Non-invasive diagnosis of steatosis and fibrosis

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Abstract

The prognosis and management of liver disease greatly depends on the amount of liver fibrosis. Non-alcoholic fatty liver disease (NAFLD), ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), is emerging as a major cause of liver disease in Western countries because of the increasing prevalence of obesity and type 2 diabetes. A key issue in patients with NAFLD is the differentiation of NASH from simple steatosis. It is particularly important to identify NASH patients as they are at greatest risk of developing complications such as cirrhosis, liver failure and hepatocellular carcinoma. The limitations of liver biopsy (invasive procedure, sampling errors, interobserver variability and non-dynamic fibrosis evaluation) have stimulated the search for non-invasive approaches for the assessment of steatosis and liver fibrosis in patients with NAFLD. A variety of methods, including serum markers, imaging techniques such as ultrasound, CT, MRI and measurement of liver stiffness by transient elastography, have been proposed for the non-invasive assessment of steatosis and hepatic fibrosis. This review discusses the advantages and limitations of these different methods in clinical practice.

Résumé

Diagnostic non invasif de la stéatose et de la fibrose hépatiques

L’importance et la progression de la fibrose hépatique conditionnent à la fois le pronostic et la prise en charge des maladies chroniques du foie. La stéatose hépatique non alcoolique (NAFLD), qui va de la stéatose simple jusqu’à la stéatohépatite non alcoolique (NASH), est une cause émergente de maladie du foie dans les pays occidentaux, en raison de la prévalence croissante de l’obésité et du diabète de type 2. Différencier la NASH de la stéatose simple est d’une importance fondamentale chez les patients atteints de NAFLD. En effet, les patients atteints de NASH sont les plus à risque de développer d’une part, une fibrose hépatique progressive et, d’autre part, des complications telles qu’une cirrhose, une insuffisance hépatique ou un carcinome hépatocellulaire. Les limite de la biopsie hépatique (examen invasif avec biais d’échantillonnage et variabilité interobservateur qui ne permettent pas une évaluation dynamique de la fibrose) ont stimulé la recherche d’approches non invasives pour évaluer la stéatose et la fibrose hépatiques chez les patients atteints de NAFLD. Plusieurs méthodes comprenant des marqueurs sériques, des techniques d’imagerie comme l’échographie, le scanner ou l’IRM, et plus récemment la mesure de l’élasticité hépatique par élastométrie impulsionnelle (FibroScan), ont ainsi été proposées. Cette revue a pour but de discuter les avantages et les limites respectives de ces méthodes en pratique clinique.

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Keywords: Type 2 diabetes; Obesity; Non-alcoholic steatohepatitis; Liver fibrosis; Steatosis; Non-invasive procedure; Transient elastography (FibroScan); Serum markers; Liver biopsy; Review.

Mots clés : Diabète de type 2 ; Obésité ; Stéatohépatite non alcoolique ; Fibrose hépatique ; Stéatose ; Méthode non invasive ; Élastométrie (FibroScan) ; Marqueurs sériques ; Biopsie hépatique ; Revue.
1. Non-invasive diagnosis of steatosis

1.1. Imaging techniques

Non-invasive techniques such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (1H-MRS) can detect hepatic steatosis, but currently cannot distinguish between simple steatosis and NASH.

1.1.1. Ultrasound

Hepatic ultrasound is a simple, non-invasive technique that is widely used in clinical practice to detect fatty infiltration of liver. Hepatic steatosis causes increased echogenicity on ultrasound, making the liver appear brighter than the cortex of the right kidney. Several studies have shown that ultrasound for detecting hepatic steatosis has a sensitivity of 60% to 94%, and a specificity of 84% to 95% [10]. The sensitivity of ultrasound increases with increasing degrees of fatty infiltration. However, ultrasound is unable to provide a precise grading of hepatic fat content. Also, its sensitivity is reduced in the morbidly obese, and its performance is highly operator-dependent.

1.1.2. Computed tomography

Non-contrast-enhanced CT is the most accurate CT technique to detect and characterize hepatic steatosis [11]. The CT diagnosis of hepatic steatosis is made by measuring the difference in liver and spleen attenuation values in Hounsfield units. In subjects with steatosis, as the mean attenuation value of the liver is lower that of the spleen, the liver appears darker than the spleen. Although non-contrast-enhanced CT is useful for the qualitative diagnosis of macrovesicular steatosis of 30% or greater, there is conflicting evidence as to whether or not it can accurately quantify hepatic fat content. In addition, it exposes subjects to ionizing radiation.

