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Metabolic Steatosis & Fibrosis: Review of the Non-Invasive Tools for Diagnosis and Screening

Miette Véronique, Abdennour Meriem, Sandrin Laurent and Sasso Magali
Echosens,
France

1. Introduction

Hepatic steatosis affects 20% to 30% of the general adult population in affluent countries. Metabolic syndrome and its components—type 2 diabetes mellitus and central obesity—lead to the development of nonalcoholic fatty liver disease (NAFLD), in part due to insulin resistance, apoptosis and altered adipokine and cytokine pathways. Indeed, NAFLD is now considered the hepatic manifestation of metabolic syndrome. Globally, NAFLD is a common cause of chronic liver disease and, as the obesity epidemic continues, the prevalence of NAFLD is anticipated to increase. Non Alcoholic Fatty Disease (NAFLD) encompasses a histological spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), the latter characterized by steatosis plus necroinflammation. NASH can have different stages of fibrosis ranging from absent (stage F0) to cirrhosis (stage F4). Currently, the technique of choice for determining hepatic fat deposition and the stage of fibrosis is liver biopsy. However, it is an invasive procedure and its use is limited. It may also be subject to sampling error. Non-invasive techniques such as ultrasound, computerized 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 steatohepatitis, or stage the degree of fibrosis accurately. Four new non-invasive methods received independent validation to assess NASH diagnosis (serologic tests) or to stage advanced fibrosis (blood tests, magnetic resonance elastography (MRE) and vibration controlled transient elastography (VCTE)).
The chapter is organized as follows: section 2 describes natural history of NAFLD and NASH. The epidemiology is detailed section 3 and the risk factors in section 4. Than the limitations of liver biopsy are explained section 5; section 6 contains a description of non invasive tools clinically used or at the contrary new development not yet clinically evaluated. Advantages and limitations of these devices are detailed. Their applications in NAFLD diseases are described. Eventually, discussion and conclusion are drawn in section 6 and section 7, respectively.

2. Natural history: NASH and NAFLD

Ludwig et al. about 30 years ago described a liver disease that resembled alcoholic hepatitis in its histopathologic features but without a history of significant alcohol consumption (Ludwig et al. 1980). Since then, literature about non-alcoholic fatty liver disease (NAFLD)

has expanded exponentially in attempts to understand its pathophysiology and treat the condition.

The term NAFLD is used to describe a condition of fat accumulation in the liver in the absence of excessive alcohol consumption and any other specific causes of hepatic steatosis (Ludwig et al. 1980; Bedogni et al. 2006). In the majority of the cases, NAFLD is of primary origin and its etiology is not yet completely understood, even if it is strictly related to the presence of insulin resistance, and thus frequently occurs as the initial part of the metabolic syndrome, and accompanies obesity, type 2 diabetes and dyslipidaemia.

NAFLD is a chronic liver condition that spans a spectrum of abnormalities ranging from simple hepatic steatosis to a predominantly lobular necroinflammation, with or without centrilobular fibrosis (termed non-alcoholic steatohepatitis or NASH), which can eventually lead to cirrhosis and its associated complications. Around 30% of NAFLD may progress to NASH (Farrell & Larter 2006). At the time of diagnosis, the presence of severe fibrosis in liver biopsies in patients with NASH has been noted in 15 to 50% of patients and 7% to 26% with cirrhosis (Angulo & Lindor 2002). Undiagnosed NASH is the most likely cause for cryptogenic cirrhosis (Bugianesi et al. 2002). Hepatocellular carcinoma is also a recognized complication of fatty liver disease and emerging evidence suggests that cardiovascular disease may also be more common.

A first phase is responsible for the accumulation of fatty acids in hepatocytes, which weakens the adaptive phase hepatocytes and makes them more susceptible to attacks. The second phase, lipid peroxidation secondary to oxidative stress, is ultimately responsible for cellular damage and the appearance of liver fibrosis.

In fact, the mechanisms and causes of the initial lesions of steatosis are less known. It is recognized that insulin resistance plays a central role in pathogenesis, occurring probably in a favorable genetic background. Adipocyte-related factors (leptin, resistin, adiponectin), particularly from the visceral fat, have been implicated in causing insulin resistance and fatty lesions. The existence of genetic factors predisposing to the development of these lesions has also been suggested (mitochondrial abnormalities, genetic polymorphism of inflammatory cytokine synthesis).

3. Epidemiology

Epidemiological studies can be separated into two categories: selected population studies and general screening population studies. The studies using highly selected populations suffer from bias but they have high specificity because histology was used to diagnose presence of NAFLD and its severity. The general population screening studies provides more representative prevalence rates but cannot identify the type of NAFLD because of the limitations of their diagnostic technique (Farrell et al. 2005).

3.1 General screening population studies

NAFLD is related to modern lifestyle and with expanded clinical importance because of the rising incidence of type 2 diabetes mellitus and obesity. In fact NAFLD affects 20-25% of the general population (Bedogni et al. 2006; Nomura et al. 1988) and 20-30% in North America and western countries (Angulo & Lindor 2002; Williams 2006), thus it is becoming one of the commonest liver disease worldwide . NAFLD continues to rise and affects about 30% of the adult population in the US now have NAFLD and 3-6 % have NASH (Torres & Harrison 2008).

In China, with the increasing pandemic of obesity, the prevalence of NAFLD has approximately doubled in the past decade. Among more affluent regions of China, the community prevalence of NAFLD is about 15% (Machann et al. 2006), the community prevalence of NAFLD in India varies from 5% to 28%, and varies from 9 to 30% in Japan (Amarapurkar et al. 2007).
The frequency of hepatic steatosis varied significantly with ethnicity (45% in Hispanics; 33% in whites; 24% in blacks), the higher prevalence of hepatic steatosis in Hispanics was due to the higher prevalence of obesity and insulin resistance in this ethnic group (Browning et al. 2004).

3.2 Selected study population

3.2.1 Obese

NAFLD occurs in the majority of subjects with severe obesity, NAFLD was present in 55-90% of severely obese patients while NASH occurred in 37% (Farrell & Larter 2006; Machado et al. 2006) and 25% has fibrosis (Gholam et al. 2007).

3.2.2 Children

NAFLD is the commonest cause of paediatric chronic liver disease in North America (Patton et al. 2010), NAFLD was observed in 10% of children in the USA (Adams) and when population of obese children are analyzed, NAFLD was found in 40% (Pacifico et al. 2010).

3.2.3 Healthy young adults

Nonalcoholic steatosis was discovered in 20% of healthy young adults who are evaluated for possible right lobe donor hepatectomy transplantation in adult-to-adult living donor transplantation (Marcos et al. 2000)

3.2.4 Patients who had random deaths

The liver of subjects died in airplane crashes or traffic accidents showed prevalence of 16% and 24% for NAFLD and 2.1% -2.4% for NASH (Hilden et al. 1977; Ground 1982).
All these are strictly selected populations, which do not reflect the true prevalence.

4. Risk factors

Nonalcoholic fatty liver disease (NAFLD) is associated with obesity and insulin resistance; it is considered the hepatic manifestation of metabolic syndrome. The metabolic syndrome is defined by the presence of 3 or more of the following criteriasummarized in Table 1: elevated waist circumference, high fasting glucose, hypertension, elevated triglycerides and decreased high density lipoprotein concentration. Features of the metabolic syndrome, Insulin resistances and systemic hypertension are independently associated with advanced forms of NAFLD. NASH is associated with obesity, diabetes mellitus and dyslipidemia.
Predictors of NASH are: a raised index of insulin resistance(odds ratio OR = 9.3, Cl=3.4 –26, systemic hypertension (OR = 5.2, Cl =2.0–13.5), and raised alanine aminotransferase (OR 8.6, Cl = 3.1–23.5) (Dixon et al. 2001). The insulin resistance is the strongest predictor of NASH(Bugianesi et al. 2002).
Only a fraction of patient with simple steatosis will progress into the more severe NASH. This implies that other factors metabolic, environmental and genetic variables participate in the pathogenesis of the disease.

