Review Article

Nonalcoholic Fatty Liver Disease: Noninvasive Methods of Diagnosing Hepatic Steatosis

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ABSTRACT

Hepatic steatosis is the buildup of lipids within hepatocytes. It is the simplest stage in nonalcoholic fatty liver disease (NAFLD). It occurs in approximately 30% of the general population and as much as 90% of the obese population in the United States. It may progress to nonalcoholic steatohepatitis, which is a state of hepatocellular inflammation and damage in response to the accumulated fat. Liver biopsy remains the gold standard tool to diagnose and stage NAFLD. However, it comes with the risk of complications ranging from simple pain to life-threatening bleeding. It is also associated with sampling error. For these reasons, a variety of noninvasive radiological markers, including ultrasound, computed tomography, magnetic resonance spectroscopy, and the controlled attenuation parameter using transient elastography and Xenon-133 scan have been proposed to increase our ability to diagnose NAFLD, hence avoiding liver biopsy. The aim of this review is to discuss the utility and accuracy of using available noninvasive diagnostic modalities for fatty liver in NAFLD.

Key Words: Hepatic steatosis, nonalcoholic fatty liver, noninvasive methods for hepatic steatosis assessment

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Nonalcoholic fatty liver disease (NAFLD) is a spectrum of disease characterized by macrovesicular steatosis of the liver. It ranges from simple fatty liver (steatosis) to nonalcoholic steatohepatitis (NASH), which is a state of hepatocellular inflammation and damage in response to the accumulated fat. NAFLD is usually a diagnosis of exclusion made in patients who have not consumed alcohol in amounts considered to be harmful to the liver.[1] NASH carries a risk for progressive fibrosis, cirrhosis, and ultimately end-stage liver disease. It is currently the third most common cause of liver transplantation and is projected to be the leading cause in 2020.[2] An estimated 20-33% of American adults, approximately 90 million people, have NAFLD.[3] NAFLD prevalence has been reported to be 5-30% in the Asia Pacific region, which is lower than that in Western countries.[4] Hepatic steatosis, which is the simplest stage in NAFLD, occurs in approximately 30% of the general population and in as much as 90% of the obese population in the United States.[5] The clinical implication of NAFLD is that it is not limited to cause serious injury to the liver alone; it is a strong predictor of cardiovascular disease. It frequently occurs with features of the metabolic syndrome, including obesity, diabetes mellitus, dyslipidemia, and hypertension. The pathophysiological distinctive feature of NAFLD is insulin resistance, associated with mediators of oxidative stress and inflammatory cytokines.[6,7] One of the biggest challenges in many individuals with either NAFLD or NASH is the asymptomatic nature of the disease, making early detection difficult. When symptoms do occur, they are nonspecific such as vague right-upper-quadrant abdominal pain, fatigue, and malaise. Rarely, pruritus, anorexia, and nausea can develop.

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The occurrence of ascites with abdominal distension, variceal hemorrhage, or hepatic encephalopathy is indicative of progression to decompensated cirrhosis. Tests measuring liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) are performed routinely. Unfortunately, such markers may fail to detect the presence of hepatic steatosis due to the fact that many individuals have completely normal transaminases even those with significantly advanced steatosis.[8] A retrospective study by Fracanzani *et al*. showed that NASH was diagnosed in 59% and 74% of the patients with normal and increased ALT, respectively.[9] Liver biopsy is still used as the gold standard because it is considered the best diagnostic and staging tool in most studies. Liver biopsy comes with a risk of procedure-related complications ranging from simple pain to life-threatening bleeding.[10] In addition, it is associated with a sampling error that is in part related to the patchy histological changes of NAFLD and NASH as biopsies sample only approximately 1/50,000 of the total mass of the liver.[11,12] For these reasons, a variety of noninvasive serum and radiologic markers have been proposed to increase our ability to distinguish between simple hepatic steatosis, which is benign, and NASH. It is also important to grade the severity of hepatic steatosis, as it is associated with a long-term prognosis in NASH as compared to the general population. Therefore, grading steatosis will enhance the follow-up management of patients with NAFLD. The aim of this article is to provide a comprehensive evidence-based review of those noninvasive techniques to diagnose NAFLD.

