Chapter

Diagnosis of Nonalcoholic Steatohepatitis

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Abstract

The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased in the last years up to 25% in the adult population. This disease includes a large spectrum of disorders, from simple fatty liver disease to cirrhosis and Hepatocellular Carcinoma (HCC), and they are related to chronic metabolic conditions. NAFLD is characterized by the presence of at least 5% of hepatic steatosis without evidence of hepatocellular injury. The diagnosis of this disease should be of exclusion and focused on its progression, treatment, and identification of the prognosis. The European Association for the Study of the Liver (EASL), the National Institute for Health and Care Excellence (NICE), the Italian Association for the Study of the Liver (AISF), and the American Association for the Study of the Liver (AASLD), published their Clinical Guidelines that have identified the criteria for the diagnosis of NAFLD, several, using imaging or histological diagnostic methods, although they imply a different approach and screening. The Fatty Liver Index and the NAFLD Liver Fat Score are used by 3 out of 5 Guidelines and they are easily calculated using blood tests and clinical information. Other non-invasive scales for NAFLD are the NAFLD fibrosis score (NFS), Fib-4, AST/ALT ratio index; also the ELF panel, Fibrometer, Fibrotest, Hepascore; and some imaging techniques that include transient elastography, magnetic resonance elastography (MRE), and shear wave elastography. Finally, proteomic's and glycomic's technologic biomarkers are currently under investigation and recent use, such as Cytokeratin 18 and Sirtuin 1. Still, liver biopsy remains the gold standard to distinguish between steatohepatitis and simple steatosis, using the histological classification and staging scoring systems of NAFLD Activity Score (NAS) and the Steatosis Activity Fibrosis (SAF), to evaluate the disease's activity.

Keywords: non alcoholic liver disease, no invasive diagnosis, diagnosis

1. Introduction

In the last years, the prevalence of non-alcoholic fatty liver disease (NAFLD) has raised at a worldwide level, affecting up to 25% of the adult population [1].

The prevalence of type 2 diabetes, cardiovascular diseases, cancer associated with obesity, and advanced hepatic diseases (liver cirrhosis and liver cancer), have increased together with the growth of the prevalence of NAFLD [1–4].

The broad spectrum of disorders that involve NAFLD range from simple fatty liver to nonalcoholic steatohepatitis, and the increasing of fibrosis that concludes in cirrhosis [5, 6]. Among the most relevant metabolic conditions related to this disease, are obesity, insulin resistance, dyslipidemia, and type 2 diabetes [5–7].

Furthermore, the European Association for the Study of the Liver (EASL) and the Asia-Pacific Guidelines point out the relation between Hepatocellular Carcinoma (HCC) and NAFLD, since it can occur in patients with NAFLD but without cirrhosis [8, 9].

2. Definition

Nonalcoholic fatty liver is characterized by the presence of at least 5% of hepatic steatosis without evidence of hepatocellular injury (ballooning). On the other hand, the definition of NASH (non-alcoholic steatohepatitis) is the appearance of at least 5% of hepatic steatosis and inflammation, hepatocytic injury (eg. ballooning) with or without fibrosis [10].

3. Diagnosis

The diagnosis' approach should focus on the non-invasive evaluation to first identify NAFLD in patients with metabolic risk factors, and then, monitor the progression of the disease, the treatment, and the response, in order to identify early patients with a worse prognosis [6, 11].

The risk with NAFLD is that it is a silent entity that is diagnosed incidentally, because abnormal liver enzymes are reported in liver biochemistry or through images, such as in ultrasound with steatosis reported. NAFLD is a diagnosis of exclusion, therefore once it is suspected, the diagnosis should be confirmed by ruling out other possible causes of steatosis; for example, alcoholic hepatitis and NASH are clinically indistinguishable. For this exclusion, it is necessary to evaluate if there is a significant consumption of alcohol, which is generally considered of more than 20 g per day [12]; also, it is important to carry out a good clinical record to identify risk factors for liver disease, such as the use of medications or a family history of liver disease. Several Clinical Guidelines have identified criteria for the diagnosis of NAFLD (**Table 1**).