Measure	Abnormal level
Elevated waist circumference	Population- and country specific definitions
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator)	≥ 150 mg/dL (1.7 mmol/L)
Reduced HDL-cholesterol (drug treatment for reduced HDL-cholesterol an alternate indicator)	In Males: < 40 mg /dL (1.0 mmol/L) In Females : < 50 mg/dl (1.3 mmol/L)
Elevated blood pressure (antihypertensive drug treatment in a patient with history of hypertension in a alternate indicator)	Systolic ≥ 130 and/or diastolic ≥ 85mmHg
Elevated fasting glucose (drug treatment is alternate indicator)	≥ 100 mg/dL

Table 1. Criteria for Clinical Diagnosis of the Metabolic Syndrome from (Alberti et al. 2009).

5. Liver biopsy: a limited gold standard

In the precedent section, we saw that typical histologic features of NASH primarily include macrovesicular steatosis, a mixed lobular inflammation, and hepatocellular ballooning. Thus, for the diagnosis of NASH to be established, a liver biopsy is still required and therefore remains the gold standard. Only information from biopsy allows grading and staging of the disease.

5.1 Non-alcoholic fatty liver disease: an histological diagnosis

Non-alcoholic fatty liver disease (NAFLD) is a complex metabolic liver disease. The clinical spectrum of NAFLD ranges from benign steatosis to steatohepatitis (Farrell 2004), named NASH (non-alcoholic steatosis hepatitis). NASH defines a sub-group of NAFLD patients where steatosis coexist with liver-cell injury and inflammation (Ratziu et al. 2009). NASH is a progressive form of liver injury that may lead to liver fibrosis which can result in cirrhosis, liver failure and hepatocellular carcinoma (Farrell 2004; Ratziu et al. 2009).

The diagnosis of NAFLD is clinicopathological. NAFLD or NASH can be defined as significant steatosis or steatohepatitis not resulting from alcohol, drugs, toxins, infectious agents or other exogenous causes (Farrell 2004). NASH was first described in 1980 by Ludwig *et al.* (Ludwig et al. 1980) who described a series of patients with chronic liver disease and no history of significant alcohol intake in whom the liver histology was similar to patients with alcoholic liver disease.

The diagnosis of NAFLD is usually suspected in patients with asymptomatic and persistent elevation of aminotransferase, radiological finding of fatty liver and unexplained persistent hepatomegaly (Angulo & Lindor 2002). However the elevation of liver enzyme has a poor predictive value (Angulo & Lindor 2002; Clark et al. 2002) and no clinical or biochemical abnormality permit an accurate diagnosis of NAFLD (Angulo & Lindor 2002). Liver imaging is useful to determine the presence of fatty infiltration but cannot determine the presence and severity of liver damage (Angulo & Lindor 2002).

A liver biopsy is the gold standard to diagnose NAFLD/NASH (Nugent & Younossi 2007) and determine the stage of hepatic fibrosis (Nugent & Younossi 2007). Liver biopsy is the only way to establish definite diagnosis of NASH (Nugent & Younossi 2007) and is the only technique which can distinguish simple steatosis from steatohepatitis (Farrell 2004).

A liver biopsy can assess all liver features associated with NAFLD *e.g.* steatosis, hepatocellular injury, inflammation, fibrosis, etc. (Angulo & Lindor 2002) and can make the

distinction with chronic liver disease from other cause (Ratziu et al. 2009). Liver biopsy provides information about the stage of hepatic fibrosis which is the most crucial clinical prognostic information.
However despite all advantages of liver biopsy, its role for NAFLD patients in clinical practice remains controversial (Nugent & Younossi 2007).

5.2 When to consider a liver biopsy for NAFLD patients

A liver biopsy is essential for the diagnosis and staging of NAFLD. However given its invasiveness, its potential severe complication and the lack of treatment for NAFLD patients (Nugent & Younossi 2007), the decision for the hepatologist to refer to liver biopsy might be difficult. Furthermore, NAFLD patients are asymptomatic and are reluctant to undergo a liver biopsy (Nugent & Younossi 2007).
Decision for the hepatologist to refer to a liver biopsy is made on individual basis (Ratziu et al. 2009; Nugent & Younossi 2007). According the 2010 position statement on NAFLD/NASH of the European Association for the Study of the Liver (EASL) (Ratziu et al. 2009), a liver biopsy should be performed in patients:

- with disorders of the metabolic syndrome for whom non-invasive methods suggest advanced fibrosis or yield discordant results,
- with chronic liver disease not related to NAFLD and evidence of metabolic risk factors, insulin-resistance and steatosis at ultrasound,
- undergoing bariatric surgery and cholecystectomy.

Furthermore, in patients with disorders of the metabolic syndrome with both increased alanine transferase and steatosis at ultrasound, liver biopsy could be the first-line procedure until non-invasive methods becomes extensively and independently validated (Ratziu et al. 2009).

5.3 NAFLD liver histology

5.3.1 Histological liver lesions associated with NAFLD

The zonal location of liver lesions is an important feature for histological evaluation. Briefly, liver is histologically divided into lobules which take the shape of a hexagon (Dancygier 2010), as represented in Figure 1.

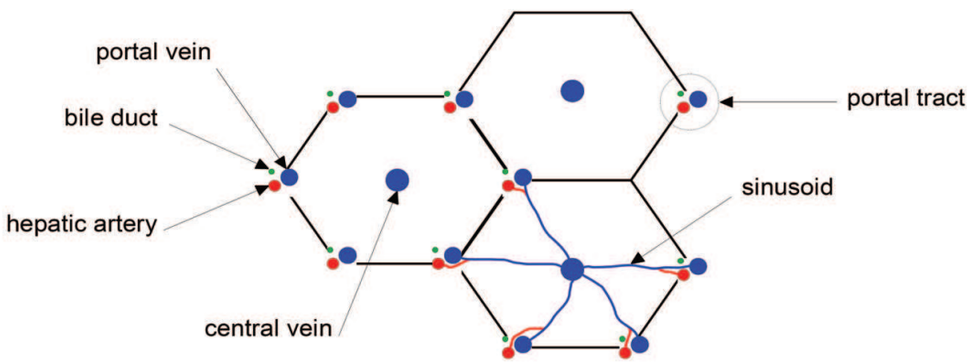


Fig. 1. Liver lobule organization.

At the centre of the lobule is the central vein. At the periphery of the lobular are portal tract. The portal tract is an island of connective tissue containing branches of the portal vein, hepatic artery and bile duct. Portal vein and hepatic artery empty into the sinusoid, from which blood flows to the central vein.

The liver lobule can be divided into three zones: the periportal zone which encircles the portal tract, the centrolobular zone which encircles the central vein and the zone in between named midlobular zone.
In NAFLD/NASH, steatosis, necroinflammation, Mallory's hyaline and fibrosis are typically concentrated in the centrolobular zone (Angulo & Lindor 2002).

5.3.1.1 Steatosis

Steatosis is characterized by the accumulation of fat droplets in the liver. Steatosis is necessary for the diagnosis of NAFLD except for patients with cirrhosis for whom steatosis can be absent. The minimal threshold to diagnose steatosis is 5% of hepatocytes containing fat droplets.
Steatosis in NAFLD patients is mainly macrovesicular (Farrell 2004) which means that fat is a single droplet that fill in the hepatocyte and displace its nucleus at the periphery of the cell. Sometimes microvesicular steatosis can also be seen but in a smaller proportion and in that case, the hepatocytes contain a large number of tinier droplets surrounding the central nucleus (Farrell 2004).
In NAFLD early or mild steatosis is seen in centrolobular zone (Farrell 2004).
Simple steatosis is a reversible condition in a matter of days to weeks (Farrell 2004).