NONINVASIVE TESTS TO DISTINGUISH BETWEEN HEPATIC STEATOSIS AND NASH

Serum markers

There is no single biochemical marker that distinguishes the stages of NAFLD (simple steatosis, NASH, and cirrhosis). However, there are some groups that are trying to validate some markers to distinguish simple steatosis and NASH. Cermille *et al*. studied the miRNAs by extracting intracellular and extracellular RNA using miRNeasy extraction kit from NAFLD patients. They found that miR-122 levels were increased by 7.3-fold in NAFLD patients compared with healthy controls. They also found that miR-122 and miR-34a levels were higher in NASH group compared with patients with simple steatosis.[13]

Cytokeratin-18 fragments in blood have been shown to be significantly elevated in patients with NASH (median 516.7 U/L) as compared with fatty liver or healthy controls (median 234 U/L). Therefore, it suggests that noninvasive monitoring of hepatocyte apoptosis in the blood of patients with NAFLD is a reliable tool to differentiate positive and negative for NASH in patients with suspected NAFLD. A meta-analysis of 10 studies showed that cytokeratin-18 fragments had area under the receiver–operating characteristic (ROC) curve of 0.8 to diagnose NASH.[14]

Magnetic resonance

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) works on the same physical principle. MRI provides the anatomical information, whereas MRS provides the biochemical component. Both have an advantage over ultrasound (US) and computed tomography (CT) in that they are able to detect small changes in liver fat content. MRS can be performed as an adjunct to whole body MRI, as part of the same examination, allowing a comparison to be made between hepatic fat content and whole body adipose tissue distribution in the same subject.[15]

Magnetic resonance imaging

MRI techniques use the frequency difference between water and lipid signals for generation of in-phase (IN) and opposed-phase (OP) images.[16] This method acquires MR images at echo times in which fat proton and water proton signals are either in-phase (fat and water signals cancel each other) or out-of-phase (fat and water signals will add up). By comparing the two phases: Signal loss on the out-of-phase means that fat is present in liver, whereas no signal loss proposes the absence of fat. The most widely used method is the Dixon method, where he applied this principle with a modified echo technique.[17] Many researchers continue developing modifications to the original Dixon method to reduce its limitations. Such improvements include better postprocessing algorithms, faster scan time, improved T2/T1 compensation, reduce the effect of field inhomogeneity, and decrease the ambiguity between fat and water.[18] Advantages of MRI include no radiation exposure as compared with CT and a greater ability to differentiate tissue characterization than CT and US.[19] MRI has a sensitivity of 80% and specificity of 95% to detect moderate/severe steatosis. It also has a sensitivity of 85% and specificity of 100% to detect mild steatosis.[20] Hepatic MRI correlated well with histology (r=0.773, P<0.001) in a prospective study that was done on individuals with known or suspected liver disease such as Hepatitis C, NAFLD, and chronic hepatitis with unknown etiologies. MRI correlated better with macrovesicular steatosis (r=0.920, P<0.001) than mixed steatosis (r=0.605, P<0.05). NAFLD had higher fat fraction, which is calculated from fat and water proton densities determined at spectroscopy and was equivalent to tissue triglyceride concentration, than Hepatitis C.[21] The use of MRI clinically in the diagnosis and monitoring of patients with hepatic steatosis has been limited partly because of its relatively high cost, reliance on patient cooperation, and long imaging time.[21]

Proton magnetic resonance spectroscopy

MRS directly measures proton signals from the acyl groups of hepatocyte triglyceride stores. It offers a quantitative

assessment of fatty infiltration of the liver. Previous studies found that steatosis measured by MRS closely correlated with biochemical and histological assessment of liver triglyceride content.[22,23]

Clinical MRS became feasible with the development of a rapid, inexpensive, and automated technique that could be easily integrated with the MRI exam.[24] However, it is still considered a research tool.

Recently, hepatic phosphorus-31 MRS (^{31}P MRS) was proposed as a potential marker for chronic liver disease as it shows distinct biochemical changes in different NAFLD states in some studies. Moreover, it may be a useful future direction of research.[25]

Proton density fat fraction (PDFF) with MRI, is used for quantifying the liver fat content. A retrospective study that was done by Idilman *et al*. showed that PDFF with MRI had area under the curve of 0.95 when discriminating moderate or severe hepatic steatosis from mild or no hepatic steatosis. It also showed that PDFF results were affected by the presence of fibrosis.[26]

