The consensus conference statements recommend liver biopsy in the management of almost all patients with the most frequent chronic liver diseases: chronic hepatitis C (HCV) and B (HBV), non-alcoholic fatty liver disease (NAFLD), with its risk of non alcoholic steato-hepatitis (NASH), and alcoholic fatty liver disease (AFLD) with its risk of non alcoholic steato-hepatitis (ASH).1,5 but also underline the necessity of developing reliable non-invasive tests. 2,3 Numerous studies strongly suggest that due to the limitations and risks of biopsy, 4–6 as well as the improvement of the diagnostic accuracy of biochemical markers, 7–12 liver biopsy should no longer be considered mandatory. 13

Diagnostic Values of Non-invasive Alternatives

Among the non-invasive alternatives to liver biopsy, several studies have demonstrated the predictive value of two combinations of simple serum biochemical markers: FibroTest™ (or HCV-FibroSURE™ in the US) for the quantitative assessment of fibrosis and ActiT est™ (or HCV-FibroSURE ™ in the US) for the quantitative assessment of necroinflammatory occurrences. 10–12 Similar results have not been obtained with other diagnostic tests. 10–14

Since September 2002 FibroSURE-FibroTest-ActiT ests (FT-AT) have been used in several countries as an alternative to liver biopsy. Several systematic reviews15,16 and independent prospective studies17–25 have validated these panels of tests.

The diagnostic values of FT were similar for the four most frequent fibrotic liver diseases: HCV, 10,12,14,15,19–30 HBV, 11,13,15 NAFLD 31,32 and AFLD35 (see Table 1 and Figure 1). The diagnostic values were similar between intermediate or extreme stages as assessed by area under the receiver operator characteristic (AUROC) between all stage combinations.10 It has been also demonstrated that biomarkers may provide a more accurate (quantitative and reproducible) picture of fibrogenic and necrotic events occurring within the liver than a low-quality liver biopsy. A prospective study observed that 18% of discordances were attributable to biopsy failure (mostly due to small length) and 2% to the failure of the tests.11 The prospective follow-up of these patients during five years has also demonstrated that the prognostic value of FibroSURE was greater than that of biopsy for predicting mortality and morbidity.12

Since June 2006 three new combinations of biomarkers have completed the spectrum of non-invasive liver biomarkers: SteatoTest™, for the diagnosis of steatosis (fatty liver), 31 NashTest™ for the diagnosis of NASH, 36 and AshTest™ for the diagnosis of ASH.27 In the US, Nash-FibroSURE™ included FibroTest-SteatoTest and NashTest; Ash-FibroSURE™ included FibroTest-SteatoTest and AshTest.

Status in Patients with Hepatitis C, Hepatitis B, Non-alcoholic, and Alcoholic Fatty Liver Disease

Sensitivity and Specificity

In chronic liver diseases, the liver biopsy is far from a true gold standard and a high percentage of so-called false-negative and false-positive results could be due in fact to errors of biopsy.10–12 The risk of failure of FT has been studied on 32,527 tests carried out in patients, 54.6% of which were male and 16% were older than 65. The most frequent abnormal value observed during post-marketing follow-up was haptoglobin lower than 0.12g/l in 1,589 patients (4.89%). Among these patients, there were 272 cases with high-risk profile of false positive results (0.84%), of which the other components were not concordant in favor of significant fibrosis. Patients with extremely low haptoglobin, especially when the rest of the examinations were hardly modified, could have had hemolysis. High-risk profile of false positive results due to possible Gilbert syndrome was observed in 409 cases (1.26%). The most frequent cause of abnormally elevated values for bilirubin was Gilbert disease, while for alpha-2-macroglobulin and haptoglobin it was acute sepsis. In the presence of acute inflammation, i.e. sepis, or of acute hemolysis, FT-AT analysis must be postponed.10
Alternative Tests—Overview of Biomarkers

No available tests have been extensively validated for the diagnosis of both fibrosis and activity. Different combinations of liver tests have been suggested with small studies, without extensive studies, including the analytical variability, as well as the risk of false positive or negative results in large community-based populations. No available test has demonstrated a continuous and linear correlation with fibrosis stage and fibrosis grades. 3,5,10

Serum alanine aminotransferase (ALT) was the most commonly investigated marker, whose sensitivity was too low, ranging from 61 to 71%. The diagnostic value was lower than a combination of markers in all direct comparisons. Among the extracellular matrix tests, hyaluronic acid correlated the best with fibrosis stage overall, but has been demonstrated only for extensive fibrosis. The AUROCs for extensive fibrosis range from 0.65 to 0.86. Procollagen type III peptide and tissue inhibitors of metalloproteinase-1–4 were less predictive than hyaluronic acid. 10

One panel of biomarkers combining alpha-2-macroglobulin, hyaluronic acid and the tissue inhibitor of metalloproteinases-1 (TIMP-1) is also on the US market (Fibrospect I and II™), with only few abstracts and one full publication. 3 This panel is designed only for fibrosis diagnosis without diagnostic value for necroinflammatory activity, steatosis, ASH, or NASH. No study has been presented in a community-based population and the risks of false negative and false positive results has not been identified, along with separate studies in different chronic liver diseases.

