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Quantitative Imaging Biomarkers of NAFLD

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Abstract

Conventional imaging modalities, including ultrasonography (US), computed tomography (CT), and magnetic resonance (MR), play an important role in the diagnosis and management of patients with nonalcoholic fatty liver disease (NAFLD) by allowing noninvasive diagnosis of hepatic steatosis. However, conventional imaging modalities are limited as biomarkers of NAFLD for various reasons. Multi-parametric quantitative MRI techniques overcome many of the shortcomings of conventional imaging and allow comprehensive and objective evaluation of NAFLD. MRI can provide unconfounded biomarkers of hepatic fat, iron, and fibrosis in a single examination—a virtual biopsy has become a clinical reality. In this article, we will review the utility and limitation of conventional US, CT, and MR imaging for the diagnosis NAFLD. Recent advances in imaging biomarkers of NAFLD are also discussed with an emphasis in multi-parametric quantitative MRI.

Keywords

Nonalcoholic fatty liver disease; Steatosis; Proton density fat fraction; Imaging biomarker; Magnetic resonance imaging

Introduction

Hepatic steatosis, or accumulation of excess fat within intracellular vesicles, is the defining histopathological feature of all fatty liver diseases, including nonalcoholic fatty liver disease (NAFLD). Excess fat accumulation causes abnormal appearance of the liver parenchyma on radiological imaging such as ultrasonography (US), computed tomography (CT), and

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Compliance with ethical standards

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magnetic resonance imaging (MRI). Imaging plays an increasingly integral role in the diagnosis and management of patients with NAFLD. In patients with clinically suspected NAFLD, steatosis detected on imaging can confirm the diagnosis [1, 2]. In patients with no known liver disease, an incidental finding of steatosis on imaging may be the first presentation of NAFLD. However, conventional imaging modalities are limited as imaging biomarkers of NAFLD for various reasons. Multi-parametric MRI techniques overcome the shortcomings of conventional imaging and allow comprehensive evaluation of NAFLD by providing unconfounded biomarkers of hepatic fat, iron, and fibrosis in a single examination—a virtual biopsy has become a clinical reality. In this article, we will review utility and limitation of conventional US, CT, and MR imaging as biomarkers of NAFLD. Recent advances in imaging biomarkers of NAFLD are also discussed with an emphasis in multi-parametric quantitative MRI.

Conventional Imaging

Ultrasonography

On US, normal liver parenchyma is slightly less echogenic (i.e., darker) than both the adjacent kidney and the walls of porta hepatis. In a steatotic liver, however, lipid-laden vesicles cause increased sound wave scatter and attenuation, resulting in (a) increased parenchymal echogenicity compared to the kidney, i.e., the “bright liver” sign, (b) “blurring” of the intrahepatic vessels and bile ducts, and (c) poor visualization of the “deep” structures, including the diaphragm, due to increased attenuation of sound transmission [3]. Examples of characteristic US findings of hepatic steatosis are illustrated in Fig. 1.

When one or more of these features are recognized by subjective evaluation, a diagnosis of steatosis can be made. In moderate to severe steatosis, these features are grossly apparent, and US diagnostic sensitivity and specificity are high, ranging 78.4–90.8 and 76.9–90.9 %, respectively [4]. For mild steatosis, however, these sonographic features are more subtle and the diagnostic sensitivity drops to 62.2–82.1 % with specificity of 76.2–90.1 % [4]. Based on the subjective assessment of the degree of imaging abnormality, steatosis is typically graded using an ordinal scoring system as: none, mild, moderate, and severe [5, 6]. Steatosis grading by US has shown a wide range of correlation with histological grading, with correlation coefficients ranging from 0.33 to 0.80 [7–9].

The main advantages of US are its low cost and ease of access. US is commonly available in clinics and can be used as a point-of-care test during a clinic visit. US can be safely performed in patients of all ages regardless of underlying medical conditions, including pregnancy. However, the quality and accuracy of US are dependent on the operator, equipment and patient factors and can be hampered by operator inexperience and anatomical constraints, e.g., overlying bowel gas and large body habitus [10]. Inter- and intra-reader agreements are moderate at best, even in the hands of the experts [11, 12]. The sonographic features are also confounded in advanced NAFLD due to coexisting fibrosis and inflammation [13, 14]. Therefore, conventional US is typically not recommended when an early and accurate diagnosis (particularly mild steatosis) and/or precise severity grading is required.

