Review Article

Noninvasive Quantitative Detection Methods of Liver Fat Content in Nonalcoholic Fatty Liver Disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) ranges from simple steatosis to NAFLD-related liver cirrhosis and is a main cause of chronic liver diseases. Patients with nonalcoholic steatohepatitis and fibrosis are at a great risk of the progression to cirrhosis or hepatocellular carcinoma, both of which are tightly associated with liver-related mortality. Liver biopsy is still the gold standard for the diagnosis of NAFLD, but some defects, such as serious complications, sampling error and variability in histologic evaluation among pathologists, remain problematic. Therefore, noninvasive, repeatable and accurate diagnostic methods are urgently needed. Ultrasonography is a well-established and lower-cost imaging technique for the diagnosis of hepatic steatosis, especially suitable for population census, but limited by its low sensitivity to diagnose mild steatosis and being highly operator-dependent. Computed tomography also lacks the sensitivity to detect mild steatosis and small changes in fat content, and presents a potential radiation hazard. Controlled attenuation parameter based on the FibroScan® technology is a promising tool for noninvasive semiquantitative assessment of liver fat content, but the accuracy rate depends on the operator's expertise and is affected by age, width of the intercostal space, skin capsular distance and body mass index. Magnetic resonance imaging and magnetic resonance spectroscopy are regarded as the most accurate quantitative methods for measuring liver fat content in clinical practice, especially for longitudinal follow up of NAFLD patients. In this review, we mainly introduce the current imaging methods that are in use for evaluation of liver fat content and we discuss the advantages and disadvantages of each method.

Keywords: Nonalcoholic fatty liver disease; Hydrogen-1 magnetic resonance spectroscopy; Noninvasive; Liver fat content.

Abbreviations: 1H-MRS, hydrogen-1 magnetic resonance spectroscopy; CAP, controlled attenuation parameter; CT, computed tomography; IP, in-phase; mDixon, modified Dixon; MR, magnetic resonance; MRI, magnetic resonance imaging; MRI-PDFF, magnetic resonance imaging-estimated proton density fat fraction; MRS, magnetic resonance spectroscopy; MRS-FF, magnetic resonance spectroscopy-fat fraction; NAFLD, nonalcoholic fatty liver disease; OP, out-of-phase; PDFF, proton density fat fraction; US, ultrasonography.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is an important cause of chronic liver disease in many developed countries, with prevalence rate being up to 90% in obese patients and 70% in diabetics.[1–3] NAFLD ranges from nonalcoholic fatty liver and nonalcoholic steatohepatitis to fibrosis and cirrhosis, and even up to hepatocellular carcinoma.[4,5] Nowadays, NAFLD has become the third most common cause of liver transplantation.[6] Epidemiological studies have demonstrated that nearly 30% of people with NAFLD show evidence of histologic progression; in particular, 15–20% of NAFLD patients develop cirrhosis and 30–40% suffer from liver-related morbidity and mortality.[7,8] NAFLD is also associated with features of insulin resistance and metabolic syndrome, which include obesity, dyslipidemia, and diabetes mellitus type 2.[9,10] Finally, NAFLD is related to premature death, along with obesity, diabetes and cardiovascular disease,[11,12] and individuals with NAFLD are associated with a higher risk of cardiovascular disease, as compared to the general population.[13,14]

The diagnosis of NAFLD (EASL–EASD–EASO Clinical Practice Guidelines for the Management of NAFLD) requires exclusion of both the secondary causes and a daily alcohol consumption of ≥30 g for men or ≥20 g for women.[15] Liver biopsy is regarded as the gold standard for the diagnosis and histological assessment of NAFLD; however, biopsy has its own limitations, such as sampling error, invasiveness, and interobserver differences.[16,17] Up to now, no valid laboratory test methods can be used for the noninvasive quantification of liver steatosis, and some significant indexes of liver damage, such as alanine aminotransferase and aspartate aminotransferase levels, have been shown to be elevated in severe cases only.[18,19]

In clinical practice, ultrasonography (US) is a common method to diagnose fatty liver. US is a highly operator-dependent screening tool, with poor reproducibility in quantification of liver fat; the threshold of US for detection of fat infiltration with liver tissue is 30%, and not sensitive enough to detect mild steatosis.[20–23] Computed tomography (CT), on the other hand, as a NAFLD diagnostic method, also lacks the sensitivity to detect mild steatosis and small changes in fat content.