NONINVASIVE DIAGNOSIS OF HEPATIC STEATOSIS IN NAFLD

Measurement of body fat and fat distribution

Studies have shown that obesity is strongly associated with hepatic steatosis.[27] Therefore, the usual management of NAFLD includes gradual weight reduction and increase in physical activity.[28] However, it remains uncertain whether excessive food consumption *per se* causes fatty liver or diets that are enriched in certain types of food are more likely to cause hepatic steatosis.[27,29] Body mass index (BMI) has been directly linked with the prevalence of NAFLD.[30] This leads to the speculation that a greater BMI in patients with NAFLD will lead to a more severe degree of hepatic steatosis.[31] NAFLD now occurs in the range of 65-92.3% of morbidly obese patients (BMI > 40 kg/m^2).[32] Waist circumference (WC) is a simple and inexpensive tool for assessing body fat distribution. It correlates well with abdominal obesity and it is associated with increased risk for adiposity-related morbidity and mortality.[33] WC and waist/hip ratio (WHR) are used as markers of abdominal obesity as they reflect central obesity.[34] It has been proposed that WHR and/or WC are more related to NAFLD than BMI.[30] In summary, WHR and WC are simple tools that can be applied as important anthropometric indicators to screen populations with a high risk for NAFLD.[35]

Serum markers

There is no single biochemical marker that confirms the diagnosis of NAFLD. However, several groups have proposed noninvasive models to diagnose hepatic steatosis in NAFLD:

Steatotest

Steatotest (ST) is a logistic regression module consisting of 12 components–ALT, α_2-macroglobulin (A2M), apolipoprotein A-I (ApoA1), haptoglobin, total bilirubin, gamma glutamyl transferase (GGT), cholesterol, triglycerides, glucose, age, gender, and BMI. Poynard *et al*. evaluated the ST for the diagnosis of hepatic steatosis in patients with chronic liver diseases secondary to hepatitis C virus (HCV) infection, alcoholic fatty liver disease (ALD), and NAFLD. This prospective study was tested on 884 subjects and it predicted >30% steatosis with 90% sensitivity, 90% specificity, 93% negative predictive value (NPV), and 63% positive predictive value (PPV).[36] ST has been validated in HCV patients before and after treatment but not validated in ALD and NAFLD patients. Another study showed that ST had PPV of 92% for the diagnosis of steatosis greater than steatosis grade 0, but ST was not able to differentiate between steatosis greater than 66% versus 33-66%.[37] However, ST has several advantages, in that it is non-invasive, easy to use, and the 12 components of ST are readily available.

Fatty liver index

It is a validated algorithm derived from the serum triglyceride (TG), BMI, waist circumference, and GGT levels. It was validated in a large group of subjects with or without suspected liver disease with an accuracy of 0.84 (95% CI) in detecting fatty liver. Fatty liver index varies between 0 and 100. An index <30 rules out fatty liver and an index ≥60 rules in fatty liver.[38] Zelber-Sagi *et al*. cross-sectional study of the subject on the general population showed that fatty liver index has notable agreement with ST compared with a moderate agreement with US.[39]

Imaging techniques

Ultrasound

US is currently the preferred method for screening asymptomatic patients with elevated liver enzymes or those at high risk of having NAFLD. US is accepted as an initial screening for fatty liver because it is noninvasive, inexpensive, and widely available.[40,41] Hepatic steatosis will lead to an increase in the echogenicity of the liver parenchyma on ultrasound that will show the liver appearing brighter than the cortex of the kidney.[42] A Japanese study conducted on the general population shows that ultrasound scanning has a sensitivity of 94% and a specificity of 84% for detecting liver steatosis.[43] Another prospective study done by Saadeh *et al*. showed a sensitivity of 100% and PPV of 62% for detecting steatosis >33%.[44] Palmentieri *et al*'s prospective study on patients suspected of having liver disease of various etiologies undergoing ultrasound and liver biopsy also showed that B-model ultrasound had 91% sensitivity, 89% specificity, 89% NPV, and 94% PPV.[40] The sensitivity of US decreases in morbidly obese patients to 86% sensitivity and 68% specificity.[45] It also decreases if the degree of fat

infiltration is ≤30%.[46] Dasarathy *et al*. showed that US is better in detecting macrovesicular hepatic steatosis of any degree with a sensitivity of 61% and a specificity of 100% compared with microvesicular fat with a sensitivity of 43% and a specificity of 73%.[47] US has several limitations; (1) diffuse hepatic steatosis and diffuse fibrosis can have similar sonographic appearance and therefore, sometimes it is difficult to distinguish between them;[48] (2) it is an operator-dependant modality with varying results between operators; (3) its inability to precisely quantify hepatic fat content (ie, grading: Mild, moderate, and severe steatosis); and (4) its inability to detect small changes in liver fat content with time, which makes its use in follow up challenging.[49]