To overcome the subjectivity of the US diagnostic/grading criteria and problems of intra- and inter-reader agreement, several quantitative US techniques have been proposed, including US backscatter and attenuation value [15], attenuation and backscatter far-field slope value [16], and hepato-renal attenuation ratio [17]. A closely related, but a non-imaging technique is controlled attenuation parameter (CAP). Of these, CAP is the most validated and commercially available through Echosens (Paris, France), the manufacturer of FibroScan®. CAP is an objective measure of ultrasound attenuation and can be performed using the appropriately selected FibroScan probe to measure liver stiffness and steatosis simultaneously [18, 19]. A preliminary study in a mixed population of diffuse liver disease reported high correlation of CAP with histological steatosis grade (correlation coefficient 0.81) with excellent severity grading performance and high reproducibility [20]. In a NAFLD population, validation data are still incomplete, but CAP has thus far shown promise as a standardized quantitative US biomarker for steatosis [19, 21]. However, further validation is needed, including evaluation of newer probes optimized for obese patients.

Computed Tomography

On conventional CT, the “brightness” of the pixels corresponds to the X-ray attenuation value (or density). By convention, attenuation is calibrated to a standardized quantity called Hounsfield unit (HU): air = −1000 HU, water = 0 HU, and bone = 1000 HU. On unenhanced CT (i.e., no intravenous contrast administered), normal liver parenchyma is slightly denser (or brighter in appearance) than the spleen as well as intrahepatic vessels and bile ducts, with Hounsfield units, approximately 60 ± 10 HU. Subcutaneous adipose tissue is much less dense (or darker in appearance), approximately −90 HU. With increasing fat accumulation, liver parenchymal density decreases and liver appears darker relative to spleen and blood vessels (Fig. 2). Therefore, the entire spectrum of steatosis severity can be represented by a continuous range of the CT density, from that of the normal liver to that of adipose tissue.

Liver density <40 HU [22], liver-minus-spleen ($L - S$) density difference less than −10 HU, and liver-to-spleen (L/S) density ratio <0.9 on unenhanced CT have been proposed as thresholds for detecting moderate or greater (≥ 30 %) steatosis at histology [23, 24], with sensitivity 59.7–81.7 % and specificity 88.1–97.7 %, depending on the criteria used in different studies [4]. As to US, the sensitivity of CT in detection of low-grade steatosis is lower compared to that of moderate- to severe-grade steatosis [25, 26]. Image quality and accuracy of HU values can be limited in morbidly obese patients or those who are unable to comply with breath-hold instructions.

The main advantage of CT for steatosis evaluation is its wide availability, moderate cost and inherently standardized and quantitative nature of CT density as a steatosis surrogate. CT is less prone to anatomical constraints than US allowing complete visualization of the entire liver within a short breath-hold. Further, CT is largely operator-/equipment-independent. However, evaluation of steatosis using CT density can be confounded by other factors that may affect the X-ray attenuation of the liver. Hounsfield unit calibration may vary across vendors and also is dependent on acquisition parameters [27]. Accumulation of materials including water (edema), collagen (fibrosis), glycogen (glycogen storage disease), iron (hemochromatosis and hemosiderosis) [28], copper (Wilson’s disease), and iodine

(intravenous CT contrast agent, certain drugs) alter the liver parenchymal attenuation and confound steatosis evaluation.

In particular, iodinated contrast agents, which are used in the majority of abdominal CT examinations, complicate the diagnosis of steatosis. The CT density of the liver, as well as that of the reference structures, changes dynamically as the injected contrast circulates and distributes in the body. This change is dependent on many additional factors including iodine concentration of the administered contrast agent, volume and rate of injection as well as timing of the CT image acquisition. Correlations of HUs of the liver and reference structures on contrast-enhanced CT have been shown to be lower compared to unenhanced CT [24, 29, 30]. Therefore, unenhanced CT currently remains the preferred technique for steatosis evaluation. Dual-energy CT with material-specific decomposition is an emerging technology, which in principle allows reconstruction of an iodine-subtracted image from the enhanced CT image, i.e., a “virtual unenhanced CT.” However, early validation studies have shown conflicting results, some promising [31] and others not [32]. This technique remains to be further validated in future studies with larger patient population.

