Editorial

MR quantitative biomarkers of non-alcoholic fatty liver disease: technical evolutions and future trends

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis as the earliest manifestation and hallmark, and ranges from benign fatty liver to non-alcoholic steatohepatitis (NASH). Liver biopsy (LB) is considered the reference standard for NAFLD diagnosis, grading and characterization, but it is limited by its invasiveness and observer-dependence. Among imaging surrogates for the assessment of hepatic steatosis, MR is the most accurate. ^{1}H MR spectroscopy (MRS) provides a quantitative biomarker of liver fat content (LFC) called proton density fat fraction (PDFF), but it is time-consuming, not widely available and limited in sample size. Several MR imaging (MRI) techniques, in particular fat suppression and in-opposed phase techniques, have been used to quantify hepatic steatosis, mainly estimating LFC from water and fat signal intensities rather than proton densities. Several technical measures have been introduced to minimize the effect of confounding factors, in particular a low flip angle, a multiecho acquisition and a spectral modeling of fat with multipeak reconstruction to address respectively T1 effect, T2* effect, and the multifrequency interference effects of fat protons, allowing to use MRI to estimate LFC based on PDFF. Tang *et al.* evaluated MRI-estimated PDFF, obtained by applying the above-mentioned technical improvements, in the assessment of hepatic steatosis, using histopathology as the reference standard. The identification of PDFF thresholds, even though to be further explored and validated in larger and more diverse cohorts, is useful to identify steatosis categories based on MRI-based steatosis percentages. MRI, with the new refined techniques which provide a robust quantitative biomarker of hepatic steatosis (PDFF) evaluated on the whole liver parenchyma, is a promising non-invasive alternative to LB as the gold standard for steatosis diagnosis and quantification.

Key Words: Non-alcoholic fatty liver disease (NAFLD); Magnetic Resonance Imaging (MRI); proton density fat fraction (PDFF); confounding factors; histopathology; PDFF thresholds

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Non-alcoholic fatty liver disease (NAFLD), with a prevalence of approximately 20-30% in adult population, is increasingly recognized as the most common cause of chronic liver disease in Western Countries, and it is typically associated with obesity, type II diabetes and hyperlipidemia as the most important risk factors (1). The term NAFLD refers to a complex spectrum of conditions ranging from benign fatty liver, to non-alcoholic steatohepatitis (NASH), characterized by necro-inflammation and fibrosis and may further progress into cirrhosis and its complications, including hepatocellular carcinoma (HCC) (1).

The earliest manifestation and hallmark of NAFLD is hepatic steatosis, which is defined as intracellular accumulation of triglycerides in hepatocytes. Since an early diagnosis of NAFLD is recommended (2), patients with liver enzymes abnormalities in serum or sonographic findings of increased hepatic echogenicity, particularly in presence of metabolic risk factors, are directed to

liver biopsy (LB). Histopathology is considered the reference standard for NAFLD diagnosis, grading and characterization, being capable to identify intracellular triglyceride accumulation, classify the degree of steatosis into semi-quantitative categories and distinguish among the different conditions which constitute NAFLD spectrum (3). However, LB has several drawbacks, mostly including its observer-dependence and, of course, its invasiveness, which limits its usefulness especially in the follow-up of NAFLD patients.

For these reasons, imaging surrogates for the assessment of hepatic steatosis have been investigated, not only for early detection of disease, but also to reliably quantify the severity of disease, using histology as the reference standard. Among non-invasive imaging techniques, MR is the most accurate for NAFLD diagnosis and grading, both with respect to MR spectroscopy (MRS) and MR imaging (MRI) (4).

^{1}H MRS allows the *in-vivo* study of liver molecular composition, usually showing the two dominant peaks of water and methylene protons of fatty acids, thus allowing to calculate the proportion of hepatic proton density which is attributable to fat. This MR quantitative biomarker of liver fat content (LFC) is called proton density fat fraction (PDFF) (5). Unfortunately, ^{1}H MRS is time-consuming and not available on all clinical scanners, remaining mainly a research tool radiologists are not used to work with. Furthermore, single voxel-MRS has the same limitation of LB when considering the limited sample size.

Several MRI techniques have been used for the assessment of hepatic steatosis, all with the objective to obtain water and fat separation. Fat suppression techniques, based on the use of selective saturation or excitation pulses, or on short-tau inversion recovery (STIR) imaging, have been used since the 1980s. T2-weighted fast spin-echo MRI with and without fat suppression have shown controversial results in the quantification of LFC calculated as the percentage of relative signal intensity loss of the liver on fat-saturated images, usually using spleen as the tissue of reference (6,7). These techniques are limited by their sensitivity to B0 and B1 inhomogeneities or by reduced signal to noise ratio. Besides, they are affected by low scan time efficiency (8).

In-opposed-phase MRI techniques rely on the water/fat chemical shift difference to achieve water and fat separation by using different TEs (echo times). Since the first of these methods was introduced by Dixon in 1984 (9), many researchers have made modifications to the Dixon method to decrease its sensitivity to magnetic field inhomogeneity, e.g., with the "three-point" method proposed by Glover *et al.* (10), and to reduce scan time, particularly with the introduction of fast Gradient Echo techniques (11). Opposed-phase liver signal intensity loss, obtained by using T1-weighted dual-phase gradient-echo sequence, has been largely investigated as a biomarker of hepatic steatosis, showing quite accurate results (6,7,12).