In addition, CT involves ionizing radiation, which is not beneficial for a longitudinal study.[4]

An ideal method for liver steatosis assessment must be widely available, noninvasive, safe, sensitive, accurate and repeatable. In recent years, some improved diagnostic methods such as controlled attenuation parameter (CAP), hydrogen-1 magnetic resonance spectroscopy (1H-MRS) and magnetic resonance imaging (MRI), have been applied in the diagnosis of fatty liver. In this review, we discuss several of the new noninvasive quantitative detection methods of liver fat content in NAFLD and provide some referable opinions for the clinical diagnosis of NAFLD (Table 1).

CAP in the diagnosis of liver fat content

CAP based on FibroScan® (Echosens, Paris, France) is a promising tool for noninvasive semiquantitative assessment of liver fat content.[24] CAP measures the ultrasound attenuation at the center frequency of the FibroScan M probe (3.5 MHz), with values range from 100 to 400 dB/m. Besides CAP being able to provide an instantaneous assessment of liver steatosis, some advantages, such as quantificational accuracy and ease of performance (which provide for instantaneous results), as well as inexpensive cost and reproducibility are highlighted when compared with other imaging methods.[25] In a prospective study of 153 patients, the areas under the receiver operating characteristics curves of CAP for ≥5%, >33% and >66% steatosis were 0.79, 0.76 and 0.70, respectively.[26]

Although CAP is a useful diagnostic method in detecting liver fat content, some defects still exist. In a multicenter prospective cohort study of 152 Chinese patients, CAP appeared to be a promising tool for the noninvasive quantification of hepatic steatosis, but was limited by the body mass index.[27] A study by Naoyuki and colleagues[28] showed that CAP was correlated with liver fat content in patients whose body mass index was less than 28 kg/m^2, particularly less than 25 kg/m^2. In this case, CAP was equipped with a new XL probe and the measure failure rates were reduced subsequently, but the probe cannot be used in many countries. An interesting discovery was that the diagnostic accuracy of the XL probe seems to be similar to that of the M-probe in patients who are not very obese (mean body mass index = 28.1 kg/m^2); however, a recent study showed that the CAP values with the XL probe and the M-probe were inconsistently associated with the waist circumference and the serum triglycerides levels.[2,29] The diagnostic accuracy of the XL probe and the M-probe in CAP measurement need further study. Furthermore, larger skin capsular distance can also affect the diagnostic accuracy, as a recent research reported that skin capsular distance ≥25 mm may lead to an overestimation of liver steatosis.[30] Other factors such as age, width of the intercostal space, ascites and visceral fat could also affect the diagnostic accuracy of CAP.[2]

Magnetic resonance (MR) in the diagnosis of liver fat content

MR is based on the common isotope of hydrogen, that is 1H. This isotope is distributed widely throughout the human body, and is mainly observed through water, fat and protein molecules, for which MR possesses the highest sensitivity, especially for water and fat.[10] The spectrum of fat often displays as a single peak, with a 3.3 ppm higher chemical shift than water, or a 210 Hz and 420 Hz lower resonance frequency than water at 1.5 T and 3.0 T MRI, respectively.[31]

MR as a noninvasive diagnostic method possesses many special advantages. Firstly, MR is a noninvasive test only. Secondly, different from CT, the imaging principle of MR is based on the hydrogen ions, rapidity and radiation-free imaging techniques, so urgent adoptions were made in this methodology for the pediatric population.[32] Moreover, the noninvasive character of the MR measures is convenient for observers to conduct related longitudinal studies, where long-term analysis on the same subjects is interesting.[33] Conventional MRI technique is limited by T1 bias, T(2)* decay, and

Table 1. Several noninvasive quantitative detection methods of liver fat content in NAFLD