Finally, the use of ionizing radiation must also be considered carefully, particularly in radiosensitive patients such as pregnant women, children, and young adults. Given the availability of other modalities with a higher safety profile (US and MRI), CT is generally not recommended for the purpose of steatosis evaluation alone. Despite the appeal of quantitative standardized metric, the utility of CT is generally limited to retrospective evaluation in patients undergoing CT for other clinical indications.

Magnetic Resonance Imaging

In a normal liver without steatosis, the observed proton MR signal of the liver parenchyma arises from the mobile water within the tissue. In a steatotic liver with abnormal fat accumulation, however, the observed signal arises from an admixture of the water and triglyceride molecules. The protons (i.e., the hydrogen atoms) in the fat and water molecules resonate at unique and distinct resonance frequencies, aka chemical shift. This property can be exploited to measure the proton signals of fat and water signals separately. Serendipitously, signal from protons in normal fat, such as that bound to cholesterol, sphingolipids, and phospholipids bound in cell membranes are invisible on conventional MRI systems. Thus, only the abnormal accumulation of triglycerides (i.e., steatosis) will be detected by MRI.

Several MR imaging techniques are available, but by far the most widely used in clinical practice is called in-phase (IP) and opposed-phase (OP) imaging, also known as chemical shift imaging or two-point Dixon technique [33, 34]. This technique is universally available on all modern clinical 1.5 and 3 Tesla (T) systems and included in most clinical abdominal MR examinations. In IP–OP imaging, two sets of images are acquired, one such that the water and fat signals are approximately in-phase (the IP image), and the other such that the two signals are opposed in phase (the OP image). Thus, IP image represents the sum of water and fat signals of the liver and OP image their difference (water–fat). In a non-steatotic liver, no fat is present, and therefore, liver signal in OP and IP is nearly the same as only water contributes to the liver signal. With increasing steatosis, the liver parenchyma

becomes darker on the OP images. If the IP–OP acquisition is T1 weighted, the liver signal in the IP image will also increase, because the T1 of fat is shorter than the T1 of water. The IP–OP signal difference is often visually assessed for steatosis diagnosis (Fig. 3).

The IP–OP images also provide an opportunity for crude numerical evaluation; the so-called liver signal fat fraction (FF) is commonly used and defined as the proportion of the fat signal divided by the total (water + fat) signal, $FF = \text{fat signal} / (\text{fat signal} + \text{water signal})$. It is a continuous-scale metric ranging from 0 % (all water, no fat) to 100 % (no water, all fat). Because IP and OP signals are arithmetic sum and differences of fat and water signals, liver signal FF can also be calculated using a formula: $FF = (IP - OP) / 2 \times IP$. By calculating signal FF values pixel by pixel, a cross-sectional FF map of the liver can be reconstructed. The sensitivity and specificity of MRI have been reported to be good to excellent even for detection to mild-grade steatosis with 63.7–92.2 and 81.0–94.9 %, respectively, but were somewhat variable across studies [35–37].

This variability in diagnostic performance, for the most part, is attributable to lack of technical standardization (e.g., choice of imaging parameters, field strength) and coexisting confounding biological factors (e.g., fibrosis/cirrhosis and iron overload) that modulates the relative signal strengths (but not the resonance frequencies) of the fat and water protons. For these reasons, the signal FF calculated from conventional IP–OP MRI remains crude and semiquantitative at best. New quantitative MRI techniques, described later in this article, address these shortcomings and provide a standardized imaging biomarker of steatosis that is reproducible across protocols, platforms, vendors, and field strength.

The main advantage of MRI is its direct chemical specificity to triglycerides, accumulation of which is the histopathological signature of steatosis. This is in contradistinction to both US and CT whose imaging features correlate only empirically with steatosis. Thus far, MR is the only imaging modality shown repeatedly to have high sensitivity and specificity to detect mild-grade steatosis. The ability to detect mild-grade steatosis may be important as the severity of steatosis may in fact decrease with progression of NASH [38]. Furthermore, a recent study showed that the clinically relevant threshold for adolescent girls is about 3 % [39]. MRI does not use ionizing radiation and considered safe in all ages including children and young adults. The main disadvantage of MRI remains to be the relatively high cost. Access to MRI, previously thought to be a major limiting factor, is now less of an issue in developed countries, as most modern imaging centers are now equipped with high-field MRI (1.5 or 3 T) capable of IP–OP imaging. Another shortcoming of the IP–OP imaging is that the calculated signal FF values have a limited dynamic range of FF 0–50 %; in markedly severe steatosis, FF could exceed 50 %, but this situation is thought to be rare. Some histological features of steatosis cannot be evaluated by MRI due to the inherently different spatial scale of imaging and microscopy. These include the distinction between microvesicular versus macrovesicular steatosis (i.e., the droplet size) and zonality of the fat droplet distribution.