5.3.1.2 Hepatocyte injuries

Hepatocyte injuries that frequently occur in NAFLD are: hepatocellular ballooning, liver cell death, and Mallory's hyaline (Farrell 2004).
Hepatocellular ballooning is a structural manifestation of severe cell injury (Schattner & Knobler 2008). Ballooned hepatocytes are enlarged and have a pale cytoplasm as a result of fluid retention (Farrell 2004), with a rarefaction of the cytoplasm (Schattner & Knobler 2008). Ballooning degeneration is reversible (Farrell 2004) but is likely a form of necrosis (Schattner & Knobler 2008). Hepatocellular ballooning is seen most of the time in the centri-lobular zone, mixed or adjacent to regions with steatosis (Schattner & Knobler 2008).
Liver cell death can be seen in the form of necrosis or apoptosis and is not reversible (Farrell 2004). Apoptotic hepatocytes cells are seen as shrunken cells with densely condensed nuclei (Farrell 2004). Necrotic hepatocytes are not usually prominent but mixed inflammatory infiltrate can be seen is the site where necrotic hepatocytes have disappeared (Farrell 2004).
Mallory's hyaline is seen as a ropy inclusion with the cytoplasm of hepatocytes, especially in cells showing ballooning degeneration (Farrell 2004). It develops as a result of an impaired proteosomal degradation of cytoplasmic proteins (Schattner & Knobler 2008).
Other lesions can be seen in NAFLD: glycogenated nuclei, lipogranulomas, Kupffer cells, mitochondrial abnormalities, fatty cyst, etc. (Schattner & Knobler 2008; Brunt 2005).

5.3.1.3 Inflammation

The hallmark of inflammation in NAFLD is the lobular inflammation (Schattner & Knobler 2008). Lobular inflammation is characterized by the presence of typically mild mixed inflammation, close to ballooned hepatocytes (Schattner & Knobler 2008), and includes a small number of lymphocytes, a small number of macrophages, Kupffer cells, but also a small number of polymorphonuclear leukocytes (Brunt 2005). Portal inflammation can also be seen in NAFLD (Brunt 2005).

5.3.1.4 Fibrosis

In NASH, fibrosis is first seen in the centrilobular zone (Angulo & Lindor 2002; Farrell 2004). This fibrosis is characteriscally perisinusoidal (Brunt 2005). This fibrosis can progress portal fibrosis, central-portal and portal-portal septum and eventually cirrhosis (Angulo & Lindor

2002). Around 20% of NASH patients may progress to cirrhosis (Angulo & Lindor 2002; Farrell 2004). In patients with cirrhosis, the features of steatosis and necroinflammatory activity may no longer be present (Angulo & Lindor 2002).

5.3.2 Histological description of NAFLD/NASH

There is currently no evaluation system massively admitted by experts for the evaluation of NAFLD (Angulo & Lindor 2002; Farrell 2004; Nugent & Younossi 2007; Brunt 2007). For instance, some authors (Farrell 2004; Brunt et al. 1999) but not all (Farrell 2004; Diehl 2002) consider ballooning as an absolute requisite for the diagnosis of NASH, it can be the same for other histological features such as Mallory's hyaline (Brunt 2005).
The early description of NAFLD described four subtypes: fatty liver, fatty hepatitis, fatty fibrosis and fatty cirrhosis (Adler & Schaffner 1979).
Ludwid *et al.* characterized NASH specimen by the presence of steatosis, necrosis, lobular inflammation and in most specimens Mallory's hyaline and fibrosis (Ludwig et al. 1980).
Matteoni *et al.* (Matteoni et al. 1999) proposed in 1997 the classification summarized in Table 2.

Type of NAFLD	Histological description	Prognosis
Type 1	Simple steatosis	Begnin
Type 2	Steatohepatitis : steatosis plus lobular inflammation	Probably begnin
Type 3	Steatonecrosis: steatosis and ballooning	Bad prognosis
Type 4	Steatonecrosis plus fibrosis: steatosis, lobular inflammation, ballooning, Mallory's hyaline and/or fibrosis	Bad prognosis

Table 2. Types of NAFLD according to (Matteoni et al. 1999).

Brunt *et al.* (Brunt et al. 1999) have proposed in 1999 a grading and staging system for NASH. This system includes a necroinflammatory grade and a fibrosis score. The necroinflammatory grade is a combination of features of steatosis, ballooning and inflammation since no single feature can determine activity. The three grades are summarized in Table 3.

Grade	Histological characteristics
Grade 1 – mild	Steatosis: up to 66%. Ballooning: mild, occasional Lobular inflammation: scattered and mild Portal inflammation: absent to mild
Grade 2 – moderate	Steatosis: any degree Ballooning: moderate, obvious in centrolobular zone Lobular inflammation: mild to moderate Portal Inflammation: mild to moderate
Grade 3 – severe	Steatosis: any degree Ballooning: severe, marked in centrilobular zone Lobular inflammation: severe Portal inflammation: mild to moderate

Table 3. Necroinflammatory grading system for NASH from (Brunt et al. 1999).

In addition, Brunt *et al.* (Brunt et al. 1999) proposed a staging system for fibrosis in NASH. This staging is summarized in Table 4.

Stage	Histological description
0	No fibrosis
1	Centrilobular perisinusoidal fibrosis
2	Centrilobular perisinusoidal fibrosis and portal/periportal fibrosis
3	Bridging fibrosis
4	Cirrhosis

Table 4. Staging of fibrosis for NASH, according to (Brunt et al. 1999).

Eventually, by Kleiner *et al.* from the NASH clinical research network developed (Kleiner et al. 2005) in 2005 an approach for the assessment of NAFLD. This scoring system grade three histological features: steatosis, inflammation and ballooning according to standardized histological evaluation summarized in Table 5.

Histological feature	Histological evaluation	Score
Steatosis	< 5%	0
	5-33%	1
	34-66%	2
	> 66%	3
Lobular inflammation	No foci	0
	1~2 foci per 200xfield	1
	2-4 foci per 200xfield	2
	>4 foci per 200xfield	3
Ballooning	None	0
	Few cells	1
	Many cells/prominent	2

Table 5. Scoring system for steatosis, lobular inflammation and ballooning for NAFLD (Kleiner et al. 2005).

Then, the score of each feature (steatosis, lobular inflammation and ballooning) are summed up to constitute the NAFLD activity score (NAS score) which ranges from 0-8. According to the NAS score, the diagnosis of NASH can be made or excluded as described in Table 6.

NAS score	Diagnosis
≤ 2	No NASH
3-4	Borderline
≥ 5	NASH

Table 6. Diagnosis of NASH upon the NAFLD activity score (NAS), according to (Kleiner et al. 2005).

In addition, Kleiner *et al.* (Kleiner et al. 2005) have proposed a staging system of fibrosis for NAFLD patients, which is summarized in Table 7.

Stage	Histological description
0	No fibrosis
1	Perisinusoidal or periportal fibrosis
1A	Mild centrilobular perisinusoidal fibrosis
1B	Moderate centrilobular perisinusoidal fibrosis
1C	Portal/periportal fibrosis
2	Perisinusoidal and portal/periportal fibrosis
3	Bridging fibrosis
4	Cirrhosis

Table 7. Staging of fibrosis, according to (Kleiner et al. 2005).

The Kleiner score is very fetching since it provides a standardized approach for diagnosis of NASH and this score tend to be widely used, especially for clinical studies of patients with NASH. However, despite the practical side of NAS score, it has some limitations and does not perfectly correlate with the definite diagnosis of NASH (Brunt et al. 2011).

5.4 Advantages and drawbacks of liver biopsy

5.4.1 Advantages

Despite its limitations, liver biopsy is the only method for:

- diagnosis : to distinguish simple steatosis from steatohepatitis (Farrell 2004),
- prognosis: grading and staging of NAFLD.

5.4.2 Drawbacks

Liver biopsy is invasive, often painful procedure which can result in severe complications (Grant & Neuberger 1999). Its invasiveness implies that it cannot be performed repeatedly to monitor NAFLD or NASH after intervention.

Liver biopsy can only be performed in selected patients, according to the type of liver biopsy. The mains liver biopsy procedures are summarized in the next paragraph, together with their contraindications and possible complications.