The hepatorenal ratio (HRR) is a US index for quantifying liver steatosis. Normal liver shows an echostructure similar to that of renal parenchyma. In fatty liver, the increased hepatic echogenicity creates hepatorenal contrast.[50] Webb *et al*. studied 111 patients with hepatitis B, hepatitis C, NAFLD, or unexplained elevation of liver enzymes that were referred for sonographically guided liver biopsy. Hepatorenal sonographic index had a 100% sensitivity, 91% specificity, NPV 88%, and PPV of 100% for the diagnosis of hepatic steatosis>5%.[51] An observational study on healthy volunteers showed that HRR has 92.7% sensitivity and 92.5% specificity compared with liver biopsy.[52] Marshal *et al*. studied 101 patients, excluding renal disease patients and liver masses patients, who underwent liver biopsy; they observed that HRR of 1.28 or greater had a 100% sensitivity and 54% specificity.[53] Hepatorenal sonographic index is a promising tool for the follow-up patients with steatosis.

Controlled attenuation parameter

Transient elastography, (Fibroscan or Echosens) is a technique used to measure tissue elasticity based on ultrasound technology; it is used as a noninvasive assessment of hepatic fibrosis. The Fibroscan is a simple and low-cost device that may be performed by physicians or even nonphysicians after a short training period.[54] It has recently been proposed for measuring liver stiffness. Liver stiffness measurement (LSM) appears to be a reliable tool to identify hepatic fibrosis and cirrhosis mainly in patients with chronic hepatitis C (CHC) but limited data are available in patients with NAFLD.[54] BMI is the only factor associated with failure of Fibroscan in NAFLD. In overweight or obese patients, the fatty thoracic belt attenuates elastic and US waves, rendering liver stiffness measurement impossible, which may lead to underestimation of liver damage.[55] Controlled attenuation parameter (CAP) is a novel proprietary algorithm and a noninvasive tool based on US attenuation. It has been developed for use with the fibroscan to measure steatosis. It is only measured on the same validated measurements according to the same criteria used for LSM and on the same signals. The final CAP value, which ranges from 100 to 400 decibels per meter (dB/m), is the median of individual measurements. A prospective study in overweight and obese patients with chronic liver disease showed that CAP at a cutoff of 283 dB/m has 76% sensitivity and 79% specificity to detect steatosis.[56] In another prospective study by De Ledinghen *et al*., on patients with chronic liver disease (HCV and NAFLD) to evaluate the diagnosis of steatosis, showed that CAP, area under the ROC was 0.84 for the diagnosis of steatosis≥S1, 0.86 for the diagnosis of steatosis≥S2, and 0.93 for the diagnosis of steatosis≥S3.[57] CAP is a semi-quantitative method to assess steatosis that is operator/machine independent. It is less influenced by sampling error compared with liver biopsy because it explores liver volume 100 times larger.[58] However, only a few studies on CAP have been published on patients with chronic liver disease, and none on the general population. Furthermore, CAP is not available in the measurement with XL probe. Therefore, CAP needs further validation and further development.[59]

Computed tomography

CT provides an accurate and a reliable visualization of the whole liver, so that not only diffuse but also focal fatty infiltrations of the liver parenchyma can be accurately diagnosed.[60] The CT diagnosis of hepatic steatosis is made by measuring the difference in liver and spleen attenuation values in Hounsfield units.[61] Hepatic steatosis is best visualized in nonenhanced CT images, which presents as decreased attenuation values of the parenchyma (ie, hypodense liver) due to the inverse relationship between hepatic fat content and hepatic attenuation.[62] It has 73-100% sensitivity and 95-100% specificity to detect moderate-to-severe steatosis.[63] Park *et al*.'s prospective study on 158 living donors underwent same-day unenhanced CT using liver-to-spleen attenuation ratio and liver-to-spleen attenuation difference to detect steatosis>30% and ultrasonography-guided liver biopsy. It showed that CT had 73-82% sensitivity and 100% specificity in the assessment of macrovesicular steatosis.[64] Another study suggested that 0% of steatosis in histology findings showed 1.296 liver-to-spleen ratio, which would be beneficial as a cutoff value to exclude clinically important liver steatosis.[65] Enhanced CT has a limited role in the diagnosis of steatosis due to the influence of contrast injection rate and the timing of analysis on liver attenuation.[63] Widespread application of CT scan in patients with NAFLD is limited for many reasons, such as the risk of radiation exposure, high cost that makes it difficult to use in follow-up. In addition, its ability to detect steatosis decreases as the severity of steatosis increases.[60]