1.1.3. MRI and proton MR spectroscopy

Chemical-shift MRI uses the difference in resonance frequency of water and lipid to differentiate tissue containing only water from those containing water and lipid, known as the Dixon method. Several studies have recently demonstrated a good correlation between the severity of hepatic steatosis on MRI and liver biopsy [12,13]. Multiecho imaging may also be a promising method [14]. Similarly, in vivo 1H-MRS is a fast and safe technique for the quantitative assessment of hepatic steatosis. Several studies have shown a good correlation between quantification of hepatic fat content by H-MRS and liver biopsy [13,15]. Both techniques will be useful tools in the future.

1.2. Serum markers

So far, the only serum test that has been proposed to detect steatosis is the SteatoTest [16]. This test includes the 6 parameters of FibroTest–ActiTest plus BMI, serum cholesterol, triglycerides and glucose adjusted for age and gender. It has been constructed from a training group of 310 patients with various chronic liver diseases, using the presence of steatosis (> 5%) on liver biopsy as the reference, and validated in three different groups of patients with hepatitis C and alcoholic liver disease (n = 434). At a cutoff of 0.3, the sensitivity of SteatoTest ranged from 85% to 100% whereas, at a cutoff of 0.7, the specificity ranged from 83% to 100%. Validation of this test in other groups of patients (including NAFLD) by independent studies is awaited.

More interest has been focused on whether or not non-invasive serum tests can differentiate NASH from simple steatosis among patients with NAFLD. Several groups have proposed tests, including the NashTest [17], and scores combining age, gender, AST, BMI, AST/ALT ratio and hyaluronic acid [18] or adiponectin, HOMA-IR, and serum type IV collagen [19] (Table 1).
under the receiver operating characteristic (AUROC) curve, which plots the sensitivity over 1 – specificity, with optimal values being as close to 1.0 as possible. The diagnostic performances of most of the proposed indices are summarized in Table 3. Importantly, the results of the training set were confirmed in an independent validation set in only a few studies [27–31]. In addition, most of these studies included small numbers of patients with heterogeneous scoring systems and endpoints for fibrosis assessment. Some indices such as the FibroTest have also been proposed for the screening of fibrosis in large populations at risk of developing fibrosis such as diabetics [34] or hyperlipidemic patients [35].

Three indices are protected by patents and are currently commercially available: the FibroTest® in Europe (BioPredictive, Paris, France) or FibroSURE® in the USA (LabCorp, Burlington, NC, USA); FibroMeters® (BioLiveScale, Angers, France); and ELF® (Enhanced Liver Fibrosis Test, iQur Ltd, Southampton, UK).

### Table 1

<table>
<thead>
<tr>
<th>Markers</th>
<th>N</th>
<th>Parameters</th>
<th>Endpoint</th>
<th>AUROC</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NashTest [17]</td>
<td>257</td>
<td>Age, gender, BMI, triglycerides, cholesterol, α-2-macroglobulin, γGT, AST, ALT, haptoglobin apolipoprotein A1, total bilirubin</td>
<td>NAS ≥ 5</td>
<td>0.79*</td>
<td>29</td>
<td>98</td>
<td>91</td>
<td>71</td>
</tr>
<tr>
<td>Palekar index [18]</td>
<td>80</td>
<td>Age ≥ 50 yrs; female gender; AST ≥ 45 U/L; AST/ALT ratio ≥ 0.8; BMI ≥ 30 kg/m²; hyaluronate ≥ 55 mcg/l</td>
<td>NASH</td>
<td>0.76</td>
<td>74</td>
<td>66</td>
<td>68</td>
<td>71</td>
</tr>
<tr>
<td>Shimada index [19]</td>
<td>85</td>
<td>Serum adiponectin level; HOMA-IR; serum type IV collagen 7s level</td>
<td>NAS ≥ 5</td>
<td>ND</td>
<td>ND</td>
<td>94</td>
<td>74</td>
<td>94</td>
</tr>
</tbody>
</table>

NAS: NAFLD activity score.
AUROC: area under ROC curve; Se sensitivity; Sp specificity; PPV and NPV: positive and negative predictive values.
*Validation group: performances correspond to validation group.
**Control group.

### Table 2

| Proposed serum indices for non-invasive evaluation of fibrosis in NAFLD. |
|-----------------------------|-------------------------------------------------------------------------|
| – BAAT score (BMI, age, ALT, triglycerides) | |
| – NAFLD Fibrosis Score (NFS) (age, hyperglycemia, BMI, platelet count, albumin, AST/ALT ratio) | |
| – European Liver Fibrosis score (ELF) (age, hyaluronate, MMP-3, TIMP-1) | |
| – FibroMeter NAFLD (age, weight, platelet count, ferritin, glucose, AST, ALT) | |
| – FibroTest (α-2-macroglobulin, γGT, apolipoprotein A1, haptoglobin, total bilirubin, age, gender) | |
| – NS score (type IV collagen 7s, hyaluronate) | |

2. Non-invasive diagnosis of fibrosis in NAFLD

Scoring of liver fibrosis by histology is used in a variety of scoring systems, including some that have been specifically designed for NAFLD, such as the Brunt [20] and Kleiner scores [21], and others, such as META VIR [22] and Scheuer [23], designed for the scoring of fibrosis in the context of viral hepatitis. The most attention has been focused on whether or not non-invasive tests can detect advanced fibrosis (F3-F4) or cirrhosis (F4). Such an approach is clinically relevant because the presence of advanced fibrosis or cirrhosis is an indication for specific monitoring of complications related to portal hypertension and to the increased risk of developing hepatocellular carcinoma.