5.4.3 Type of liver biopsy: procedure, contraindication and complications

The three main types of liver biopsy are the percutaneous liver biopsy, transjugular liver biopsy and laparoscopic liver biopsy (Grant & Neuberger 1999; Bravo et al. 2001).

Laparoscopic liver biopsy is rarely used (Bravo et al. 2001) and therefore will not be described hereafter. Its complications include those of laparoscopy itself (Bravo et al. 2001).

5.4.3.1 Percutaneous liver biopsy

Percutaneous liver biopsy is the commonest liver biopsy technique.

Patients should lie in bed and are monitored for at least six hours after the biopsy (Bravo et al. 2001). Patient should be driven home from the hospital and reliable person should stay with the patient the night after the biopsy to provide care and transportation if necessary (Bravo et al. 2001). Patient should be hospitalized after liver biopsy if there is evidence of bleeding, bile leak, pneumothorax (Bravo et al. 2001).

The contraindication for a percutaneous liver biopsy include: uncooperative patients, history of unexplained bleeding, extrahepatic biliary obstruction, bacterial cholangitis, abnormal coagulation indexes, cardiac liver, ascite, blood for transfusion unavailable,

suspected vascular tumor, suspected cyst in the liver (Grant & Neuberger 1999; Bravo et al. 2001).
Most of complications occur within 24 hours after the procedure (Piccinino et al. 1986) and sixty percent of complications occur within 2 hours. Commonest complications are pain and vasovagal episodes. Major complications are significant haemorrhage, haemobilia, puncture of other viscera and pneumothorax (Grant & Neuberger 1999). Mortality rate is around 0.1‰ (Bravo et al. 2001). The main cause of mortality after liver biopsy in intraperitoneal bleeding (Grant & Neuberger 1999).

5.4.3.2 Transjugular liver biopsy

Transjugular liver biopsy is recommended for most patients for whom percutaneous liver biopsy cannot be performed (Grant & Neuberger 1999) that is with cogulapathy, ascite, massive obesity, suspected vascular tumors and in patients for whom percutaneous liver biopsy failed (Bravo et al. 2001).
Procure last around on hour. A catheter is inserted in the right internal transjugular vein and guided via fluoroscopy (live X-ray) to the right hepatic vein. A needle biopsy of the liver is then performed through the catheter (Bravo et al. 2001).
Complications occurs in around 1 to 20% of patients and include: abdominal pain, neck hematoma, cardiac arrhythmias, fistula from the hepatic artery to the portal vein or biliary three and perforation of the liver capsule (Bravo et al. 2001). Mortality rate is around 0.1% to 0.5% (Bravo et al. 2001).

5.4.4 Sampling, intra/inter observer variability & effect of liver biopsy sample length

5.4.4.1 Sampling variability

A limitation of liver biopsy is its sampling variability (Ratziu et al. 2009; Qayyum et al. 2005). Sampling variability is a relevant limitation of liver biopsy due to the fact that the liver biopsy specimen represent only a very limited part of the whole liver (Bravo et al. 2001) and that histologic lesions of NAFLD/NASH are likely to have a very unevenly distribution in the liver (Brunt 2008), even at a macroscopic level.
Indeed, in Merriman *et al.* (Qayyum et al. 2005), 41 subjects underwent intraoperative liver biopsies from both right and left lobes. The κ coefficient was assessed and shown: excellent agreement for steatosis (κ = 0.88), moderate for fibrosis staging and lobular inflammation (κ = 0.53 and κ = 0.32, respectively) and slight for ballooning and portal inflammation (κ = 0.20 and κ = 0.19, respectively).
In Ratziu *et al.* (Ratziu et al. 2009), 2 samples of liver biopsy were successively collected in 51 patients. The κ reliability test was assessed and shown: substantial agreement for steatosis (κ = 0.64), moderate agreement for fibrosis staging and ballooning (κ = 0.47 and κ = 0.45, respectively), fair agreement for Mallory's hyaline (κ = 0.27) and poor agreement for lobular inflammation (κ = 0.13).
The heterogeneous distribution of histological lesions of NAFLD/NASH is particularly accentuated for lobular inflammation and ballooning that are key features in NASH diagnosis.
To avoid as much as possible potential sampling variability error, large specimen are required for reliable evaluation of NAFLD patients (Brunt 2007; Vuppalanchi et al. 2009). Usually, most of the pathologists recommend a specimen of at least 1.5 cm with at least six to eight portal tracts (Bravo et al. 2001), and preferably a specimen of at least 25 mm is preferable (Vuppalanchi et al. 2009).

5.4.4.2 Intra/inter-observer variability

Intra/inter-observer variability is an important cause of error when staging and grading liver biopsy (Ratziu et al. 2009; Marcellin et al. 2009; El-Badry et al. 2009) which can yield poor reproducibility even when performed by experts pathologists (El-Badry et al. 2009).
In the study by El-Badry *et al.* (El-Badry et al. 2009), 4 established expert pathologist from different institutions worldwide were asked to grade steatosis from 46 NAFLD patients. Concomitant computerized morphometric analysis was performed on the same slides. Poor agreement was found among pathologists for the evaluation of total steatosis: intra-class correlation agreement ICC = 0.57, macrovesicular steatosis ICC = 0.55. Failed agreement was found for the evaluation of micro-vesicular steatosis. When compared with computerized morphometic analysis, poor agreement was found for 3 pathologists (Spearman rank correlation coefficient ρ = 0.22, 0.28 and 0.38) and good agreement was found for one pathologist (ρ = 0.82). In addition, features of NASH (lobular and portal inflammation, ballooning, Mallory's hyaline) were assessed by the 4 pathologists as present or absent, as well as diagnosis of NASH, and a strong disagreement was found for all parameters including the overall diagnosis. Evaluation of NAFLD/NASH is therefore strongly observer-dependent and therefore seems weakly reproducible.
Even when performed by the same operator, variability can be important. Indeed, in Ratziu *et al.* (Ratziu et al. 2009), intra-observer variability was assessed on 50 liver biopsies and intra-observer κ reliability test yield to substantial agreement for steatosis (κ = 0.74), moderate agreement for ballooning (κ = 0.62), perisinusoidal fibrosis (κ = 0.53), fair agreement for Mallory's hyaline (κ = 0.39) and lobular inflammation (κ = 0.37). Overall staging of fibrosis was substantial (κ = 0.69) and grading of NAFLD was moderate (κ = 0.55).

5.4.4.3 Other cause of variability

Some other parameters such as tissue fixation and staining methods can also influence the evaluation and be a cause of variability in the assessment of NAFLD/NASH (DiDonato & Brasaemle 2003; Fukumoto & Fujimoto 2002).

5.4.4.4 Impact of variability

Sampling error, intra and inter-observer variability and other causes of variability may induce misdiagnosis and substantial staging inaccuracy that might have significant implication in the clinical management of NAFLD/NASH patients.

6. Non invasive tools: how to detect transition between steatosis and fibrosis?

Hepatic steatosis refers to the excessive accumulation of fat within hepatocytes. The most common form of steatosis is nonalcoholic fatty liver disease (NAFLD), which comprises a wide spectrum of liver damage, ranging from simple steatosis (`fatty liver') to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis. Several systems of grading and staging of NAFLD have been proposed, but only a few have been validated; these typically include the degrees of steatosis, cytological ballooning, infammation, and fibrosis. To replace the biopsy, the ideal non invasive tool has to detect and quantify the same parameters than biopsy. The most important of a clinical point of view is to detect transition between simple steatosis and beginning of hepatitis. This review discusses of a part of current non invasive tools used to clinical diagnosis NAFLD and/or NASH and then these tools are compared to biopsy efficiency.

6.1 The past: non invasive tools currently used in clinical practice

6.1.1 Ultrasound

Ultrasound imaging is an established imaging modality in the diagnosis of hepatic steatosis, both clinically and in large scale studies. Several grading system have been proposed, studied, evaluated for the assessment of steatosis using ultrasound but no consensus has been achieved.