All of these considerations imply a different approach to NAFLD detection by Scientific Societies. Only the recommendations of the Asia-Pacific Associations, EASL and NICE (National Institute for Health and Care Excellence) [13] recommend screening, in particular, of high-risk groups (**Table 2**). In contrast, the AASLD (American Association for the Study of the Liver) recommends a concept of surveillance in the metabolic risk factor populations since there is no cost-effectiveness evidence to support a test to determine NAFLD in adults [6, 14].

3.1 Liver biochemistry

The liver biochemistry of NAFLD usually presents within normal parameters, although a slight increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) or gamma-glutamyl transpeptidase (γGT) can occur. However, since liver enzymes are not a sensitive screening test, all the recommendations agree that their normal values may not exclude NAFLD [13]. Besides, liver enzyme

	EASL	NICE	Asia-Pacific	AISF	AASLD
Criteria	Steatosis in>5% of hepatocytes by imaging or histology. There are no other causes of steatosis. Insulin resistance	Excessive fat in the liver. There are no other causes of steatosis. No significant alcohol consumption.	Hepatic steatosis by imaging or histology. There are no other causes of steatosis. No significant alcohol consumption.	Hepatic steatosis in images or histology. There are no other causes of steatosis. No significant alcohol consumption.	Evidence of hepatic steatosis by imaging or histology. There are no other causes of steatosis. No significant alcohol consumption. Non-coexisting chronic liver disease.
Alcohol consumption limit (males)	30 g/d	30 g/d	2 standard drinks / day 140 g / week	30 g/d	21 standard drink / week 294 g / week
Alcohol consumption limit (females)	20 g/d	20 g/d	1 standard drink / day 70 g / week	20 g/d	14 standard drinks / week 196 g / week

Translated from Leoni S. World J Gastroenterol. 2018 Aug 14;24(30):3361–3373. EASL: European Association for the Study of the Liver, NICE: National Institute for Health and Care Excellence, AISF: Italian Association for the Study of the Liver, AASLD: American Association for the Study of the Liver.

Table 1.
Diagnostic criteria for NAFLD according to various clinical guidelines.

	EASL	NICE	Asia-Pacific	AISF	AASLD
Generalized screening	No	No	No	No	No
Screening in high-risk groups Screening type	Yes Obesity Metabolic syndrome Altered liver enzymes Yes, hepatic enzymes	Yes Obesity Type 2 diabetes No, hepatic enzymes. Yes, ultrasound.	Yes Obesity Type 2 diabetes No, hepatic enzymes Yes, ultrasound If transient elastography	Not mentioned	No (active surveillance)

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Table 2.
Comparisons of recommendations for screening of NAFLD.

abnormalities can mask another cause of liver disease, in which steatosis is a coexisting condition. Also, abnormalities in laboratory tests (such as ferritin or autoantibodies) do not always diagnose the presence of another liver disease but could be an epiphenomenon of NAFLD with no other clinical consequence. In particular, according to the AASLD guidelines, elevated serum ferritin and low autoimmune antibody titers (especially antinuclear and smooth muscle antibodies) are frequent features in patients with NAFLD and may not demonstrate hemochromatosis or autoimmune liver disease [6, 15, 16].

3.2 Non-invasive techniques

Currently, the absence of highly specific and sensitive non-invasive markers that can predict inflammation and fibrosis has increased the efforts in the identification of new markers of the disease's progression and the development of clinical scores of disease's severity. To evaluate steatosis, the Fatty Liver Index (FLI) and the NAFLD Liver Fat Score are used by the EASL, the Asia Pacific Association, and the Italian guidelines. These scores can be calculated easily by using common blood tests and simple clinical information. For instance, FLI is calculated from triglyceride levels, body mass index, waist circumference, and gamma-glutamyltransferase, while NAFLD liver fat score is determined by evaluating the presence/absence of the metabolic syndrome and type 2 diabetes, fasting serum insulin, and aminotransferases. Both of them have been validated in a cohort of severely obese patients and in the general population, which can predict the presence of steatosis, but not its severity [6, 17–19].

