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EDITORIAL

Noninvasive imaging assessment of non-alcoholic fatty liver disease: Focus on liver scintigraphy

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Abstract

Noninvasive diagnoses of nonalcoholic fatty-liver disease (NAFLD) involve the use of serologic markers and imaging methods, such as conventional ultrasonography (US), computed tomography, and magnetic resonance imaging. Although these methods are reliable for the noninvasive detection of moderate to severe fatty changes in the liver, they are not reliable for detecting nonalcoholic steatohepatitis (NASH) and fibrosis. New imaging technologies, such as US-based transient elastography, acoustic radiation force impulse and magnetic resonance-based elastography, can reportedly be used to determine the severity of liver fibrosis associated with NASH. In this context, the field of nuclear medicine through liver scintigraphy has recently been proposed, and is being explored for use in the diagnosis of NASH. More importantly, nuclear medicine may contribute to the distinction between simple steatosis and NASH. For example, the enhanced release of cytokines and the decrease in the phagocytic activity of Kupffer cells play important roles in the pathogenesis of NASH. Removal of technetium-99m colloid from circulation by Kupffer cell phagocytosis therefore provides a valuable imaging technique. Thus, nuclear medicine is poised to provide useful tools for the evaluation of patients with NAFLD. However, the evidence is still scarce, and more studies with larger samples are needed to identify their role before they are used in clinical practice.

Key words: Liver fibrosis; Liver scintigraphy; Nonalcoholic fatty-liver disease diagnosis; Nonalcoholic steatohepatitis; Noninvasive methods

Core tip: Noninvasive methods used to diagnosis nonalcoholic fatty-liver disease (NAFLD) include ultrasonography (US), computed tomography, and magnetic resonance imaging. Although these methods are reliable for the noninvasive detection of moderate to severe fatty changes in the liver, they are not reliable for detecting nonalcoholic steatohepatitis and fibrosis. New imaging technologies, such as US-based transient elastography,

acoustic radiation force impulse and magnetic resonance-based elastography, may be used to determine the severity of liver fibrosis. Liver scintigraphy has recently been proposed to evaluate the diagnosis of nonalcoholic steatohepatitis, and has potential for the evaluation of patients with NAFLD.

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INTRODUCTION

Nonalcoholic fatty-liver disease (NAFLD) is the most common form of chronic liver disease in developed countries. It can present as simple steatosis, which does not progress to more advanced disease and with a better prognosis, or as nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and hepatocellular carcinoma[1-3]. Moreover, patients with NASH have an especially high death rate due to associated cardiovascular disease, extrahepatic cancer, and liver disease[4]. In a recent retrospective cohort study that followed-up patients with biopsy-proven NAFLD for 150 mo, a global death rate of 39.8% was observed, mainly due to these associated conditions[5]. Furthermore, although overall and cardiovascular mortalities were similar between steatosis and NASH patients, liver-related deaths were six times more frequent in patients with NASH. Therefore, the assessment of liver disease severity and identification of patients with NASH is of utmost importance.

Liver biopsy is the gold standard to distinguish among the different presentations of NAFLD, but it is an invasive procedure associated with severe complications in 0.3%-3.0% of cases, and leads to death in 0.01% of cases[4,6-8]. In addition, biopsy findings may be misrepresentative, causing misdiagnosis depending on the experience of the pathologist. Therefore, there is ongoing interest in developing noninvasive methods for diagnosing NAFLD and its various forms of presentation.

The noninvasive methods currently in use, including serologic markers, are intended for diagnosis of patients with NASH and those with fibrosis. Conventional imaging methods are reliable for the detection of moderate to severe fatty changes in the liver, though they are not reliable for detecting NASH and fibrosis. New imaging technologies show promise for determining the severity of liver fibrosis associated with NASH. In this context, the field of nuclear medicine through liver scintigraphy has been proposed and is also being explored.

ULTRASONOGRAPHY

Ultrasonography (US) US is a noninvasive imaging method used to detect NAFLD, particularly hepatic steatosis[9]. US shows a sensitivity for detecting steatosis of between 60% and 94%, depending on steatosis degree[10]. One study reported a sensitivity of US of 91% and specificity of 93% in 235 patients with $\geqslant$ 30% steatosis on biopsy[11]. In the same manner, a prospective study by Dasarathy *et al*[12] reported 90% sensitivity with US when steatosis was > 20% on biopsy. However, the sensitivity is low when the degree of steatosis is < 20%-30%[12,13]. Moreover, the sensitivity and specificity of US are considerably reduced in the presence of obesity[14]. de Moura Almeida *et al*[15] found a sensitivity of 64.9% using US for the diagnosis of hepatic steatosis in 105 severely obese patients. The presence of underlying chronic liver diseases can also reduce the accuracy of US in the diagnosis of hepatic steatosis, as hepatic fibrosis can increase liver echogenicity[16]. Another limitation of US is that it cannot be used to quantify the amount of fat or provide a differential diagnosis between simple steatosis and NASH. Moreover, it is operator dependent with significant intra- and inter-observer variability[17].