Methods		Advantages	Disadvantages
CAP		quantificational accuracy; easy to perform; instantaneous results; inexpensive and reproducible	affected by body mass index, larger skin capsular distance, age, width of intercostal space, ascites, and visceral fat
MR	1H-MRS	measures proton signals directly; accurate quantification of liver fat content	expensive and complicated; difficult to acquire parameters; complicated analytical method
	Dixon-MRI	shortened scan time; provision of spatial resolution; noninvasive	affected by liver iron deposition; accuracy could be underestimated;
	mDixon-MRI and mDixon-TSE	flexible choice of echo times; acquiring images in a single scan	expensive
	MRI-PDFF	obtaining all data in a breath-hold; calculating the fat fraction in any segment of liver; measuring fat mapping of the entire liver; lower cut-off value	accuracy could be affected by fibrosis; accuracy could be affected by severe steatosis

Abbreviations: 1H-MRS, hydrogen-1 magnetic resonance spectroscopy; MR, magnetic resonance; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; MRI-PDFF, magnetic resonance imaging-estimated proton density fat fraction; TSE, turbo spin echo.

multifrequency signal-interference effects of protons in fat and eddy currents, and the liver fat content may not be measured accurately.[20] With the development of this methodology, four MR-based diagnostic methods—1H-MRS, proton density fat fraction (PDFF), Dixon MRI technique, and modified Dixon type (mDixon)—are used in clinical practice and epidemiologic studies for accurate and quantitative liver lipid contents in patients. In the next paragraphs, we mainly discussed the quantitative detection methods of liver fat content based on the MR.

1H-MRS in the diagnosis of liver fat content

1H-MRS is performed using a multiecho sequence. The chemical shift of protons in water at 4.65 ppm and in the main lipid peak at 1.3 ppm is a common definition of the hepatic fat percentage.[33] 1H-MRS parameters' analyses includes that of the lipid peak, areas under the lipid peak, fat peak/water peak ratio, and the ratio of the areas under the lipid peak to the areas under the water peak.[23] MRS can determine the ratio of water to fat in tissue accurately and has been regarded as a reference imaging method for the assessment of liver lipid content.[34,35]

One of the most representative methods is the 1H-MRS, which can accurately measure proton signals directly from the acyl groups of triglycerides accumulated in liver cells and analyze the hepatic fat fraction quantitatively.[36] Keese *et al.*[23] showed that the 1H-MRS could be used to detect and quantify liver fat content in rats with acute fatty liver, in *ex vivo* as well as *in vivo* models. However, the 1H-MRS diagnostic method is expensive and complicated, as it requires sophisticated post-processing program, and not every MRI is routinely equipped with MRS capabilities.[18] Moreover, the spatial distribution of fat in the entire liver is difficult to understand by liver biopsy or MRS-fat fraction (MRS-FF), since the fat distribution in liver is nonuniform.[35] In addition, the complicated acquisition parameters, method of analysis, and location of the volume assessed can affect the accuracy of its evaluation.[7] Although these drawbacks exist, 1H-MRS is still an effective and non-invasive technique that can be used for diagnosis and quantitative analysis of hepatic steatosis.

Dixon-MRI in the diagnosis of liver fat content

As early as 1984, Dixon had suggested that four images could be obtained by simple summation of water and fat signals of the image and subsequent subtraction, being 180° out-of-phase (OP), from the in-phase image. The four images were named in-phase (IP), out-of-phase (OP), water-only and fat-only.[37] The chemical shift-based water and fat separation Dixon MRI method had been widely used by analyze the characteristics of the resonance frequency difference of hydrogen atoms between water and fat molecules.[14] The Dixon method may be considered as a restricted chemical shift imaging method and is a T1-weighted gradient echo sequence that achieves fat suppression using two different echo times, namely the IP and opposed-phase of water and fat. This method includes two steps: firstly, obtaining uniform fat suppression across the fields of view in pre- and postcontrast gradient-echo imaging; secondly, reconstructing water, fat, IP and OP images from a single, multiecho acquisition.[38] The robust algorithms for reliable water–fat separation, powerful reconstruction hardware for their fast actuation, and high-performance gradient systems for rapid chemical shift encoding has facilitated wide use of the Dixon method in clinical and experimental research.[31]