Respectively, there has been an increase in the investigation of different tools in this regard, that include non-invasive scales (NAFLD fibrosis score (NFS), FIB-4, AST/ALT ratio index), serum biomarkers (ELF panel, Fibrometer, Fibrotest, Hepascore), and techniques of imaging, such as transient elastography, magnetic resonance elastography (MRE), and shear wave elastography. According to the NICE guideline, the best cost–benefit ratio in identifying patients with advanced fibrosis stages was demonstrated by the liver fibrosis (ELF) blood test, and therefore, these tests should be offered to all patients with an incidental diagnosis of NAFLD. On the contrary, the EASL and Italian guidelines suggest the use of the NAFLD fibrosis score (NFS) and the FIB-4 as non-invasive scores to identify patients with different risks of advanced fibrosis. Both scores predict liver-related mortality and cardiovascular disease since they have been validated in several ethnically NAFLD patients. Furthermore, in a recent study of the AASLD is highlighted that both NFS and FIB-4 present the best predictive value for advanced fibrosis in NAFLD patients with histological diagnosis (**Table 3**) [20–22].

3.3 Proteomics, glycomics and microRNA

The new technology in proteomics, glycomics, and microRNA (miRNA) can tell us about the pathophysiology of NAFLD/NASH [23].

Sirtuin 1 (Sirt 1) is a heat shock protein that is related to toxic immune reactions, antimicrobial activity, and mitophagy. Mitophagy is very important in NAFLD along with other diseases, therefore there is an increasing interest in maintaining the regulation and homeostasis of the mitochondria, due that it is necessary for the survival of many tissues [24]. The nuclear receptor of Sirt 1 is a nicotinamide adenine dinucleotide (NAD+) dependent class III histone deacetylase (HDAC) that modifies the gene expression to the metabolic activity of transcription factors, such as p53, and deacetylation of nuclear receptors. Its functions involve the metabolism of cholesterol, fatty acids, glucose, and xenobiotics, as well as the expression of p450 in the hepatic metabolism [25]. This is why the regulation of the nuclear receptor Sirt 1 is crucial to prevent NAFLD and other metabolic diseases. The proteome blood clinical analysis for the proteomic biomarkers, especially Sirt 1, with its measurement in plasma, cytoplasm, and nucleus, is the key to detect, evaluate and determine mitochondrial apoptosis and the progression of the disease [24, 25].

The most studied biomarker is cytokeratin 18 that is used to evaluate the presence of inflammation. There is a lot of research about its circulating levels as a signal of hepatocellular apoptotic activity and as a specific feature of NASH [6, 26].

Validated diagnostic panels to predict hepatic steatosis				
Panel	**Study**	**Biomarkers**	**Sensitivity (%)**	**Specificity (%)**
SteatoTest	Poynard et.at, 2005	α-MG, Haptoglobin, Apolipoprotein A1, Total Bilirubin, GGT, Glucose, Triglycerides, Cholesterol, ALT, Age, Gender, and BMI	90	70
FLI	Bedogni et al. 2006	Triglycerides, BMI, GGT, waist circumference	87	86
NAFLD-LFS	Kotronen et al. 2009	Mets, DT2, AST, ALT, insulin	95	95
LAP	Bedogni et al. 2010	Waist circumference, triglycerides	NA	NA
Diagnostic dashboards to predict NASH				
NASH Test	Poynard et al. 2006	NASH panels Undisclosed formula, α-MG, Haptoglobulin, Apoliprotein A1, Total Bilirubin, GGT, AST, Triglycerides, Cholesterol, ALT, Age, Gender, Weight and Height	33	94
Nash Diagnosis	Younossi et al. 2008	Undisclosed formula, CK18-M30, CK 18-M65, adiponectin and resistin	72	91
Apoptosis Panel	Tamimi et.al 2011	Cytokeratin 18 fragments, Fas ligand, soluble Fas	88	89
Diagnostic panels to predict fibrosis in NASH				
NAFLD fibrosis score	Angulo et al.2007	Age, glucose, BMI, platelets, albumin, AST / ALT	82	98
Fibrotest	Ratziu et al. 2006	Age,,α2-macroglobuline, Total bilirubin, GGT and apolipoprotein A1	77	98
BARD	Harrison et al. 2008	BMI ≥ 28 Kg/m^2, AST/ALT≥0.8, DT2	NA	NA
FibroMeter	Cales et al. 2009	Glucose, AST, ferritin, platelets, ALT, weight, age	79	96
FIB-A	McPherson et al. 2011	Age, AST / platelets, ALT	85	65