COMPUTED TOMOGRAPHY

The capability of diagnosing hepatic steatosis with computed tomography (CT) is similar to that of US. Unenhanced CT shows low attenuation of the steatotic liver in contrast to the spleen, and the severity of steatosis correlates with the liver-spleen attenuation ratio[18,19]. However, misdiagnosis can occur when other diffuse liver conditions are present, such as hemochromatosis[20]. Furthermore, CT cannot detect the degree of fibrosis, and cannot distinguish NASH from simple steatosis[21]. With the additional issue of radiation exposure, CT is not an appropriate modality for routine diagnosis.

MAGNETIC RESONANCE IMAGING

Conventional magnetic resonance imaging (MRI) is an accurate and comparatively superior technique to US for detecting minor steatosis[22]. As water and fat protons produce different frequencies in a magnetic field, MRI can be used to qualitatively and quantitatively diagnose fatty infiltration. The most commonly used quantitative method is the so-called in- and out-phase imaging, in which the signal from fat protons is added or subtracted, respectively, from the signal from protons in water. Reduction of the out-phase signal on T1-weighted images is an accurate predictor of hepatic fat content compared with the histologic assessment[23]. Although many

MRI techniques have been developed to improve its performance in the diagnostic spectrum of NAFLD and provide a quantitative assessment of hepatic fatty infiltration[23-25], these methods are limited in their ability to detect coexisting inflammation or fibrosis[24]. Moreover, MRI is costly, time consuming, and motion artifacts, such as from respiration, can affect image quality[26,27].

Magnetic resonance-based proton magnetic resonance spectroscopy (1H-MRS) provides a quantitative biomarker of liver fat, termed proton density fat fraction, which enables identification of tissues that contain a significant proportion of intracellular lipids. Furthermore, 1H-MRS facilitates examination of resonance frequencies of all hydrogen nuclei (protons) within a region of interest. Although the absolute differences in resonance frequencies with this method are quite small, they can be separated along a spectrum. The concentration of any given molecule in a sample is represented by the area under the specific resonance peak within the spectrum. Quantification of hepatic fat requires evaluation of the two dominant peaks within the unsuppressed MR spectrum, namely water at 4.7 ppm and lipids at 1.0-1.5 ppm[28]. Livers with fatty infiltration show an increased intensity within the lipid resonance peak. As 1H-MRS allows direct measurement of the area under the lipid resonance curve, it may also provide a quantitative assessment of fatty infiltration of the liver[28]. Although results from this method correlate well with those obtained by CT and liver biopsy[25,29,30], 1H-MRS remains a research tool, despite the fact that MRI scanners have 1H-MRS capabilities[31,32].

An additional magnetic resonance-based technique uses gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB) as a contrast agent. As this contrast agent is partially secreted by hepatocytes into the bile duct, it can be used to assess liver function and liver fibrosis[33]. However, there are no studies to date evaluating its use in the diagnosis of NAFLD.

US-BASED TRANSIENT ELASTOGRAPHY

Transient elastography (TE) (FibroScan; Echosens, Paris, France) uses ultrasonic elastography principles for the noninvasive assessment of liver fibrosis. This technique applies low-frequency shear waves created by a vibrating probe to the skin overlying the liver. The shear wave velocity of ultrasound signals is used to calculate the elastic modulus, expressed in kilopascals (kPa)[34]. A meta-analysis showed that the mean areas under the receiver operating characteristic curves (AUROCs) for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis were 0.84 (95%CI: 0.82-0.86), 0.89 (95%CI: 0.88-0.91), and 0.94 (95%CI: 0.93-0.95), respectively, independent of the cause of the liver disease[34].