The clinical and biological variables most commonly associated with advanced fibrosis in patients with NAFLD are: increasing age; elevated BMI; presence of diabetes; presence of the metabolic syndrome; increased homeostatic insulin resistance (HOMA-IR); increased aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio; decreased platelet count; and hyaluronic acid [24].

2.1. Serum markers

Compared with hepatitis C [25], only a limited number of serum markers have been evaluated for their ability to assess liver fibrosis in patients with NAFLD. They include the BAAT score [26], NAFLD score [27], ELF score [28, 29], FibroMeters [30], FibroTest [31], hyaluronic acid [32] and NS score [33] (Table 2). Markers have been validated against the current clinical gold-standard liver biopsy using, as an expression of effectiveness, the area under the receiver operating characteristic (AUROC) curve, which plots the sensitivity over 1 – specificity, with optimal values being as close to 1.0 as possible. The diagnostic performances of most of the proposed indices are summarized in Table 3. Importantly, the results of the training set were confirmed in an independent validation set in only a few studies [27–31]. In addition, most of these studies included small numbers of patients with heterogeneous scoring systems and endpoints for fibrosis assessment. Some indices such as the FibroTest have also been proposed for the screening of fibrosis in large populations at risk of developing fibrosis such as diabetics [34] or hyperlipidemic patients [35].
2.2. Transient elastography

Transient elastography (TE) (FibroScan®, Echosens, Paris, France) has recently been proposed for measuring liver stiffness [36]. Briefly, an ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisitions are used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness, expressed as the elastic modulus: the stiffer the tissue, the faster the shear wave propagates.

TE is painless, rapid (less than 5 min) and easy to perform at the bedside or in the outpatients clinic. The results are immediately available and expressed in kilopascals (kPa), corresponding to the median value of 10 validated measurements and ranging from 2.5 to 75 kPa, with normal values being around 5.5 kPa [37]. The main limitation of TE in clinical practice is the impossibility of obtaining any liver stiffness measurements in around 5% of cases, mainly obese patients, which may represent a concern for its use in NAFLD patients.

TE has been shown to be reliable in the assessment of liver fibrosis initially in patients with chronic hepatitis C [38,39], with a strong correlation of liver stiffness values with META-VIR fibrosis stages, and with AUROCs ranging from 0.79 to 0.83 for the diagnosis of significant fibrosis and from 0.95 to 0.97 for cirrhosis. So far, only two studies have investigated TE in patients with NAFLD [40,41] (Table 4). However, these TE results should be interpreted with caution as these studies were conducted in a Japanese [40] and a pediatric population [41], with low mean BMIs (26.6 ± 4.2 and 26 ± 4, respectively) and small sample sizes (97 and 52, respectively). This may be an explanation for the rather low LSM failure rate in these two studies (5% and 4%, respectively), similar to that reported in patients without NAFLD.

Liver stiffness values may be influenced by the metabolic syndrome even in the absence of biological features of NAFLD. Indeed, in a recent study conducted in 429 healthy Western subjects without overt causes of liver disease and normal liver enzymes, liver stiffness values were significantly higher in subjects with the metabolic syndrome (n=59; 13.7%) than in those without (6.5 ± 1.6 vs 5.3 ± 1.5 kPa, respectively; *P< 0.0001) [42]. Interestingly, in four of the seven subjects with the metabolic syndrome who had liver stiffness values above 8 kPa and who underwent liver biopsy, all had NASH lesions with portal fibrosis, but mild or absent steatosis, suggesting that TE may be a sensitive tool for the detection of fibrosis. More data are awaited regarding the use of TE in NAFLD patients.

Table 3
Diagnostic performance of currently available non-invasive indices of liver fibrosis in NAFLD.