Multi-parametric Quantitative MRI

Due to inherent procedural risk and non-negligible cost, the utility of liver biopsy for comprehensive evaluation of NAFLD is limited to those who meet criteria in regard to both medical necessity and safety [40, 41]. Ethical considerations also narrow the scope of clinical trial design and target study population. Perhaps even more importantly, biopsy's inherent sampling variability (and its repeatability implications) limits its utility for grading accuracy and longitudinal monitoring [42, 43]. This is a fundamental limitation of taking a small sample (1/50,000th) of an organ with a microscopically heterogeneous disease. For these reasons, the development and validation of noninvasive quantitative biomarkers of NAFLD have become a major research focus in recent years. Desirable features of noninvasive biomarkers include [1] high sensitivity to screen individuals for the need for further invasive testing (e.g., biopsy), [2] high specificity to confirm the diagnosis without biopsy, and/or [3] high precision (low variability) for longitudinal monitoring of disease progression or therapy response assessment. Currently, multi-parametric quantitative MRI offers the most comprehensive set of NAFLD biomarkers for clinical care and research, not only allowing objective quantification of fat, but also iron and fibrosis, in a single examination—i.e., a *virtual liver biopsy*. In the remainder of this article, we will review the basic concepts and emerging validation data of MR biomarkers of NAFLD.

FAT: Proton Density Fat Fraction

Steatosis is the histomorphological manifestation of intracellular accumulation of fat in the form of vacuoles, or “droplets” within hepatocytes. Because accumulated fat is stored as triglycerides, the degree of steatosis is best measured by the molecular quantity of triglyceride. Proton density fat fraction (PDFF), defined as the fraction of mobile protons (H^1) belonging to triglyceride relative to those to water, has been proposed as a standardized MR biomarker of steatosis. By design, PDFF is an unconfounded and universal measure of triglyceride concentration, overcoming previously described flaws in conventional US, CT, and MRI. The use of a *fraction* is necessary, in order to normalize the fat signal, relative to the total water and fat signals. The absolute water and fat signals from the MR system are scaled by multiple factors including amplifier gain and receive coil sensitivity.

Specialized MRI techniques have been developed to measure PDFF (Fig. 4), which include both magnitude- and complex data-based algorithms on 1.5 and 3 T systems. While technical details vary, the common strategy is to acquire imaging data at multiple different echo times (TEs) and perform time-domain analysis (curve fitting) to estimate the signals originating from triglyceride and water. If confounding biophysical factors (T1-bias and T2*-decay, multiple resonance frequency components of triglyceride) as well as technical factors (noise bias, eddy currents, and field strengths) are minimized or corrected, the signals from triglycerides and water directly reflect the concentration of protons in these two species. A detailed discussion of how each confounder can be addressed is beyond the scope of this article but reviewed elsewhere [44, 45].

A closely related non-imaging technique is proton MR spectroscopy (MRS), an *in vivo* extension of nuclear magnetic resonance (NMR) that is commonly used in analytical chemistry. Spatially localized NMR spectra are measured within a small volume of the liver

(usually 1–8 cm³) at progressively longer echo times. Frequency-domain analysis of the MRS data determines the relative strength of fat and water signals. Taking similar approaches as imaging to account for confounding effects of T1 and T2 relaxation, known multiple resonance frequencies of triglyceride, and *j*-coupling, PDFF can also be determined from the frequency-domain data. The technical details of MRS acquisition and analyses are also beyond the scope of this review, but can be found elsewhere [46]. MRS has long been considered the reference standard for in vivo tissue fat quantification [47] and therefore is often used to validate MRI techniques. Liver fat quantification using MRS, however, requires a skilled technologist or physicist to set up and acquire data, and a dedicated spectral analysis software to analyze data. Single-voxel MRS is also limited in its sampling volume [48], whereas MRI can cover the entire liver in a single breath-hold. Therefore, for the purpose of PDFF measurement, MRI is generally more practical than MRS in most clinical settings [49].