However, there is ongoing debate regarding the diagnostic accuracy and feasibility of TE, especially in obese patients[35]. A body mass index (BMI) > 28 kg/m^2 was found to be an independent risk factor for failure of the method, and successful measurements were obtained using a standard probe in only 75% of patients with a BMI $\geqslant$ 28 kg/m^2[36]. Because of the poor accuracy of this probe in detecting liver fibrosis in overweight/obese patients, a larger probe (XL) has been developed, and failure occurred less frequently with this new probe than with the standard probe (1.1% *vs* 16.0%)[37]. Although the probes have comparable accuracy, lower liver stiffness cutoffs are necessary when the XL probe is used. Liver stiffness measurement is possible in 91.2% of patients with comparable diagnostic accuracy[38]. In clinical practice, the standard probe could be used as a first step for liver stiffness measurement, and the XL probe reserved for when invalid or unreliable measurements are obtained. This result could be useful for the assessment of liver fibrosis in NAFLD and/or obese patients.

A recent meta-analysis evaluated the use of several noninvasive methods for the diagnosis of NAFLD[35]. Favorable results were obtained by many of the included studies[36,39-45] that compared TE with liver biopsy. All studies had high-quality data, similar baseline characteristics, used similar cutoffs, with no heterogeneity factors identified. The overall results suggested that TE enables the diagnosis of F3 (85% sensitivity, 82% specificity) and F4 (92% sensitivity, 92% specificity), with moderate accuracy for F2 (79% sensitivity, 75% specificity) stage fibrosis[35].

Obesity is the main reason for failure of TE, which can be overcome using the XL probe[46,47]. Thus, TE is useful as a screening test to exclude advanced fibrosis, with high negative predictive value (NPV) and modest positive predictive value (PPV). Liver biopsy may be considered in NAFLD patients with liver stiffness of $\geqslant$ 7.9 kPa[36].

Although TE has emerged as a useful tool for the assessment of liver fibrosis, it also suffers from several limitations. For example, the technique is limited to imaging within an acoustic window through an intercostal space, thereby only allowing a small portion of the liver to be examined[48]. Additionally, TE should be carefully applied when used as an alternative measurement of liver stiffness instead of liver biopsy, as liver stiffness measurements can be influenced by conditions such as steatosis, obesity, lower degrees of hepatic fibrosis, necroinflammation of hepatocytes, cholestasis, elevated central venous pressure, and even postprandial conditions[49].

ACOUSTIC RADIATION FORCE IMPULSE

Acoustic radiation force impulse (ARFI) is a form of tissue elastography integrated within a conventional high-end ultrasound machine (S 2000; Siemens, Munich, Germany). With this method, the elasticity of the liver is calculated from short-duration acoustic pulses of a fixed frequency targeted to one region. As

with TE, the result is expressed in kPa[35,50].

ARFI has been suggested for patients with a high BMI[51], which can affect the accuracy of liver stiffness measurements. AUROCs reported with ARFI for the diagnosis of F3-F4 in NAFLD ranged from 0.74 to 0.97[43,50,52,53]. One study evaluating liver stiffness in 54 patients with biopsy-confirmed NAFLD and 10 healthy volunteers found a significant correlation between ARFI and TE[43]. Furthermore, there was a significant positive correlation between ARFI and severity of liver fibrosis in patients with NAFLD, and the results were similar to those of TE[43]. Another study evaluating 172 NAFLD patients used a predictive shear stiffness threshold of 4.24 kPa and found that shear stiffness distinguished low (0-2) from high (3-4) fibrosis stages with a sensitivity and specificity of 90% (AUROC of 0.90)[50]. In that study, BMI > 40 kg/m^2 was not a limiting factor for ARFI imaging, and no correlation was noted between BMI and shear stiffness. As a result, the authors concluded that ARFI is a promising imaging modality for detecting advanced fibrosis in patients with obesity-related liver disease[50]. TE and ARFI using the standard or the XL probes were also compared for the diagnosis of liver fibrosis in 61 patients with NAFLD/NASH[53]. Liver steatosis was evaluated using a controlled attenuation parameter, which correlated with liver histology. Although histologic liver fibrosis significantly correlated with TE results, the ARFI results did not.

Sporea *et al*[54] found that F2 fibrosis could be detected using a combination of the two elastography methods (ARFI and TE) with 60.5% sensitivity, 93.3% specificity, 96.8% PPV, 41.4% NPV, and 68% accuracy, with 84.9% sensitivity, 94.4% specificity, 84.9% PPV, 94.4% NPV, and 91.8% accuracy for predicting cirrhosis. Thus, the authors suggest that the use of TE in combination with ARFI is highly specific for predicting significant fibrosis, and may decrease the need for liver biopsy.