α-MG: alpha 2 macroglobulin, FLI: liver fat index, LAP. Lipid accumulation product, NA: not applicable. Translated from Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. J Hepatol 2013;58(5):1007–1019.

Table 3.
Different scores and models to predict steatosis, NASH, and fibrosis.

The Asia Pacific Association guidelines recommend that elevated levels of cytokeratin18 have a good predictive value for NAFLD in comparison to healthy livers, but it makes no difference between NASH versus simple steatosis. However, the EASL recommendations highlight that serum levels of cytokeratin 18 has an inverse relation with the histological improvement, although its predictive value is no better than ALT in identifying histological responders [6, 27–29].

3.4 Liver ultrasound and imaging techniques

The first line of diagnosis of hepatic steatosis is liver ultrasound because it is inexpensive, non-invasive, and widely accessible. Also, it is used currently in clinical practice and is quite accurate with an overall sensitivity of 85% and a specificity of 94% [30]. On the ultrasound can be observed that there is usually a visual decrease in the vascular margins, a loss of definition of the diaphragm, hepatomegaly, and hyperechogenicity of the liver parenchyma, as well as focal fat deposition in the hyperechoic area. If hepatocyte steatosis is not inferior to 31%, the transabdominal ultrasound is very effective [31].

There is a consensus for the use of abdominal ultrasound (USA). On the other hand, it can miss the diagnosis when the fat hepatic content is <20% because the sensitivity of USA among patients with morbid obesity (BMI > 40 kg/m2) is low [6, 32, 33].

Transient elastography has been recently approved by the United States (US) Food and Drug Administration (FDA) as a diagnostic tool for adult and pediatric patients with liver disease. Its cut-off value for advanced fibrosis in adults with NAFLD has been established at 9.9 KpA with a sensitivity of 95% and a specificity of 77%. Particularly, for clinically significant fibrosis, the elastography score has been shown to have good diagnostic accuracy with an AUROC of 0.93 (95% CI: 0.890.096) for advanced fibrosis (F3) and cirrhosis, and a negative predictive value of 90% in ruling out cirrhosis when a cutoff of 7.9 kPa is used. Although, it has a weaker capacity to make a difference between F2 and F3. Due to this high rate of false positive results, the EASL and the Asia Pacific recommendations mention that its low specificity limits its use in daily practice in the diagnosis of the advanced degree of fibrosis and cirrhosis, as well as a high failure rate. Moreover, the EASL highlights that it should not be used only as a first-line screening tool to identify advanced fibrosis or cirrhosis because of the unreliable results among patients with high BMI and thoracic fold thickness. However, by using M or XLprobe, the performance can improve and increase the success rate. For the identification of different degrees in fibrosis in NAFLD patients, especially in the intermediate stage, the US guidelines recommend magnetic resonance elastography (MRE), since it has a better performance than transient elastography in this regard, but shows the same predictive value for advanced stages of fibrosis. As a result, the AASLD concludes that ERM and transient elastography are useful tools to identify NAFLD patients with advanced liver fibrosis. Although, like transient elastography, shear wave elastography seems to be inadequate to distinguish between intermediate stages of fibrosis and to provide reliable results in 73% of patients with a BMI of 30 kg/m^2 [34–37].