Ultrasound is the commonest technique used in clinical practice to detect fatty infiltration of the liver due to its simplicity, low cost, noninvasive nature, and widespread availability (Karcaaltincaba 2007). However, accurate quantification of steatosis is not feasible with the current technology. The basic principle underlying the sonographic detection of steatosis is that the degree of tissue brightness, the so-called echogenicity, directly depends on fat composition of the tissue (Quinn et al. 1985). Hence, a fatty liver is hyperechogenic with fine and tightly packed echoes and appears brighter on ultrasound examination (`bright liver') when compared with the echogenicity of other fat-free internal organs such as the kidneys or spleen (Joseph AE, et al.1979, Vaalls et al, 2007, ,Joy D; et al., 2003). The 'quantification' of steatosis depends on the experience of the operator: mild steatosis is characterized by 'mild' increase in liver echogenicity. Moderate steatosis can be diagnosed with increased liver echogenicity compared to the spleen for example, echogeneicity that obscures visualization of hepatic and portal vein wall. However, ultrasonographic evaluation of steatosis does not exactly match histopathologic quantification of steatosis. Furthermore, fat is less penetrable by ultrasound leading to attenuation of the signal, which causes posterior darkness (i.e., acoustic shadowing leading to hypoechogenicity in the far field) and loss of definition of the diaphragm and portal and hepatic veins, giving rise to a relatively bland and featureless appearance of the liver. It's the criteria to diagnose steatosis ≥30% (Palmentieri B. et al., 2006). Evaluation of steatosis in patients with hepatitis can be difficult due to accompanying inflammation and fibrosis. Fibrosis may also appear hyperechoic, but most of the time fibrosis and fatty infiltration co-exist in cirrhotic patients which is called fatty–fibrotic pattern (Joseph AEA, 1991). Still, despite the fact that several attempts have been made to generate scoring systems in order to provide better semi-quantitative information on the degree of steatosis (Hamaguchi M, 2007, Mehta SR 2008), ultrasonography remains largely a qualitative method for detecting NAFLD rather than a quantitative one for measuring liver fat. Several studies have examined the ability of ultrasound in recognizing fatty liver among patients suspected of having liver disease, using liver biopsy as the comparison standard, and reported sensitivity values between 60 and 94%and specificity values between 84 and 95%(Joy D. et al, 2003). the sensitivity is 55% when intrahepatic fat content is 10±20% and rises to 80% when intrahepatic fat content is greater than 30% (Ryan CK. Et al,

Hence, its ability to detect longitudinal changes is poor; it has been estimated that even a reduction of intrahepatic fat content from 40 to 20% following a successful intervention would alter the sonographic appearance of the liver by little, if any (Fishbein et al., 2005). Moreover, both the sensitivity and specificity of ultrasound decrease sharply in morbidly obese patients (Mottin CC. et al. 2004). Technical factors such as transducer frequency and instrument settings also affect the sonographic appearance of the liver, and hence the performance of ultrasound (Yeh WC et al. 2005, Garra B.S. et al.1887).

Sonography has the advantages of ease of acquisition, ability to assess the whole liver. The drawback are: subjectivity of the operator, no precise quantification, no follow-up of progression of the disease, poor agreement of intraobserver reproducibility (0.4-0.51) and interobserver (0.58) (Strauss S et al. , 2007).

We can conclude that abdominal ultrasound is a moderately specific and sensitive tool for diagnosing hepatic steatosis (> 33 %) but is not predictive for NASH.

6.1.2 Computed tomography (CT)

Noncontrast-enhanced computed tomography (CT) is widely used for the evaluation of NAFLD, by means of measuring tissue density as a function of radiographic attenuation. Tissue fat deposition results in lower attenuation (Kawata R et al., 1984), hence fatty tissues are less dense and appear darker than fat-free tissues (Piekarski J et al. 1980). Attenuation and density on CT imaging can be objectively measured on the Hounsfield scale. Fibrosis does not cause any effect on the attenuation of liver (Joy D et al. 2003). The diagnosis of fatty liver by contrast-enhanced CT imaging is more cumbersome, because contrast type and injection rate and timing of measurements can significantly influence the optimal liver-to-spleen attenuation difference for diagnosing NAFLD. Hence, unenhanced hepatic scanning remains the optimal CT technique for the detection of fatty liver (Kodama Y et al. 2007). Unenhanced CT images are used for qualitative evaluation and spleen is used as the reference organ for comparison. Spleen to liver attenuation ratio or difference between attenuation of spleen and liver can be used for the evaluation of steatosis. Studies that have evaluated CT imaging against liver biopsy have reported that the liver CT attenuation value and the ratio of liver-to-spleen CT attenuation values (Longo R et al. 1993, Oliva MR et al. 2006) correlate well with the degree of steatosis by histological analysis. Attenuation of spleen is approximately 8–10 HU less than the liver in a normal patient. Iwasaki et al. suggested cut-off value of 1.1 (spleen to liver attenuation ratio) for exclusion of moderate steatosis based on their correlative findings on 194 patients (Iwasaki M et al., 2004). The sensitivity and specificity of noncontrast-enhanced CT in the detection of moderate and severe hepatic steatosis (intrahepatic fat >30%) have been reported to range between 73% and 95%, respectively. However, at lower degrees of steatosis, the diagnostic performance of unenhanced C T for the quantitative assessment of intrahepatic fat is not clinically acceptable (Park SH et al. 2006) nor is C T able to evaluate hepatic fibrosis. In their study, liver/spleen attenuation ratio of 0.8 and difference of 9H between liver and spleen attenuation had similar sensitivity. Use of suggested criteria can be helpful in avoiding biopsy in moderately steatosic livers (Brancatelli G, 2006). In another study, Limanond et al. 2004 also concluded that unenhanced CT quantified the degree of steatosis relatively well in liver donor patients, but stated that most of the time liver biopsy is necessary to exclude fatty liver, co-existing iron deposition and parenchymal disease.

It should be noted that radiographic attenuation through the liver is non-uniform due to many factors that cannot be measured by CT, such as iron, copper, and glycogen concentrations and presence of fibrosis or oedema. For example, up to 40% of patients with NASH may have hepatic iron overload (George DK et al. 1998), which would alter hepatic CT attenuation independently of intrahepatic fat content. Recently, xenon CT, which is widely used to quantify and visualize tissue blood flow, was evaluated for its ability to assess fatty infiltration and changes in blood flow throughout the entire liver; it was found that xenon solubility was strongly and positively correlated with both grade of steatosis and each 10% range of histological fatty infiltration (r value > 0.8) and inversely associated with the liver-to-spleen CT attenuation ratio (Kobayashi M et al. 2009). Hence, xenon CT appears to provide a more objective measure of the severity of hepatic steatosis than conventional CT. However, the major limitation of CT is the exposure of patients to ionizing radiation, which makes longitudinal assessment of NAFLD impractical, especially among sensitive populations.