Supersonic shear wave imaging is another method that has been used in the evaluation of liver fibrosis. It is a new, shear wave-based US elastography technique that employs a larger, fan-shaped region of interest than other modalities[49]. Liver stiffness measurements were obtained using supersonic shear wave imaging in patients with many chronic liver diseases[55-57]. The findings indicate that it is a fast, simple and reliable method for noninvasive assessment of liver fibrosis, comparable to TE and ARFI. However, there are no studies at present that have reported evaluation of this method in patients with NAFLD.

MAGNETIC RESONANCE-BASED ELASTOGRAPHY

MRE is a phase contrast-based MRI technique that produces an image using a propagating shear wave[58]. Preliminary studies suggest that magnetic resonance-based elastography (MRE) is superior to TE for diagnosing each stage of fibrosis[59], and has good accuracy for diagnosing NASH[60]. Although this technique is expensive and not widely available, it may be useful for early detection of NASH in patients with NAFLD, as necroinflammation and fibrosis have similar effects on hepatic stiffness[60,61]. In contrast, simple steatosis does not result in any significant increase in stiffness[62]. The shear wave length in fibrotic liver is much longer than it is in healthy liver, and the elastograms show that fibrotic liver is much stiffer than healthy liver[63].

MRE can be performed before or after the intravenous gadolinium injections that are routinely used for liver studies. Yin *et al*[58] showed that liver fat content, as estimated using a conventional in- and out-of-phase imaging technique, did not affect the MRE assessment of hepatic fibrosis. They also found that MRE discriminates between patients with moderate and severe fibrosis (grades 2-4) and those with mild fibrosis (sensitivity 86%, specificity 85%). Loomba *et al*[64] prospectively assessed the accuracy of MRE for predicting advanced fibrosis in 117 patients with biopsy-proven NAFLD; a threshold of > 3.63 kPa had a sensitivity of 86%, specificity of 91%, PPV of 68%, and NPV of 97%. Simple steatosis can be differentiated from NASH with an accuracy of 93% using a cutoff value of 2.74 kPa[20], whereas NASH with advanced fibrosis can be detected with 95.4% accuracy using a cutoff value of 4.15 kPa[65].

The most frequent reason for technical failure in MRE is hepatic iron overload, which can decrease hepatic signal intensity in gradient echo-based MRE sequences to unacceptably low levels[61]. However, MRE can be performed in obese patients, as it is not affected by the degree of fatty change in the liver or amount of subcutaneous fat[62]. Further studies are needed to assess cost-effectiveness of using MRE over other available modalities for the diagnosis of advanced fibrosis in patients with NAFLD.

LIVER SCINTIGRAPHY

Xenon-133 is a highly fat-soluble gas that concentrates in fat tissue, and thus was evaluated for use in the diagnosis of fatty liver[66]. In a retrospective study of 258 patients suspected of having NAFLD, Al-Busafi *et al*[66] compared the characteristics of Xenon-133 liver scans to US; of the 35/43 (81.4%) patients with biopsy-confirmed NAFLD, Xenon-133 scanning had a sensitivity of 94.3% and a specificity of 87.5%, compared to 62.9% and 75.0%, respectively, obtained with US. Furthermore, scintigraphy was accurate in a subset of overweight patients, with a sensitivity of 93.8% and a specificity of 100%. The results of this study found that the degree of steatosis as defined by Xenon-133 liver scans was strongly and significantly correlated to the histologic degree. Finally, all patients with advanced fibrosis had positive Xenon-133 scans,

Table 1 Advantages and disadvantages of imaging methods in the identification of the spectrum of nonalcoholic fatty-liver disease

Imaging method	Accuracy			Risk	Cost
	Steatosis	NASH	Fibrosis		
US	++	0	0	0	+
CT	++	0	0	+++	++
MRI	+++	0	0	++	+++
TE	++	0	++	0	++
ARFI	++	0	++	0	++
MRE	++	+	+++	++	+++
Scintigraphy	++	++	0	+	+

ARFI: Acoustic radiation force impulse; CT: Computed tomography; MRE: Magnetic resonance-based elastography; MRI: Magnetic resonance imaging; NASH: Nonalcoholic steatohepatitis; TE: Transient elastography; US: Ultrasound; 0: Absent; +: Low; ++: Intermediate; +++: High.

and all patients with moderate or severe steatosis measured by scintigraphy were diagnosed with NASH upon biopsy. However, this method is only able to detect steatosis, and cannot be sued to distinguish between subtypes of NAFLD[66].