There is a wealth of validation data to qualify PDFF as a biomarker of hepatic steatosis. PDFF measured by MRI and MRS has been confirmed to be equivalent and interchangeable in the NAFLD population [50–52]. It agrees closely to known and biochemically measured triglyceride concentrations in phantoms [53, 54] and in human liver samples [55]. It is also highly correlated with tissue histological grades in animal models of NAFLD [56, 57], as well as human subjects with NAFLD [58–60]. Proposed PDFF intervals for each histological steatosis grade in NAFLD are: grade 0 (normal) [0–6.4 %], grade 1 (mild) [6.5–17.4 %], grade 2 (moderate) [17.5–22.1 %], and grade 3 (severe) [22.2 % or greater] [58, 61]. PDFF is highly specific to steatosis and not confounded by other histological or clinical parameters, including age, sex, BMI, lobular inflammation, hepatocyte ballooning, or fibrosis [62]. In the MR research community, PDFF is now generally accepted as the reference standard for liver fat [63] and is now commercially available worldwide from major MR manufacturers, marketed under product names of IDEAL IQ (GE), mDixon-Quant (Philips), and Multiecho VIBE Dixon (Siemens), respectively. These techniques are approved by US Food and Drug Administration (FDA) and are ready for clinical use in the USA.

The practicality, reliability, and transferability of PDFF MRI have been shown repeatedly in independent clinical studies of adults and children worldwide across multiple scanner platforms and manufacturers [64–67]. These are important features of a biomarker to facilitate generalization of results from single-site studies, as well as combining data from multiple sites in multicenter studies or in meta-analyses. Compared to histopathological analysis, a distinct advantage of PDFF as an outcome metric in longitudinal studies is the ability to measure objectively the changes on a continuous scale in each subject (i.e., $\pm x$ % in absolute PDFF), which is superior to histological grading using subjective assignment of a discrete severity bracket [55]. The overall reproducibility (i.e., between-examination measurement 95 % confidence interval) of PDFF has been reported ± 1.8 %, implying a change in PDFF exceeding this threshold can be considered a real effect [68].

The use of PDFF as a NAFLD biomarker in clinical trials was first introduced in 2013 [69, 70]. Thereafter, PDFF has been used in prospective epidemiological and observational studies in patient populations in which liver biopsy is traditionally not indicated, including

the type II diabetic population in primary care setting [71] as well as in healthy children and young adults [39, 66, 72]. As the primary end-point in a randomized clinical trial for steatosis-reducing pharmacotherapy, PDFF has proven to be useful as an alternative to biopsy [69, 73, 74] as well as for cross-sectional observational studies [75, 76].

These cumulative data provide a strong foundation of support PDFF as a noninvasive biomarker of steatosis. In many cases, it may be used as an initial screening tool to determine the need for further workup, which may include liver biopsy. High sensitivity of PDFF for low-grade steatosis is advantageous, as other histological features of NAFLD, such as inflammation and fibrosis, do not necessarily follow the steatosis grade [38]. The main disadvantage of PDFF MRI is cost and access—PDFF pulse sequences may not be available in all imaging centers, although it is being rapidly adopted and is expected to be widely available in the next 1–3 years. In those undergoing MRI for clinical care, the incremental cost of adding an additional single breath-hold PDFF sequence is negligible. Some centers are performing a 5- to 10-min “abbreviated” liver MRI examinations with fat-quantification sequences only, at a reduced cost less than that of abdominal US. Other steatosis quantification techniques (e.g., ultrasound CAP and material-specific CT reconstruction) aimed at lower cost and easier access are in development; these techniques can now be validated against a noninvasive reference standard, PDFF, rather than invasive and semi-quantitative histology, making these validation studies safer, less expensive, and feasible [77, 78].

IRON: T2*

Accumulation of iron in the liver, referred to as iron overload or siderosis, is a well-known cause of chronic hepatocellular injury and can occur in a variety of liver diseases including NAFLD [79, 80]. Whether iron contributes to a more rapid disease progression in NAFLD/NASH remains controversial [81–85]. Many previous studies were limited by relatively small sample size and/or lack of quantitative assessment of liver iron concentration [86]. Because of a large number of potential covariates and confounders (e.g., age, sex, BMI ethnicity, etiology of iron overload), a large-scale well-powered clinical study with biopsy-determined liver iron has been difficult to conduct. Noninvasive quantitative imaging is therefore an appealing alternative for the evaluation of liver iron in NAFLD.