Several studies directly compared FT-AT with hyaluronic acid, the Forns index 17 and the APRI index 18,20 in the same patients. The FT had higher diagnostic values (the AUROC was significantly higher). FT was, in particular, more sensitive for discriminating between F1 and F2 and more linearly correlated to stages when compared with those three other markers. 10,18,20,24

Another weakness of aspartate aminotransferase (AST) to platelet ration index (APRI) (combining serum AST and platelets) is the absence of standardised methods and assay calibration-expression of aminotransferase or gamma-glutamyl-transpeptidase (GGT) in multiples of the upper limit of reference values should not be employed. 18,40,41 An additional weakness of the Forns index (combining age, platelets, gamma-glutamyl-transpeptidase, and cholesterol) is the inclusion of cholesterol, which varies greatly in patients with genotype 3. 17

A study using profiles of serum protein N-glycans found that a profile has a similar AUROC to the blood test for the diagnosis of compensated cirrhosis. When combined with FT, this marker had 100% specificity and 75% sensitivity for the diagnosis of compensated cirrhosis, which was not significantly different from the 92% specificity and 67% sensitivity of FT alone. 19 Numerous preliminary studies of panels for the diagnosis of fibrosis have been published, but without extensive developments so far:

- Rosenberg index combining hyaluronic acid, procollagen III peptide (PIIP), TIMP-1;
- a score named MP3 that combines TIMP-1, matrix metalloproteinases (MMP2);
- FPI (fasting plasma insulin) combining age, cholesterol, AST, insulin, alcohol consumption;
- Laine index combining hyaluronic acid, carbohydrate transferrin, alkaline phosphatase (AP) combining age and platelets;
- FibroMeter combining prothrombin time, AST, alpha-2-macroglobulin, hyaluronic acid, urea, and age;

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Table 1: Summary of Advantages and Limits of Liver Biopsy and Biochemical Markers

<table>
<thead>
<tr>
<th>Liver biopsy</th>
<th>Biochemical markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>History</td>
<td>Classical standard</td>
</tr>
<tr>
<td>Disease diagnosis</td>
<td>Fibrosis, activity, steatosis, steato-hepatitis, iron</td>
</tr>
<tr>
<td>Estimate</td>
<td>Semi-quantitative</td>
</tr>
<tr>
<td>False negative</td>
<td>Regeneration nodule, small biopsy</td>
</tr>
<tr>
<td>False positive</td>
<td>Subcapsular biopsy, small biopsy</td>
</tr>
<tr>
<td>Adverse events</td>
<td>Three deaths in 10,000, Three severe adverse events in 1,000</td>
</tr>
<tr>
<td>Sampling error</td>
<td>33% discordance in fibrosis staging, 24% discordance in activity grading</td>
</tr>
<tr>
<td>Observer error</td>
<td>Fibrosis stage discordance (20%), Activity grade discordance (40%)</td>
</tr>
<tr>
<td>Minimal requirements</td>
<td>At least 25mm size, More than five portal tracts</td>
</tr>
<tr>
<td>Hospitalisation</td>
<td>Six to 24 hours</td>
</tr>
<tr>
<td>Contraindications</td>
<td>Coagulation disorder</td>
</tr>
<tr>
<td>Cost</td>
<td>€1,032 for uncomplicated biopsy, €2,745 for complicated biopsy</td>
</tr>
</tbody>
</table>
• HepaScore combining bilirubin, gamma-glutamyltransferase, hyaluronic acid, alpha-2-macroglobulin, age, and sex; and
• FIB-4 combining age, AST, platelets and ALT.