Nevertheless, nuclear medicine may have a larger contribution regarding the distinction between simple steatosis and NASH. For example, the enhanced release of cytokines and the decrease in the phagocytic activity of Kupffer cells play important roles in the pathogenesis of NASH. Kupffer cells can be imaged using radioactive colloids, as they remove technetium-99m (^{99m}Tc) colloid from circulation by phagocytosis. Kikuchi *et al*[67] evaluated ^{99m}Tc-phytate scintigraphy in 29 patients with NASH and 8 patients with simple steatosis, diagnosed through liver biopsy according to criteria previously published by Kleiner *et al*[68]. They measured mean radioactive counts per area in liver, heart, and spleen to calculate liver/spleen and spleen/heart uptake ratios. Patients with NASH had significantly lower liver/spleen uptake ratios than patients with simple fatty livers, with an AUROC of 0.819 that was independently associated to NASH in multivariable logistic regression. With a cutoff point of 2.93, the liver/spleen uptake ratio predicted NASH with a specificity of 75.0%, a sensitivity of 99.9%, and positive and negative predictive values of 93.5% and 99.9%, respectively. Steatosis could also be distinguished from early stages of NASH (stages 0 and 1) using the liver/spleen uptake ratio. The spleen/heart uptake ratio was also significantly associated with NASH, with higher ratios in patients with NASH[67].

We consider ^{99m}Tc-phytate scintigraphy an interesting method for the evaluation of NAFLD because it is noninvasive, relatively inexpensive, and widely available. As it provides a numerical result (*i.e.*, the liver/spleen uptake ratio), the diagnosis of NASH is less subjective than for other imaging techniques. Moreover, it can be used to distinguish fatty liver from NASH, even in earlier stages. Finally, if studies in other populations validate the findings reported by Kikuchi *et al*[67], a high liver/spleen uptake ratio would rule out NASH, and patients with a low ratio could be referred for liver biopsy to confirm the diagnosis. However, only the one paper with a limited number of patients has been reported, and there are no data comparing the results with other methods.

Mitochondrial function of myocardium and skeletal muscle can be assessed with ^{99m}Tc-m-2-methoxy-isobutyl-isonitrile (MIBI) scintigraphy. Considering that hepatic mitochondrial abnormalities contribute to the pathogenesis of NASH, Masuda *et al*[69] hypothesized that ^{99m}Tc-MIBI uptake would be reduced in the liver of patients with NASH compared to those with simple steatosis. In their study, 26 cases with biopsy-proven NAFLD were classified as definitive NASH, borderline NASH, and non-NASH based on the NAFLD activity score[68], and liver fibrosis was classified according to Brunt *et al*[70]. Patients were subjected to ^{99m}Tc-MIBI liver and heart scintigraphy, and hepatic uptake was calculated as the ratio of mean counts per pixel within the right upper lobe, to correlate with the region of liver biopsy. A region of the same size in the anterolateral wall of the left ventricle of the heart was measured in order to avoid the influence of the uptake of the left lobe of the liver. Using these values, the liver/heart uptake was calculated and used as an indicator of intrahepatic uptake, as there was no suspicion of heart disease among patients[69]. Their results showed that intrahepatic uptake of ^{99m}Tc-MIBI in patients with NASH was significantly lower than that of patients with simple steatosis (1.42 ± 0.41 and 1.56 ± 0.20 in patients with definitive and borderline NASH *vs* 2.07 ± 0.29 in non-NASH patients). Moreover, the liver/heart uptake ratio was significantly correlated to the NAFLD activity score. This study demonstrates that ^{99m}Tc-MIBI scintigraphy can distinguish NASH from simple fatty liver in a noninvasive manner, while providing a nonsubjective numerical result. In addition, there is no additional cost to obtain a liver/heart uptake ratio in patients for whom ^{99m}Tc-MIBI heart scintigraphy is recommended based on cardiologic indications. However, the technique might be biased if heart uptake is impaired by non-diagnosed ischemic heart disease, which frequently occurs in NAFLD patients[4].

CONCLUSION

Table 1 summarizes the main advantages and disadvantages of the various imaging modalities for NAFLD diagnoses. New imaging methods offer promise for exclusion of advanced liver fibrosis and cirrhosis in NAFLD patients, but additional studies are needed to identify their role in prognostication and monitoring of NAFLD patients before they are used in clinical practice. Nevertheless, nuclear medicine affords exciting potential for the evaluation of patients with NAFLD.

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