included 542 overweight and obese patients. They prospectively evaluated routine and non-routine parameters in an unselected population from a single obesity center. Patients with suspected NAFLD had liver biopsy. Combining ALT, fasting insulin or C-peptide, and ultrasound had AUROC of 0.85 in the design.
Currently it is con-

In a multicenter validation study, 

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Currently this test is 

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tide of  pro-collagen III (PIIINP). Only 14% of  patients 

metalloproteinase 1 (TIMP-1), and amino-terminal pep 

of  liver collagen: hialuronic acid (HA), tissue inhibitor of 

proteins and proteinases involved in the early deposition 

This diagnostic panel combines serum levels of  three 

European liver fibrosis panel (ELF) 

Serum biomarkers and panels for predicting fibrosis 

NAFLD fibrosis score (NFS) 

This score has been useful to identify patients with fibro-

sis who will need a more close follow-up. It includes 
six variables: age, hyperglycemia/diabetes, BMI, platelet count, albumin, and AST/ALT ratio. This score can be 
calculated online at the web link http://nafldscore.com. 

Results can be interpreted with two cut-off values; the 

low cut-off value (~1.455) has a negative predictive value of 

93% to exclude advanced fibrosis and the high cut-off value (0.676) has a positive predictive value of 90% to 
predict advanced fibrosis (F3 or F4). Currently it is con-

sidered as the most extensively validated panel for detec-

tion of  advanced fibrosis in patients with NAFLD. The 

score has been validated in at least 13 studies, including 

more than 3000 patients of  different ethnic groups with 

components of  metabolic syndrome like obesity and dia-

bete. The principal limitation is that 20–58% of  patients 

fall between the cut-off values (indeterminate probabil-

ity) and is not possible to accurately classify them in the 
groups of  high or low probability for advanced fibrosis, 

and hence a liver biopsy would be indicated in order to 
appropriately stage their disease. 

European liver fibrosis panel (ELF) 

This diagnostic panel combines serum levels of  three 

proteins and proteases involved in the early deposition 
of  liver collagen: hialuronic acid (HA), tissue inhibitor of 
metalloproteinase 1 (TIMP-1), and amino-terminal pepti-

de of  pro-collagen III (PIIINP). Only 14% of  patients 

are classified in the indeterminate area for advanced 

fibrosis, reducing the need for liver biopsy in more 
than 80% of  cases. However, diagnostic accuracy for 

minimal fibrosis is poor. The combination of  NAFLD 

fibrosis score and ELF was evaluated in a group of 

91% of  patients in the original cohort of  patients, obtain-

ing a better diagnostic accuracy compared with both 
panels individually. In the validation study that included 

196 patients with NAFLD the AUROC was of  0.84, 

0.93, and 0.98 for detection of  fibrosis, moderate fibro-

sis and advanced fibrosis, respectively. 

FibroTest 

FibroTest is based on the serum quantification of alpha 

2 macroglobulin, apolipoprotein A1, haptoglobin, GGT, 

and bilirubin. Using a patented algorithm a value between 

the cut-off values of  0.3 and 0.7 is obtained. Unfortu-

nately, 33% of  patients fall in the area in which it is not 
possible to detect the presence or absence of  significant 

liver fibrosis. In a recent meta-analysis, which included 

267 patients with NAFLD, the AUROC was 0.84 to 
detect advanced fibrosis with FibroTest. This panel can 

be combined with SteatoTest and NASH test; the combi-
nation of  these three panels is known as Fibromax, which 
is commercially available. 

SUMMARY 

The three noninvasive diagnostic tests that have been 

independently validated compared with liver biopsy for 
diagnosis of  NASH and fibrosis are serum levels of  

CK-18 for diagnosis of  NASH and ultrasound elastog-

raphy and NAFLD fibrosis score for the diagnosis of 

advanced fibrosis. Serum biomarkers that have been vali-

dated (NAFLD fibrosis score, FibroTest) could be used 
as screening tools in patients with metabolic syndrome. 

Imaging techniques have been developed and could have 
a more widespread use in the future, but only ultrasound 
elastography has been validated extensively. CK-18 is a 
direct serum biomarker that could be part of  the routine 
diagnostic evaluation for NASH in the near future.

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