The Dixon method primarily differs in the number of echoes or points, including single-echo, dual-echo, triple-echo, and multiecho types.[10] All of them have their own characteristics. No evidence has yet shown that the single-echo Dixon method possesses the ability to distinguish complex water and fat signals accurately.[31] Generally, dual- or triple-echoes are acquired in qualitative imaging, whereas six or more echoes are usually sampled in quantitative imaging.[31] As the multiecho chemical shift method with T2* correction required longer scan times than the two-point Dixon method, the two-point Dixon method for MRI has been considered as an noninvasive way to measure fat deposition in the liver.[32] It is difficult to acquire thin-slice images, but imaging the entire liver at 5-mm slice thickness is feasibly using the two-point Dixon method.[35] However, the two-point Dixon method is not perfect, liver iron deposition may distort local magnetic fields for T2* shortening and may result in signal intensity loss; the multiecho technique can easily correct for T2* decay and solve this error, but when the two-point Dixon method without T2* correction is considered for patients without iron content, this will affect the accuracy of ultimate results.[28] Previous research has shown that the two-point Dixon method assumes a single peak on the fat spectrum, but the liver actually has multiple peaks for fat, suggesting that the fat fraction could be underestimated by the two-point Dixon compared with that by the multipeaks fat model.[35] Despite the calculation of multiple fat peaks having this advantage, it is available on only a few MRI machines, so the application remains limited.[4] In this regard, the two-point Dixon method could be conveniently applied and is widely used.

Regardless of the water signal, fat signal or additional free parameters, phase error caused by magnetic field inhomogeneity is inevitable.[14] Phase differences between fat and water signals are caused by differences in resonance frequencies (3.5 ppm), and the latter is dependent upon the echo time at which the signal is sampled.[4] At the same time, some advantages of the Dixon method must not be overlooked, such as shortened scan times, provision of spatial resolution, and being noninvasive.[31] McPherson *et al.*[7] reported that the methods of MRS, Dixon IP, and OP had a better accuracy for quantifying liver lipid content in patients who had no or mild fibrosis compared with patients who had moderate or severe fibrosis. After that, Hayashi *et al.*[35] carried out a retrospective study that included 106 patients who underwent liver MRI and MRS, and 201 patients who underwent the liver MRI and histological assessment. The results showed no significant confounding effects of hepatic iron, inflammation and fibrosis on the corrected MRI-FF using the two-point Dixon method, based on the NAFLD activity score. As described as above, the Dixon method is an effective way to quantify the fat and iron accumulation.

mDixon-MRI and mDixon-turbo spin echo in the diagnosis of liver fat content

mDixon is a modified version of the Dixon method, that renders images by modifying the opposing "in" and "opposed" phases of the actual measurement to fit the theoretical value.[38] This method can also acquire the four images (IP, OP, water and fat images) in a single scan, but at the same time the limits of the scan parameters can be avoid perfectly.[38]

In the clinical practice, data of Dixon are obtained during one or multiple breath-holds; however, not every patient can hold

their breath for a few seconds, such as the severely ill and pediatric patients. In addition, compared with one breath-hold, different breath-hold positions may not be obtained correctly, and a significant misalignment of the slices may miss some significant information on the true LFF.[32] To remedy the defect of the Dixon method in clinical diagnosis, the mDixon technology was developed, which uses flexible choice of echo times for water and fat separation, with the referenced seven-peak spectral model of fat in the separation. Considering the multiple spectral peaks of fat, the seven-peak spectral model seems to be a good way to improve the consistency of fat quantification and fat suppression, instead of the standard single-peak.[37] mDixon-3D-turbo spin echo sequence was used to acquire coronal sections with a 4-echo multiacquisition mode, which possesses the ability to reconstruct the 4-echo source data automatically with a single fat peak reconstruction algorithm to generate coronal water- and fat-only images.[33] mDixon could obtain the IP, OP, water and fat images quickly in a single breath-hold.[39] With the advantage of 3D-Dixon-turbo spin echo sequence, it could impede fast imaging of the trunk for feasible breath holding to avoid respiratory motion artifacts.[28]