6.2 Magnetic resonance imaging (MRI)

Moderate-to-severe hepatic steatosis can easily be detected on T1-weighted spin-echo MRI because liver fat has very high signal intensity (Danet IM et al. 2003). This sequence can be obtained with all types of MR scanners with different Tesla power including 0.5, 1, 1.5 and 3 Tesla. In the presence of steatosis signal drop is observed on out of phase images due to phase cancellation of fat and water. However, many other factors contribute to this high signal intensity such as haemorrhage, melanin deposition, high protein content, or sinusoidal dilatation. The signal intensity is much lower for mild fatty infiltration of the liver. Therefore, in clinical practice, the most common MRI modality to evaluate NAFLD is chemical-shift imaging, which utilizes the difference in resonance frequency of water andlipidhydrogens to differentiate tissues containing only water from those containing both water and lipid (Venkataraman S et al. 2002. The spleen is generally used as the internal organ of reference for signal loss. For mild degrees of steatosis (intrahepatic fat < 15%), the signal of liver on the out-of-phase images appears nearly equal to that of spleen, although demonstrating a slightly higher signal on the in-phase images; loss of signal on out-of-phase images is progressively more prominent in moderate and severe fatty infiltration. Quantification of intrahepatic fat is made possible because of the differences in resonance frequencies between fat and water; if the out-of-phase and in-phase images are acquired by using constant calibration and other scanner settings, a quantitative fat signal fraction can be calculated from the hepatic signal intensities and this can be done pixel by pixel on the image to generate a fat signal fraction map. Many limitations of this technique (*e.g.*, time-demanding, low sensitivity, disturbed image quality due to respiratory and other bodily motions, or by magnetic field heterogeneity have been overcome by new methods, such as spoiled gradient-echo pulse sequence imaging, fast gradient echo imaging, fat-saturated fast spin-echo imaging, and fast spin-echo triple-echo Dixon imaging for example. These improved techniques have been shown to accurately quantify the hepatic fat fraction, even at low or near-normal levels. Guiu et al . 2009 recently developed a method on a breath-hold triple-echo spoiled gradient-echo sequence, which has many asserted advantages such as shorter acquisition time, measurement of fat content throughout the liver instead of in one or just a few voxels, no spatial misregistration errors, as well as easier and faster processing. Generally, the sensitivity and specificity of MRI for the detection of moderate and severe steatosis are greater than 80 and 95%, respectively; in addition, MRI has agreater than 85% sensitivity and a nearly 100% specificity for mild steatosis (intrahepatic fat 5±10%) (Mazhar et al. 2009). It was confirmed by many studies which have reported very good correlations between quantitative estimations of hepatic steatosis on MRI compared with liver biopsy (Cowin et al. 2008).

Nevertheless this technique is very expensive, not always available in hospital and does not quantify fibrosis.

6.2.1 Magnetic resonance spectroscopy (MRS)

Whereas chemical shift MRI enables the identification of steatosis within whole tissues, proton MRS facilitates the examination of the resonance frequencies of all hydrogen nuclei within a tissue region of interest (localized MRS). Although the absolute differences in resonance frequencies between protons in water and fat are quite small (3.5 ppm), they can be separated out to form a spectrum on an axis of chemical shift; spectral resolution is determined by the strength of the main magnetic field (1.5 Tesla). The intrahepatic fat values

obtained by proton MRS are highly reproducible (Machann J et al., 2006) and correlate well with histological analysis of liver biopsy samples (Frimel TN et al., 2007). Moreover, the potential ability of MRS to monitor the progression of steatohepatitis by assessing saturated and unsaturated composition of fatty acyl chains in the liver has been tested in animal models, with promising results (Corbin IR, et al. 2009). MRS has been widely used in research settings for assessing the prevalence and metabolic concomitants of NAFLD among various populations including children as well as in longitudinal studies for evaluating fatty liver in response to drug treatments (Belfort R, et al. 2006) or changes in diet and physical activity.

6.3 The future: non invasive tools recently developed

6.3.1 Magnetic resonance elastography (MRE)

MR elastography (MRE) is an emerging technique for quantitatively imaging the mechanical properties of tissues. The basic approach is to generate low frequency mechanical waves (typically 20 to 200Hz) in the tissues and to use an extremely sensitive phase-contrast MR technique to quantify tissues displacements. MRE requires the addition of hardware and software to standard MR imaging system and a drumlike acoustic passive driver which is positioned against the body wall. The active driver produces acoustic vibrations which are transmitted to the passive driver, which then transmits it through the body and generate shear waves in liver. Recorded information is then processed by an inversion algorithm to generate quantitative images that depict tissues stiffness. The tissues stiffness expressed in kPa is correlated to the amount of fibrosis. Pr Ehman suggests that the increase of stiffness is a precursor of fibrosis development (Ehman et al. 2010).

A new clinical study (Talwakar et al. 2011) with 58 NAFLD patients suggests that liver stiffness evaluated with MR elastography was significantly higher in patients with NASH compared with patients with simple steatosis. Liver stiffness in patients with fibrosis (grade F>1) was significantly higher than that in patients with inflammation and no fibrosis. Liver stiffness was significantly correlated to inflammation stage and fibrosis stage. The results of this retrospective study support the hypothesis that NAFLD patients with inflammation but no fibrosis have significantly higher liver stiffness than do those with simple steatosis. Liver stiffness measured by means of MR elastography may be an accurate biomarker for detecting NASH (area under receiver operating characteristics curve, AUROC = 0.93), which suggest that MRE should be further investigated for its potential to stratify patients with NAFLD. MRE could be used to distinguish between individuals with simple steatosis and steatohepatitis.

The presence of elevated stiffness is not specific to steatohepatitis and can be generalized at all the liver disease. Moreover, this technology necessitated the use of a MR device.

6.3.2 Serologic test

The new subject of biomarkers development is to differentiate NAFLD phenotypes and to detect liver injuries (ballooning, degeneration, necrosis…) to target therapies. Several tools have been studied: biomarkers of oxidative stress, inflammation and hepatocytes apoptosis.

The most commonly used screening modality, the ALT, typically fluctuates in NAFLD and is normal in more than two-thirds of NASH patients at any given time (Wieckowska et al. 2007). For the identification of at least 5% steatosis, Poynard et al. reported that an ALT> 50 IU/L had a sensitivity and specificity of only 72% and 62% respectively (AUROC , 0.61) (Poynard et al, 2005).

Several studies explored the correlation between TBARS (thibarbituric acid-reacting substance) and steatosis (Chalasani, 2002) (Bonnefont, 2006) but there was no significant correlation. Additional studies are necessary before use in clinical context.
A new approach is the recognition of hepatocyte apoptosis. The first study correlated plasma CK-18 (cytokeratin-18) with NASH, CK-18 increased in patients with NASH compared to simple steatosis and normal biopsies (Wieckowska et al, 2006). This first study was completed recently by a cohort of 319 patients with biopsy proven NAFLD. CK-18 fragments were markedly increased in patients with NASH as compared to not NASH and borderline diagnosis (Median (Q25, Q75): 335 (196, 511), 194 (151, 270), 200 (148, 284), respectively; $P < 0.001$). On multivariable regression analysis, CK-18 fragments remained an independent predictor of NASH after adjusting for variables associated with CK-18 fragments or NASH on the univariate analysis (fibrosis, ALT, AST, age, biopsy length). AUROC curve for NASH diagnosis was estimated to be 0.83 (0.75-0.91), (Feldstein et al., 2009). The same biomarker was studied in a prospective longitudinal study with 52 patients NAFLD, with repeated biopsies at month 36. Serum cytokeratin-18 fragment level correlated well with NAS both at baseline and month 36. The change in NAS had moderate correlation with change in serum cytokeratin-18 fragment levels (r=0.51, p<0.001). Patients with increased NAS at month 36 had greater increase in serum cytokeratin-18 fragment levels (Wong et al, 2010).
Most studies would suggest that combinations of biomarkers have the highest predictive utility.

6.3.3 Composite serum markers

Several blood tests have been proposed to diagnose liver fibrosis. Some tests are simple, like the aspartate aminotransferase to platelet ratio index (APRI). Others are more complex, constructed as algorithms (regression score) like the FibroMeter (Cales et al. 2009). Most of them have been developed in chronic hepatitis C or in miscellaneous causes.