The Fibroscan is another way to physically make an estimation of the histological assessment, using elastography. Several studies have validated this technique for the diagnosis of advanced fibrosis with similar diagnostic values than FT for cirrhosis and precirrhosis stages.20 In patients with HCV infection, the sensitivity of FT seems higher for early fibrosis stages and the combination of Fibroscan and Fibrotest improves the overall diagnostic value.30

How the Blood Tests Work

FT-AT is a non-invasive blood test that combines the quantitative results of six serum biochemical markers—alpha-2-macroglobulin, haptoglobin, GGT, total bilirubin, apolipoprotein A1 and ALT—with the patient’s age and gender in a patented artificial intelligence algorithm, United States Patent and Trademark Office (USPTO) 6,631,330, to generate a measure of fibrosis stage and necroinflammatory grade in the liver.14 It is a continuous linear biochemical assessment of fibrosis stage and necroinflammatory activity grade that provides a numerical quantitative estimate of liver fibrosis ranging from 0 to 1, corresponding to the well-established METAVIR scoring system of stages F0 to F4 and of grades A0 to A3 (see Figure 1).20,42

ASH FibroSure is a non-invasive assessment of liver status for patients with AFLD. Quantitative results of 10 biochemicals, including alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT, AST, total cholesterol, triglycerides, and fasting glucose, in combination with age, gender, height, and weight, are analyzed, using a proprietary algorithm (Patent pending), to provide quantitative surrogate markers for liver fibrosis (FibroTest), hepatic steatosis (SteatoTest), and NASH (NashTest). FibroTest and SteatoTest are the same than those developed for viral hepatitis or AFLD. In a study of 171 NAFLD patients where 23% had significant NAFLD associated fibrosis (Metavir F2-F4) and 11% had cirrhosis by liver biopsy, a fibrosis result of >0.3 yielded a sensitivity of 83% and a specificity of 78% for the detection of significant fibrosis.33 The NashTest is a diagnostic assessment of the presence of NASH using three broad categories N0-N2 corresponding to “Not NASH”, “Borderline NASH”, “NASH” per the Kleiner classification (Kleiner 2005). In a population of 257 NAFLD patients, where 62% had at least borderline NASH by liver biopsy, a prediction of NASH had a sensitivity of 88% for identifying NASH, and a specificity of 50%.36

The analyses should preferably be made on fresh serum but can be carried out with plasma if necessary (blood sample on lithium heparinate). The measurements of the six parameters are made preferably on fresh serum (or plasma) or that which has been stored between +2°C and +8°C for a maximum of four days in an unlit area (for the protection of bilirubin). For deferred measurements, the serum should be quickly frozen to -80°C. After thawing, it should be centrifuged for 10 minutes at 15,000g.44 It has been prospectively demonstrated that the FT-AT can be performed on fasting or non-fasting serum samples.43

Conclusion

Several published studies and overviews have demonstrated the predictive value and the better benefit:ratio than biopsy of these biomarkers combining simple serum biochemical markers in patients with the four most frequent liver disease: patients infected with HCV and HB; patients alcoholic or NAFLD.

They allow a quantitative assessment of both fibrosis and steatosis for these patients. For chronic HCV and HBV they allow a quantitative assessment of necroinflammatory histological activity. For AFLD and
NAFLD they also allow an assessment of steato-hepatitis.

The possible causes of false-negative and false-positive results are also better identified. These tests, which are now available in more than 30 countries, can facilitate the screening and management of chronic HCV, HBV, alcoholic and NAFLD. According to its poor benefit:risk ratio, liver biopsy should be abandoned as a first-line assessment of these very frequent chronic liver disease. The non-invasive biomarkers also have limitations, although one advantage for FT-AT is that the profiles of patients at risk of false-positive or false-negative results are well identified. The analytical recommendations are also important to limit the inter-laboratory variability of these combinations. These tests can facilitate the screening of the most frequent liver diseases. The developments of biomarkers derived from proteomics or glycomics and their combinations with other technique as elastometry are likely to be important in the future.

**Key Topics**

- Numerous studies strongly suggest that due to the limitations and risks of biopsy, as well as the biochemical markers, liver biopsy should no longer be considered mandatory.

- Among the non-invasive alternatives to liver biopsy, several studies have demonstrated the predictive value and the better benefit:risks ratio than biopsy of a combination of simple serum biochemical markers in patients infected with HCV and also in HBV, alcoholic and non alcoholic fatty liver disease.

- FibroSURE™ for the quantitative assessment of fibrosis, and SteatoTest for the quantitative assessment of steatosis, in these four diseases.

- Ash-FibroSURE™ combines FibroTest, SteatoTest and AshTest for the quantitative assessment of alcoholic steato-hepatitis.

- Ash-FibroSURE™ combines FibroTest, SteatoTest and NashTest for the assessment of non alcoholic steato-hepatitis.

- A prospective study in HCV observed that 18% of discordances were attributable to biopsy failure (mostly due to small length) and 2% to FT-AT failure (haemolysis, Gilbert syndrome, and acute inflammation) and the prognostic value of FT was better than biopsy at five year.

- These tests, which are now available in more than 30 countries, can facilitate the screening and management of chronic HCV, HBV, alcoholic liver disease and metabolic fatty liver.
## References

Alternatives to Liver Biopsy for Assessing Liver Disease


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