However, with these MRI techniques, LFC is still calculated as a "fat signal fraction" from water and fat signal intensities rather than proton densities. Some technical measures have been introduced to increase MRI performance and minimize the effect of confounding factors which affect fat quantification when using in-opposed phase techniques. First, the optimal signal to noise ratio has been achieved by applying a combined gradient-echo multipoint water-fat separation known as "iterative decomposition of water and fat with echo asymmetry and least squares estimation" (IDEAL) (13). Subsequently, the use of a low flip angle has been introduced to address the effect of T1 (14), and furthermore a multiecho gradient-recalled-echo MRI has been proposed, with the multiecho acquisition allowing the estimation and the correction of T2* effect (15,16). The recent introduction of this technique is particularly remarkable because it allows to overcome the potential pitfall, typical of dual-phase imaging, due to the well known effect of iron overload on T2* decay (17). Indeed, T2* estimation can also be used as a simultaneous biomarker of hepatic iron overload, which has been reported to occur in patients with NAFLD (18). Finally, to improve separation of water and fat by addressing multifrequency interference effects of fat protons, some authors have incorporated spectral modeling of fat with multipeak reconstruction, assuming that the multiple, typically six, resonance frequencies and relative amplitudes of fat are known *a priori*, based on spectroscopy-derived measurements (18,19).

By using all of these technical precautions to address confounding factors, "fat signal fraction" turns to be equivalent to PDFF, and MRI can finally be used to estimate LFC based on proton densities. In fact, some studies demonstrated a high agreement between T1-independent T2*-corrected MRI with spectral modeling of fat and MRS (18). Therefore, it has to be further stressed that, based on the kind of technique being used, MR biomarkers of LFC reflect different physical entities, basically obtaining fat fractions which can be calculated from signal intensity or proton densities.

Tang *et al.* (20) evaluated the diagnostic performance of MRI-estimated PDFF, obtained by applying a low flip angle, multiple TEs and multipeak fat spectral modeling, in the assessment of hepatic steatosis in a relatively large population of patients with NAFLD. A remarkable aspect of their work is that, while most prior study on this topic relied on fat-water phantoms or MRS, Tang *et al.* used histopathology as the reference standard, since it is the current clinical and research gold standard for steatosis. Furthermore, performing pathological analyses allowed them to evaluate the relationship between several concomitant histological confounding factors and imaging PDFF.

One of the main elements of novelty of this study is the identification of MRI thresholds based on PDFF to classify patients into different steatosis categories, with histopathological grades as the reference standard. A few other studies indicated threshold values, but they were obtained using simpler MRI techniques (12,21). Noticeably, the obtained cut-off PDFF value to differentiate normal from abnormal fat fraction was similar, even though slightly higher, to that reported by a previous study which used MRS (22). It is well known that histological and MRI estimated steatosis percentages are not directly comparable because they refer to different entities, namely fat-containing hepatocytes and mobile fat protons. Therefore, since clinicians are used to histopathological grades, the attempt to find MRI fat fraction thresholds addresses the need to give clinical relevance to the estimated PDFF resulting from MRI examinations.

However, as acknowledged by the authors, the obtained thresholds are to be further explored and validated in larger and more diverse cohorts. In this context, it should be noted that the large majority of the studied patients were children. Moreover, PDFF thresholds could have been influenced by the use of different scanners throughout the study, since a slight discrepancy in PDFF estimates with the 1.5 and 3 T scanners has been previously reported (23). Finally, in NAFLD/NASH patients, a large variability of liver metabolites and consequently of the fat spectrum is to be expected. For this reason, it would be interesting to further analyze PDFF thresholds by introducing a spectrum self-calibration algorithm, as proposed by Yu *et al.* (19), rather than relying on a fat spectrum known *a priori*, to reduce the sensitivity to spectrum variation.

Regardless the identification of PDFF thresholds could be clinically important to obtain MRI steatosis categories, it should also be said that one of the major advantages of MR lies in providing a quantitative biomarker for steatosis assessment, i.e., PDFF, rather than giving a semi-quantitative grading as does histopathology. MR can objectively and reliably measure LFC changes which are much slighter than those assessable with histology, and with no real need for categories. In other words, if the purpose of MR steatosis measurement is to diagnose NAFLD, the use of categories can be useful and appropriate. If MR is employed during patient follow-up, with the aim to identify slight changes in LFC, the use of such wide categories can be less convenient respect to the exact steatosis percentage.

Because of the invasiveness, the observer-dependence and the semi-quantitative grading, the role of LB as a gold standard for steatosis assessment has been questioned before (24). Besides, many researchers used MRS as the reference standard for the validation of MRI techniques, or even in longitudinal clinical studies (4) and to determine the prevalence of NAFLD (22). With the new refined techniques which can reliably quantify PDFF, also MRI is an appropriate candidate for the replacement of LB in the quantification of hepatic steatosis. Furthermore, when compared to MRS, MRI is much more accessible, less time-consuming, and it allows to non-invasively evaluate the whole liver parenchyma, resulting in a more complete assessment of LFC (25). This is particularly relevant because of the inherent heterogeneity of NAFLD, which limits mostly LB (less than 1/50,000th of the liver available for histological analysis), but also single-voxel MRS, which usually includes about 8-27 g of liver parenchyma in a voxel.

In conclusion, MR, providing a robust quantitative biomarker of hepatic steatosis, is a promising non-invasive alternative to LB as the gold standard for steatosis diagnosis and quantification, especially useful in NAFLD patients who require follow-up examinations or in longitudinal studies. Unfortunately, MRI, as well as MRS, is still limited in the characterization of NAFLD, being unable to distinguish between fatty liver and NASH up to now. Research is going on in this field, with several promising techniques addressing the need to identify and grade necro-inflammatory activity and fibrosis. Indeed, to finally allow the replacement of LB in the comprehensive assessment of NAFLD, imaging biomarkers of NASH are still needed.

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