6.3.3.1 Steatosis

Bedogni *et al.* CITER developed the Fatty Liver Index (FLI) by evaluating a cohort from the Dionysos Nutrition & Liver Study. A total of 224 subjects with suspected liver disease (excluding hepatitis B and C) were selected and matched with 287 subjects without suspected liver disease. After serial analysis, four predictors (triglycerides, BMI, gamma-glutamyl transpeptidase (GGT) and waist circumference) were entered into a model to generate the FLI. The authors reported that an FLI<30 could be used to rule out and >60 to rule in hepatic steatosis. A limitation of this study is that the diagnosis of fatty liver was based on ultrasonography.
In the same way, Poynard et al. (2009) recently reported a combination of markers for steatosis referred to as the SteatoTest (BioPredictive, France) This proprietary index combines age, gender, body mass index, cholesterol, triglycerides, glucose, ALT, GGT, bilirubin, haptoglobin, alpha-2-macroglobulin, and apolipoprotein A1 in a logistic regression formula. For the prediction of steatosis ≥ 5%, the SteatoTest value had an AUROC of 0.80 in a cohort of 811 patients with NAFLD, HCV and HBV, alcoholic liver disease. Nevertheless, a substantial overlap between grades of steatosis will likely limit its widespread use; Moreover, this algorithm has yet to be externally validated. The SteatoTest (range [0-1]) was 0.14 in blood donors, 0.26 in patients without steatosis; 0.43 with [1-5%] steatosis, 0.62 with 5-33% steatosis, 0.70 with 34-66 % steatosis and 0.75 with > 66 % steatosis (Poynard et al. 2005).

6.3.3.2 NASH

Recently, Poynard and its colleaues (Poynard et al 2009)have developed a new algorithm called 'Nashtest' as a biomarker of inflammation. This proprietary tool includes the components of the SteatoTest in addition to AST, ALT, total bilirubin, height anf weight. In a study that included 257 patients with NAFLD who underwent liver biopsy, this panel was 71% sensitive and 94% specific for the diagnosis of NASH versus no NASH according to the NAFLD Activity Score. The AUROC was 0.75 (95% CI 0.67-0.82). As this index has not yet been externally validated, additional study is necessary prior its utilisation.

6.3.3.3 Fibrosis

A variety of blood tests exist to detect the NAFLD to NASH transition. For example, the NAFLD score was developed by Angulo to accurately separate patients with NAFLD with and without advanced fibrosis, rendering liver biopsy for identification of advanced fibrosis unnecessary in a substantial proportion of patients. This score was developed during a study on 733 patients (480 construction of the data basis, and 253 to validate, with NAFLD confirmed by biopsy). The NAFLD score is based on composed of routinely measured and easily available clinical and laboratory variables. The exact formula combines age, hyperglycaemia, body mass index (BMI), platelets, albumin, AST/ALT). In this study, the lower cutoff point was particularly accurate in ruling out the presence of advanced fibrosis; the NPV was 93% and 88% in the estimation and validation groups, respectively, and ranged from 87% to 98% for the prevalence of advanced fibrosis of 5% to 50%. Among these 733 patients, 439 (60%) had a negative diagnosis of advanced fibrosis, and thus a liver biopsy would have been avoided by applying the NAFLD fibrosis score; of these 439, 400 (91%) indeed had stage 0-2 fibrosis; (Angulo, 2007) However, this test was designed for severe fibrosis whereas most tests have been designed for significant fibrosis and usually for chronic hepatitis C.

Cales and its colleagues developed a regression score specifically designed to NAFLD patients called Fibrometer NAFLD (Cales et al. 2009). A study on 235 patients compared blood tests performance (NFSA (NAFLD Score of Angulo Fibrometer, Fibrometer NAFLD and APRI) and biopsy results. The score combines glucose, AST, ferritin, platelet, ALT, body weight and age. Results gave 90% negative (NPV) and positive (PPV) predictive values for significant fibrosis, when values were, respectively: FibroMeter: ≤0.611 and ≥0.715, NFSA: ≤ 0.227 and ≥0.514, APRI: ≤0.454 and ≥0.918. The ensuing proportion of patients with reliable diagnosis was: FibroMeter: 97.4%, NFSA: 86.8% ($p < 10^{-3}$ *vs* FibroMeter), APRI: 80.0% ($p < 10^{-3}$). This study thus provided an independent external validation of NFSA for significant fibrosis in a large series and for severe fibrosis. Cales and its colleagues observed a similar performance for significant fibrosis of APRI AUROC compared to the original publication in chronic viral hepatitis C. APRI AUROC was significantly inferior to that of FibroMeter and NFSA for the three diagnostic targets (except with NFSA for significant fibrosis).

All these composite serum markers are very easy to use and are able to give a first indication to the physician. But some of them are not free (Fibrometer NashTest and Steatotest for exemple) and need more external clinical validations.

6.3.4 Fibroscan® and CAP (Controlled Attenuation Parameter)

Fibroscan® (Echosens, Paris, France) is an ultrasound-based vibration-controlled transient elastography (VCTE™) device used to assess liver elasticity related to liver fibrosis (Sandrin

et al. 2003). Liver stiffness is expressed in kPa. Fibroscan® shows good results for the detection of significant fibrosis and for the diagnosis of cirrhosis in hepatitis C virus (HCV) (Ziol et al. 2005), hepatitis B virus (HBV) (Marcellin et al. 2009), biliary liver disease (Corpechot et al. 2006), alcoholic liver disease (ALD) (Nahon et al. 2008) and NAFLD (Wong et al. 2010).

Fibroscan® has high degree of accuracy and reproducibility in predicting bridging fibrosis and cirrhosis in patients with viral hepatitis (Fraquelli 2007, Wong 2008). Two independent studies of 94 (Yoneda 2007) and 120 (Tamano 2007) NAFLD patients respectively concluded that VCTE™ is a non-invasive and useful method to screen possible NASH patients, who need liver biopsy, from NAFLD populations. No correlation between stiffness values and steatosis was finded. Moreover, these results are confirmed later by Wong and their colleagues (Wong, 2010) who evaluated the accuracy of VCTE™ and biochemical tests for the diagnosis of fibrosis and cirrhosis in a large cohort of 246 NAFLD patients. They wanted to determine if liver stiffness was altered by hepatic steatosis, inflammation, and obesity. The results demonstrated the usefulness of VCTE™ to quantify fibrosis: AUROC of VCTE™ for the detection of F3 fibrosis or higher and cirrhosis was 0.93 and 0.95, respectively, and was significantly higher than that of the aspartate aminotransferase–to–alanine aminotransferase ratio, aspartate aminotransferase–to–platelet ratio index, FIB-4, BARD, and NAFLD fibrosis scores (AUROC ranged from 0.62 to 0.81, $P<0.05$ for all comparisons). At a cutoff value of 7.9 kPa, the sensitivity, specificity, and positive and negative predictive values for F3 or greater disease were 91%, 75%, 52%, and 97%, respectively. Liver stiffness was not affected by hepatic steatosis, necroinflammation, or body mass index. Discordance of at least two stages between transient elastography and histology was observed in 33 (13.4%) patient but by multivariate analysis, liver biopsy length less than 20 mm and F0-2 disease were associated with this discordance. Because of high negative predictive value and modest positive predictive value, this study concluded positively for the use of VCTE™ as a screening test to exclude advanced fibrosis. In summary, Wong and colleagues have provided valuable data regarding the use of VCTE™ in patients with NAFLD. Adam *et al.* underlined that the strength of Fibroscan® appears to be for excluding advanced fibrosis and cirrhosis; however, according to Adam, there are a number of issues that need to be clarified before it is routinely used in the clinical setting: cut-off values, utility in obese and morbidly obese populations which seem to require further validation of the dedicated obese probe (called XL probe). Nevertheless de Ledinghen et al. (2010) and Rust and al. (20110) have demonstrated VCTE™ using the XL probe for obese patients can be performed with comparable diagnostic accuracy to the standard probe and enables the examination of significantly more obese patients.