Several MRI techniques for liver iron quantification have been proposed. These include transverse relaxometry techniques based on spin-echo and gradient-echo sequences. MR images are acquired at progressively longer echo times (TE), and the rate of time-dependent signal decay (or transverse relaxation) at each pixel location is mathematically estimated based on an exponential signal decay model. This generates a cross-sectional map of T2 or T2* relaxation rates, depending on whether spin- or gradient-echo acquisitions are used. Iron accumulation in the liver causes the MR signal to decay more rapidly in a concentration-dependent manner, and therefore, T2 or T2* values are excellent candidate for liver iron biomarker. Once liver T2 or T2* value is determined, the liver iron concentration (LIC; in mg of elemental iron/g of liver tissue by dry-weight) can be estimated by using biopsy-validated calibration curves for T2-LIC [87] and T2*-LIC [88–91].

A commercially available FDA-approved technique for spin-echo-based T2 relaxometry, known as FerriScan™ (Resonance Health, Claremont, Australia), has been extensively validated for liver iron quantification and now is considered by many as standard of care for the management of genetic hemochromatosis and transfusion hemosiderosis. Translation of this technique to NAFLD has been limited, however, as it does not consider for the confounding effect of the fat on the T2 estimation. On the other hand, gradient-echo-based T2* relaxometry technique is also commercially available as this functionality is already incorporated in the PDFF MRI techniques, as part of the T2* correction algorithms for fat estimation. Simultaneous estimation of PDFF and T2* is achieved by multi-TE acquisition and curve fitting for fat and water signals, as well as T2* decay. As PDFF and T2* estimates are unconfounded by one another, liver fat and iron can be measured independently [92] (Fig. 5). The advantage of gradient-echo-based T2* technique is in contradistinction to the spin-echo-based T2 technique; the former is likely better suited for iron quantification in the setting of NAFLD, though the accuracy of T2*-LIC specifically in the NAFLD population has yet to be validated prospectively. Finally, a major advantage of gradient-echo methods that estimate PDFF and T2* is the short scan times, typically covering the entire liver within a single 20-s breath-hold. The FerriScan method, however, provides limited coverage of the liver and requires 10–20 min of scanning. This has important implications for workflow, cost, and the need for sedation in young children with iron overload.

FIBROSIS: Elasticity

Fibrosis is an important histopathological feature of advanced NAFLD/NASH and a precursor to cirrhosis. Extracellular collagen deposition in fibrosis alters the liver's mechanical property, causing loss of its normal elasticity (or equivalently, increased stiffness), which can be quantified as a mechanical stiffness parameter (shear modulus) using elastography techniques including MR elastography (MRE). We will not cover MRE in this article as detailed review on this topic can be found elsewhere in this special issue.

Summary

Conventional US, CT, and MR imaging are all capable of noninvasive diagnosis of hepatic steatosis, the histopathological hallmark of NAFLD. However, their utility as a clinical and research tool has been limited due to concerns of inadequate sensitivity/specificity (US, CT), lack of objectivity (US, MR), radiation safety (CT), and various confounding factors (US, CT, MRI). To overcome these limitations, standardized quantitative MR biomarkers have been introduced, including proton density fat fraction (PDFF) for liver fat, T2* for iron, and stiffness for fibrosis. With the availability of multi-parametric quantitative MRI for hepatic fat, iron, and fibrosis, one-stop *virtual liver biopsy* has now become a clinical reality [93–97]. While these noninvasive biomarkers do not obviate the need for liver biopsy, it may help avoid unnecessary biopsies by screening prior to invasive testing, or acting as a surrogate biomarker. A common strategy used in some institutions is to perform biopsy and quantitative MRI at the initial patient presentation and then use MRI to follow patients during the course of therapy. Noninvasive imaging biomarkers are also ideally suited as surrogate biomarkers for drug development trials where repeated accurate measures of disease features such as steatosis are needed. Reproducibility and transferability of these

imaging biomarkers are critical for future research, as they permit study designs not previously feasible, such as epidemiological and longitudinal studies, studies in children and healthy volunteers, as well as multicenter studies and meta-analyses. While these MR biomarkers are new and have only been commercially available in the last 4 years, their utilization is rapidly growing in NAFLD research trials and therefore likely to impact clinical care in the coming decades as the NAFLD epidemic continues worldwide.