MRI-PDFF in the diagnosis of liver fat content

Recently, an innovated MRI technique called magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) was developed as a novel biomarker, having demonstrated powerful correlation and equivalency with MRS.[18] The PDFF described chemical shift–based water and fat separation technique can be performed by complex-based and magnitude technique.[5] The complex-based technique uses both magnitude and phase images' on the contrary, the magnitude-based technique uses only magnitude images for PDFF.[7] The primary advantage of this method is that data acquisition can be completely obtained in a breath-hold and allows for the simple calculation of fat fraction in any segment of the liver.[13] The biases in conventional MRI, like T1 bias, T2* decay, spectral complexity of lipid, noise bias and eddy currents, could be eliminated by MRI-PDFF.[34]

Comparing with MRS, MRI-PDFF measures fat mapping of the entire liver, and it can be used with any clinical MRI platform.[40] Di Martino *et al.*[18] found that the cut-off value of MRS in the diagnosis of fatty liver was 5%, and when MRI-PDFF was used to quantify liver steatosis, a cut-off value of 3.5% was found to be more appropriate. One prior study tested 506 adults with known or suspected NAFLD, and found that six-echo parametric maps were more accurate than three, four or five echoes, and the accuracy is not meaningfully confounded by age, sex, or body mass index.[41] PDFF was able to accurately measure the liver fat content of different liver segments in mild hepatic steatosis.[7] Kang *et al.*[13] compared the accuracy between MRI-PDFF and MRS-PDFF at both 1.5T (Siemens Symphony scanner) and 3T (GE Signa Excite HD scanner). The results showed that the former is more accurate at both 1.5T and 3T, compared against the latter.[13] Therefore, the fat mapping of the entire liver could be measured by MRI-PDFF. In addition, MRI-PDF provides an effective method for the early diagnosis of liver diseases.

A good diagnostic accuracy for quantifying fat content could be observed with MRI-PDFF when compared to the liver biopsy; however, some shortcomings of MRI-PDFF still exist. One drawback is that hepatic fibrosis can reduce the correlation between biopsy results and MRI-PDFF. Therefore, the values of MRI-PDFF could be underestimated in patients with hepatic fibrosis compared to patients without fibrosis.[7] Another drawback is the correlation between biopsy and MRI-PDFF, which was lower in patients with moderate or severe forms of hepatic steatosis compared to patients with more mild forms. Besides, hepatic iron content is also a factor influencing the accuracy of estimating hepatic steatosis.[7] Although so many shortcomings exist, MRI-PDFF is still an accurate and quantitative way to measure liver lipid content. Not only because the MRI-PDFF measurement is able to assess the presence and severity of hepatic steatosis in all liver segments but also because the MRI-PDFF calculation can be obtained in less than 25 seconds, is standardization suitable for different MR imagers. On account of these advantages, MRI-PDFF is easy to perform with a professional knowledge of physics. What's more, MRI-PDFF shows the fraction of protons that are lipid versus water, while histological analysis measures the fraction of hepatocytes that show steatosis, so the difference between the MRI-PDFF and pathology could be understood.[7]

Conclusions

Quantitative detection of liver fat content is highly important in the evaluation of NAFLD stages. Liver biopsy, however, remains the gold standard for liver histological test. In consideration of the invasiveness and difficulty in repeating, and the sample bias that is due to only a fraction of the liver being measured at a time, some noninvasive diagnostic methods need to be developed. Compared to the conventional US and CT, CAP is an accurate, easily performed and noninvasive tool for semiquantitative assessment of liver fat content, and could provide instantaneous results. MRS possesses the unique ability to separate fat and water components of the acquired signal *in vivo*, but MRS, especially the 1H-MRS, as one of the accurate imaging methods, is limited by its high cost and unusual equipment. The Dixon method and MRI-PDFF belong to the MRI tools for imaging, representing a technology that is more accurate than MRS and more practical as the entire liver can be covered in assessment.

All the methods have their own advantages and disadvantages. According to the development of current technology and previous research reports, both MRS and MRI should be more reproducible and accurate for quantifying hepatic lipid content and may substitute for liver biopsy as the reference standard for further studies. Certainly, with the constant development of technology, better methods of quantitative and accurate detection of liver fat content will be found.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (SjL, SJ, YX), acquisition of data (SjL, SJ, SsL, QD), drafting of the manuscript (SjL, SJ), critical revision of the manuscript for important intellectual content (YX), study supervision (SX).

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