<table>
<thead>
<tr>
<th>Markers</th>
<th>N</th>
<th>Score</th>
<th>Fibrosis stage</th>
<th>Cut-offs</th>
<th>AUROC</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAAT [26]</td>
<td>93</td>
<td>METAVIR</td>
<td>F ≥ 2</td>
<td>2</td>
<td>0.84</td>
<td>71</td>
<td>80</td>
<td>61</td>
<td>86</td>
</tr>
<tr>
<td>NFS [27]</td>
<td>733</td>
<td>253*</td>
<td>F ≥ 3</td>
<td>≤ 1.455 &gt; 0.676</td>
<td>0.82*</td>
<td>77</td>
<td>71</td>
<td>52</td>
<td>88</td>
</tr>
<tr>
<td>ELF [28,29]</td>
<td>61</td>
<td>Scheuer</td>
<td>F ≥ 3</td>
<td>0.375</td>
<td>0.87</td>
<td>89</td>
<td>96</td>
<td>80</td>
<td>98</td>
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<tr>
<td></td>
<td>192</td>
<td>Kleiner</td>
<td>F ≥ 1</td>
<td>-0.207</td>
<td>0.76</td>
<td>61</td>
<td>80</td>
<td>81</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F ≥ 2</td>
<td>-0.1068</td>
<td>0.82</td>
<td>70</td>
<td>80</td>
<td>70</td>
<td>80</td>
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<td></td>
<td></td>
<td></td>
<td>F ≥ 3</td>
<td>0.3576</td>
<td>0.90</td>
<td>80</td>
<td>90</td>
<td>71</td>
<td>94</td>
</tr>
<tr>
<td>FibroMeters [30]</td>
<td>235</td>
<td>114*</td>
<td>METAVIR</td>
<td>F ≥ 2</td>
<td>ND</td>
<td>0.94</td>
<td>78.5</td>
<td>95.9</td>
<td>87.9</td>
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<tr>
<td>FibroTest [31]</td>
<td>170</td>
<td>97</td>
<td>Brunt/Kleiner</td>
<td>F ≥ 2</td>
<td>0.3</td>
<td>0.7</td>
<td>81</td>
<td>77</td>
<td>54</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F ≥ 3</td>
<td>0.3</td>
<td>0.7</td>
<td>88</td>
<td>77</td>
<td>54</td>
<td>90</td>
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<td></td>
<td></td>
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<td></td>
<td>0.92</td>
<td>85</td>
<td>80</td>
<td>51</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Hyaluronate [32]</td>
<td>79</td>
<td>Brunt</td>
<td>F ≥ 3</td>
<td>46.1</td>
<td>0.92</td>
<td>85</td>
<td>80</td>
<td>51</td>
<td>96</td>
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<tr>
<td>NS [33]</td>
<td>112</td>
<td>Brunt</td>
<td>F ≥ 3</td>
<td>Coll ≥ 5 or HA ≥ 50</td>
<td>ND</td>
<td>96</td>
<td>63</td>
<td>66</td>
<td>95</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coll ≥ 5 and HA ≥ 50</td>
<td>54</td>
<td>92</td>
<td>84</td>
<td>73</td>
<td></td>
</tr>
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</table>

AUROC: area under ROC curve; Se sensitivity; Sp specificity; PPV and NPV: positive and negative predictive values
* Validation group: performances correspond to the validation group.
Table 4
Diagnostic performance of transient elastography (TE) in NAFLD.

<table>
<thead>
<tr>
<th>Authors</th>
<th>N</th>
<th>Score</th>
<th>TE cut-offs (kPa)</th>
<th>AUROC</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F ≥ 1</td>
<td>F ≥ 2</td>
<td>F ≥ 3</td>
<td>F ≥ 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoneda et al. [40]</td>
<td>97</td>
<td>Brunt</td>
<td>5.9</td>
<td>0.93</td>
<td>86</td>
<td>89</td>
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<td>59</td>
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<td>0.86</td>
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<td>27</td>
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<td>0.90</td>
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<tr>
<td>Nobili et al. [41]</td>
<td>50</td>
<td>Brunt</td>
<td>5.1</td>
<td>0.97</td>
<td>97</td>
<td>91</td>
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</tr>
</tbody>
</table>

AUROC: area under ROC curve; Se sensitivity; Sp specificity; PPV and NPV: positive and negative predictive values.

2.3 Other imaging techniques

Conventional imaging techniques such as ultrasound coupled with Doppler, CT and MRI can be used for the diagnosis of cirrhosis. However, the ability to detect early and intermediate stages of fibrosis with these techniques remains limited. Novel techniques, including magnetic resonance (MR) spectroscopy, diffusion-weighted MR and MR elastography, have also emerged for detecting hepatic fibrosis [43]. The theoretical advantages of these methods include the ability to analyze nearly the entire liver and their applicability in obese patients. MR elastography has recently been suggested to have better diagnostic accuracy than TE for the diagnosis of significant fibrosis (AUROC: 0.99 vs 0.84, respectively; P < 0.05) in a series of 96 patients with liver disease (eight with NASH) [44]. Although such results are encouraging, so far, these techniques remain too expensive and time-consuming for implementation in clinical practice for screening hepatic fibrosis.

Conflicts of interest: The author has none to declare.

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