Xenon-133 liver scan

Xenon (Xe-133) gas is highly fat soluble and therefore concentrates in fatty tissues. In addition, Xe-133 gas is cheap and safe with very low radiation risk.[66] The estimated absorbed radiation dose is 155 MBq (5 mCi) of Xenon-133 for 5 min. Kitani and Winkler have confirmed the solubility of Xe-133 gas in liver tissue with varying lipid content that is

Table 1: Assortment of radiologic modalities

Advantages	Disadvantages	Sensitivity (%)	Specificity (%)
Ultrasound			
Inexpensive	Sensitivity decreased with morbid obesity	94	84
Widely available	Operator dependent,		
Can be used as a screening tool	Cannot detect small changes		
CAP			
Can detect steatosis as low as 10%	Not well studied	76	79
Immediate results			
Inexpensive			
Can grade steatosis			
Computed tomography			
Not operator dependant	Difficult to use in follow-ups due to radiation exposure	73-100 to detect moderate/severe steatosis	95-100 to detect moderate/severe steatosis
Available in almost all hospitals	Insensitivity to less than 30%		
Quantitative and qualitative			
Xenon-133 scan			
Low radiation exposure	Not well studied	94	87
MRI, MRS			
No radiation exposure	High cost	MRI: Mild and moderate steatosis: 80	MRI: Mild 100 moderate 95
Can differentiate tissue characterization	Reliance on patients cooperation		
Operator independent	Long time imaging		
Can detect fat as low as 5%-10%	Out and in phase depend on several factors such as T1, T2 relaxation		

MRI: Magnetic resonance imaging, MRS: Magnetic resonance spectroscopy

measured enzymatically.[67] Recently, a retrospective study by Al-Busafi and colleagues showed that Xe-133 scan had 94.3% sensitivity and 87.5% specificity for detecting NAFLD.[66] Xenon-133 liver scan is a promising test for the diagnosis and quantification of hepatic steatosis and can reliably rule-in or rule-out the presence of moderate to severe hepatic steatosis. One major limitation of Xe-133 scan is that it detects only fat, so it is not expected to distinguish between simple steatosis and fibrosis. The usefulness of this liver scan in the diagnosis and management of NAFLD has not been well studied.[66]

Diagnostic screening for NAFLD

According to the European Association for the Study of the Liver (EASL) NAFLD guidelines, screening for NAFLD/NASH is not recommended in the general population. However, it is recommended for high-risk groups such as patients with metabolic risk factors and/or well-characterized insulin resistance.[68] On the other hand, the American Association for the Study of Liver Diseases (AASLD) does not recommend screening for NAFLD in adults even in high-risk groups due to uncertainties surrounding diagnostic tests and treatment options, lack of knowledge related to long-term benefits, and cost effectiveness of screening.[69]

CONCLUSION

Currently, the best available diagnostic as well as staging method for both NASH and NAFLD is liver biopsy.

Table 2: Summary of diagnostic strategies and screening for hepatic steatosis

- Screening for NAFLD is not recommended in the general population. It is still controversial to screen high-risk groups such as patients with metabolic risk factors and insulin resistance
- A diagnosis of NAFLD can be achieved after excluding other causes of abnormal liver function tests and preforming a liver ultrasound
- Ultrasonography is the preferable method to diagnose steatosis
- Patients can have normal ALT and AST; therefore, it is not a reliable method to diagnose patients
- CAP is a promising method to quantify fat. It is easy and more accurate than US
- CT and MRI are not performed regularly in clinics to diagnose hepatic steatosis due to high cost and radiation exposure
- Investigation to evaluate fibrosis in patients with increased ALT and steatosis shown in US is recommended
- Liver biopsy could be performed in cases of advanced fibrosis or cases with uncertain results

NAFLD: Nonalcoholic fatty liver disease, ALT: Aminotransferase, AST: Aspartate aminotransferase, CAP: Controlled attenuation parameter, US: Ultrasound, MRI: Magnetic resonance imaging, CT: Computed tomography

Nonetheless, the associated complications and the inaccurate sampling of a liver biopsy make its diagnostic ability of NASH or NAFLD more complicated. Furthermore, problems occur in standardizing the histological staging of NAFLD. Therefore, an assortment of radiologic modalities [Table 1] can be used clinically for monitoring early changes in disease. Table 2 summarizes the diagnostic

strategies and screening of hepatic steatosis in NAFLD according to EASL.[53]

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