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Key Points

- Conventional US, CT, or MR imaging allows subjective and qualitative evaluation of hepatic steatosis in patients with or suspected NAFLD. However, their utility for objective and quantitative steatosis evaluation has been limited due to a variety of reasons.
- Multi-parametric quantitative MRI offers the most comprehensive set of NAFLD biomarkers, allowing objective and quantitative evaluation of liver fat, iron, and fibrosis in a single examination—a *virtual liver biopsy*.
- These MR biomarkers have been validated and are commercially available on standard MR scanners. Their utilization is rapidly growing in research trials and will likely impact clinical care in the coming decades.

Fig. 1.

Ultrasound of NAFLD—examples. Longitudinal ultrasound images of the right hepatic lobe and right kidney (**a–d**) and transverse images at hepatic venous confluence (**e–h**) in four patients are shown. In normal liver, the liver parenchyma is slightly more echogenic (i.e., *brighter*) than the right kidney (**a**). Posterior structures are well seen, including diaphragm (**e**). In the steatotic liver, the parenchyma becomes increasingly more echogenic than the kidney (**b–d**) and deep structures, including the diaphragm (*arrow*), which become progressively blurred (**f–h**)

Fig. 2.

CT of NAFLD—examples. Axial CT images of the liver in four patients at the level of spleen are shown. In normal liver, the parenchyma is approximately 60 ± 10 Hounsfield unit (HU) in CT density and appears brighter than the spleen and the blood vessels (*arrow*). As liver becomes steatotic, the CT density of the liver becomes closer to that of fat (approx. -90 HU). In mild steatosis, the liver may be isodense to the spleen and the blood (**b**). In moderate and severe steatosis, the parenchyma is less dense than the spleen and blood. Typical area of fat sparing is often seen in the periportal regions (**c**, **d**), near the gallbladder fossa and adjacent to the fissure for the ligamentum teres

Fig. 3.

T1-weighted in- and opposed-phase MRI of NAFLD—examples. Axial in-phase (IP) and opposed-phase (OP) T1-weighted MR images of the liver in four patients are shown. In normal liver (**a**, **e**), the liver is brighter than spleen on both T1-weighted IP–OP images. The liver signal on the IP image is similar or slightly higher in signal than the OP image (due to the earlier echo time of OP imaging). In steatotic liver, the signal of triglycerides opposes (i.e., cancels) the liver's water signal, causing net signal reduction (*darker*) on the OP images. The differences in the liver signal between the IP and OP images correlate to the severity of steatosis. *Arrows* blood vessels, *s* spleen

Fig. 4.

MRI PDFF mapping in NAFLD—examples. Axial PDFF images of the liver in four patients generated using Philips mDixon-Quant pulse sequence at 3 T. Average PDFF values of the right lobe are displayed. In normal liver (**a**), PDFF values are <6 % everywhere in the liver. In steatotic livers (**b–d**), the liver's PDFF values increase diffusely and predict borderline–mild (**b**), mild (**c**), and moderate (**d**) steatosis grade at histology

Fig. 5.

Simultaneous liver fat and iron quantification—examples. Simultaneous liver fat and iron quantification. A 24-year-old male patient with elevated transaminase, clinically suspected NAFLD (**a, d**) demonstrating normal liver FF and R2* values [and corresponding liver iron concentration (LIC) of 0.04 mg Fe/g] using Siemens VIBE q-Dixon pulse sequence. A 44-year-old female, also with elevated liver enzymes and obesity (**b, e**), demonstrating elevated liver FF but normal R2* (LIC = 0.13 mg Fe/g) using GE IDEAL IQ pulse sequence, consistent with moderate steatosis without iron overload. A 58-year-old male with elevated ferritin, diabetes, and obesity (**c, f**) demonstrating abnormal liver FF and R2* values (LIC = 3.9 mg Fe/g) using Philips mDixon-Quant sequence, compatible with mild–moderate steatosis and mild iron overload. R2*-LIC calibration based on Wood et al. [88]