So, VCTE™ based on Fibroscan® is a promising tool to quantify fibrosis but not steatosis. It's why Sasso and al. (Echosens, France) had developed a new parameter called Controlled Attenuation Parameter (CAP) based on ultrasound attenuation. This parameter can be assessed simultaneously and on the same device Fibroscan® than the liver stiffness. Knowing that fat affects ultrasound propagation, this novel attenuation parameter is based on the ultrasonic properties of the radiofrequency back-propagated signals acquired by the Fibroscan®. It is called controlled attenuation parameter (CAP) because it was devised to specifically target the liver. This control is performed by a sophisticated guidance process based on VCTE™. Therefore, CAP can be assessed by an operator who does not have any ultrasound imaging skills. Furthermore, CAP has been designed to be immediate, reproducible and operator and machine-independent. Performance of the CAP was then

appraised on 115 patients, taking the histological grade of steatosis as reference. CAP was significantly correlated to steatosis (Spearman ρ=0.81, p<10^{-16}). AUROC was equal to 0.91 and 0.95 for the detection of more than 10% and 33% of steatosis, respectively. Furthermore, results show that CAP can efficiently separate several steatosis grades. These results were confirmed by a resptropsective study an 112 patients with multiple aetiologies (HBV, HCV, NAFLD/ALD) (De Ledinghen et al. 2011). These promising results suggest that CAP is a noninvasive, immediate, objective and efficient method to detect and quantify steatosis. This parameter is available on Fibroscan®, is calculated during the same acquisition than the stiffness and than gives a steatosis evaluation in the same region of interest used for the stiffness (figure 2). Future clinical evaluations on NAFLD and NASH patients will be useful to evaluate the diagnosis performance of these 2 combined non-invasive tools and the ability to detect transition between simple steatosis and steatohepatitis.

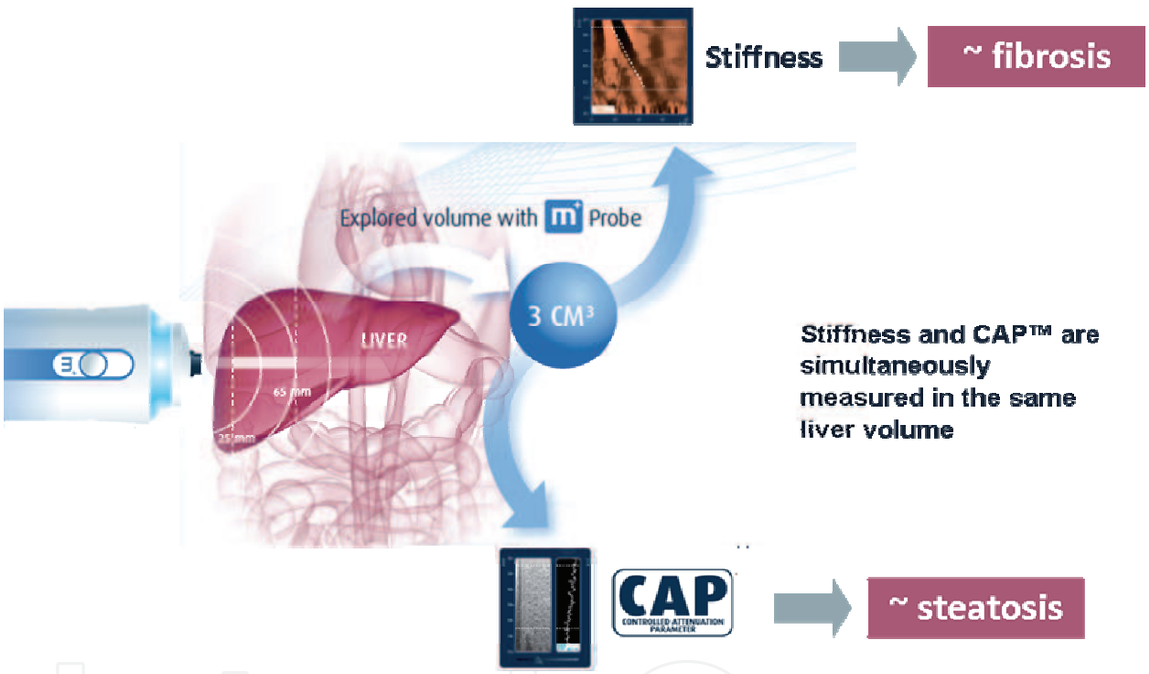


Fig. 2. Fibroscan® assess fibrosis and steatosis.

6.3.5 Discussion

According to Myers and his colleagues, the ideal non invasive tool includes 3 major goals: to diagnose NAFLD, to differentiate simple NAFLD from NASH, to determine the severity of fibrosis (Myers et al. 2009). Moreover, this ideal non invasive tool shall be:

- *Liver specific :* true for MRI, MRS, composite serum markers, serologic tests, Fibroscan® (stiffness and CAP)
- *Able to detect simple steatosis :* true for ultrasound (> 33%), CT, MRI, MRS, Fibroscan® (CAP)
- *Able to identify simple steatosis from NASH:* true for composite serum markers (shall be evaluated), Fibroscan (stiffness and CAP, shall be evaluated)
- *sensitive enough to distinguish individual stages of fibrosis:* true for serologic tests, composite serum markers, MRE, Fibroscan (stiffness)

- *easy to perform and acceptable for patients and physicians:* true for all non-invasive tools described except for CT scan cannot be repeated(ionizing)
- *Inexpensive/examination:* true for ultrasound, serologic tests, composite serum markers, Fibroscan®
- *Reproducible:* true for CT, MRI, MRS, serologic tests (shall be evaluated), composite serum markers, Fibroscan®
- *Responsive to change in disease (steatosis + fibrosis):* true for composite serum markers (shall be evaluated), Fibroscan® (stiffness and CAP, shall be evaluated)

At present only biopsy achieves most of these goals but the combination of Fibroscan® (stiffness and CAP) added with a blood marker could be a promising non invasive tool to avoid biopsy in many cases. It will be a good, simple solution, non expensive, reproducible, easy to use and very well accepted by the patients.

7. Conclusion

We have seen that NAFLD and NASH diseases are an increasing prevalence in future. The symptoms are very dangerous because of a silent, slow evolution. At present, the gold standard for the diagnosis of nonalcoholic steatohepatitis is liver biopsy; however, liver biopsy is not performed in a significant number of cases and in the absence of more-accurate imaging technologies and serum markers, the diagnosis is frequently one of exclusion. Due to the many potential errors due to sampling, inter/interobeserver variability, biopsy is the "Best" not the "Gold " standard (Bedossa & Carrat 2009) in spite of its limitations. There is also urgent need for effective and easy-to-use non-invasive methods to assess the severity of liver disease in NAFLD: while simple steatosis has a benign hepatological prognosis and may be managed with measures aiming at reducing cardio-metabolic risk, NASH may progress to end-stage liver disease and requires early hepatological referral for experimental treatment and tight follow-up. Then, the most important clinical point is to detect the transition between benign steatosis, NALD, and NASH and to be able to assess steatosis, fibrosis and inflammation. Many noninvasive tests exist to quantify either fibrosis or steatosis but few diagnose these two. Nevertheless, new technologies appear like blood markers, serologic tests, specific imaging device (MRE) or Fibroscan® device with CAP to improve the diagnosis of NAFLD and/or NASH. It is hoped that improved imaging techniques and the discovery of serum biomarkers, as well as the development of clinical algorithms, will enable a more accurate diagnosis of NASH without the need for a liver biopsy.

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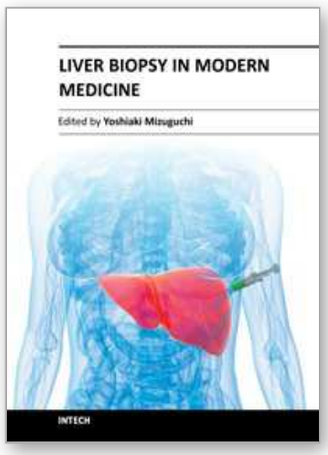
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Liver biopsy, first performed by Paul Ehrlich in 1883, remains an important diagnostic procedure for the management of hepatobiliary disorders and the candidate/donated organ for transplantation. The book "Liver biopsy in Modern Medicine" comprises 21 chapters covering the various aspects of the biopsy procedure in detail and provides an up-to-date insightful coverage to the recent advances in the management of the various disorders with liver biospy. This book will keep up with cutting edge understanding of liver biopsy to many clinicians, physicians, scientists, pharmaceutics, engineers and other experts in a wide variety of different disciplines.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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