Nevertheless, the gold standard for evaluating and quantifying hepatic steatosis and detecting the amount of liver fat as low as 5%–10% is magnetic resonance imaging (MRI), either by proton density fat fraction (1H-MRS) or by spectroscopy, although it is not commonly used in the clinical practice. This MRI is not recommended in the daily clinical setting despite its accurate precision, because of its limited availability, high costs, and long execution time [6, 38].

Another imaging technique used to quantify the fat content in the liver is transient ultrasound-based ultrasound (TE) using the continuous attenuation parameter (CAP). Due to that it simultaneously measures liver stiffness and evaluates the severity of NAFLD in the same setting, it has become a promising tool with good sensitivity [39]. However, despite its low cost and speed of implementation, its role in clinical practice has not yet been defined. In fact, according to the EASL, it has never been compared to hepatic steatosis as measured by 1H-MRS and there is limited data on its ability to discriminate different histological patterns. On

the other hand, the Asia Pacific Association proposes the CAP as a useful screening tool for the diagnosis of NAFLD, as well as to demonstrate an improvement in hepatic steatosis after the intervention in lifestyle and the reduction of the bodyweight [6].

The stiffness of the liver measured by the M probe is not always successful in obese patients. The XL probe, an improved FibroScan probe, has been demostrated to achieve better diagnostic accuracy. The cutoff values, compared to the M probe values, are approximately 1.5 to 2 kPa lower. In conclusion, in the diagnosis of fibrosis and cirrhosis, a strong alternative to liver biopsy can be ET in patients with NAFLD [23].

The optimal strategy for stratifying patients with NAFLD and monitoring disease progression has yet to be established. The EASL and the Italian guidelines mention that the combination of noninvasive scores (NFS and FIB4) and transient elastography should be used to identify patients at low risk for advanced liver disease and clinical decision making. Also, in combination, they can identify patients who must undergo a liver biopsy to confirm advanced fibrosis, and in whom a more intensive approach is needed.

3.5 Liver biopsy

The gold standard remains the liver biopsy, although it may not always be required to diagnose NAFLD, because it can distinguish steatohepatitis from simple steatosis, provide an evaluation of the degree of necroinflammatory activity, visualize fibrosis, and architectural alterations. The most widely used histological classification and staging system for NAFLD [23, 40] is the NAFLD Activity Score (NAS) and the Steatosis Activity Fibrosis (SAF) scoring systems to assess disease activity [6].

The SAF score simplified the identification of a subset of NAFLD, which includes the assessment of steatosis (S), activity (A), and fibrosis (F): NASH. The histopathologic features of NAFLD include lobular and portal inflammation, steatosis, hepatocellular ballooning, glycogenated nuclei, apoptotic hepatocytes (acidophilic bodies), deposition, megamitochondria, Mallory-Denk bodies, and fibrosis, with the characteristic pattern centered on the perisinusoidal/pericellular area. This fibrotic pattern typically originated in the adult zone, is known as chicken wire fibrosis [6, 41].

A score of ≥5 with steatosis and ballooning of hepatocytes is generally considered diagnostic of NASH, although patients may have NASH with lower NAS scores if there is the presence of steatosis and ballooning of hepatocytes [6, 40].

4. Conclusions

The incidence and prevalence of NAFLD are increasing. Clinical guidelines agree that noninvasive tests are currently not available to detect NAFLD and distinguish it from simple steatosis. Identifying people at risk of disease progression to NASH, fibrosis, and cirrhosis is extremely important because most patients are asymptomatic.

The current gold standard for the diagnosis of NAFLD / NASH is liver biopsy. Noninvasive tests such as proteomic biomarkers, transient elastography, and elastoMR to evaluate NAFLD/NASH are promising.

The most validated diagnostic panels include the NAFLD fibrosis score, FIB-4, and FibroMeter. Transient elastography is very useful in the evaluation of advanced fibrosis and cirrhosis.

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