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Original article

Magnetic resonance for quantitative assessment of liver steatosis: a new potential tool to monitor antiretroviral-drug-related toxicities

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Background: There is an increasing need for new diagnostic tools to monitor antiretroviral drug-related toxicities. Magnetic resonance (MR) imaging and MR spectroscopy are non-invasive diagnostic methods used in the detection and quantification of liver fat. The aim of this study was to compare sensitivity and specificity of different MR techniques in the quantitative assessment of liver steatosis, using liver biopsy as the reference standard, in patients with and without HIV infection.

Methods: Sequentially evaluated patients with suspected steatosis who were referred for liver biopsy at our tertiary care site were eligible. MR liver fat content (LFC) was estimated by T2-weighted and fat-suppressed T2-weighted spin-echo, dual-phase T1-weighted gradient-echo, multiecho gradient-echo and ¹H spectroscopy. Association between LFC and histological steatosis percentage was calculated by using univariate linear regressions and

Pearson's coefficient. Respective receiver operating characteristic (ROC) curves were used to compare specificity and sensitivity of MR methods in diagnosis (cutoff 5%) and in quantitative evaluation (cutoff 33%) of steatosis.

Results: A total of 28 patients were identified: 12 refused or had contraindications for liver biopsy and 16 had biopsies plus MR. LFC and histological steatosis percentage were strongly associated (fat-suppressed $r=0.86$ [$P<0.001$], dual-phase $r=0.88$ [$P<0.001$], multiecho $r=0.95$ [$P<0.001$] and spectroscopy $r=0.84$ [$P=0.01$]). MR techniques had high sensitivity and specificity in diagnosis and quantitative assessment of steatosis (areas under ROC curves ranging from 0.88 to 0.98).

Conclusions: This pilot study confirms that MR may be a sensitive non-invasive alternative to biopsy for the quantitative assessment of liver fat and a potential end point to monitor antiretroviral-drug-related toxicities.

Introduction

Fatty liver disease (FLD) refers to a spectrum of diseases characterized by accumulation of triglycerides within hepatocytes, ranging from simple steatosis (fatty liver) to steatohepatitis and liver cirrhosis with its complications, including end-stage liver disease and hepatocellular carcinoma [1,2]. FLD is highly prevalent in the general adult population, affecting up to 30% of asymptomatic individuals [3]. This condition is considered the hepatic equivalent of a systemic metabolic disease secondary to alcohol abuse, insulin resistance, drug toxicities and/or viral infections [2,4].

The gold standard for diagnosing FLD and determining hepatic steatosis severity is liver biopsy [4,5]. This procedure has several drawbacks, including small specimen size and sampling variability, invasive nature, risk of infection, bleeding and biliary leakage; reported procedure-related mortality ranges from 0.01% to 0.1% [5–8]. Several diagnostic tools have been developed to overcome these limitations, offering non-invasive diagnostic methods. Ultrasound is a highly operator-dependent screening tool with poor reproducibility in quantification of liver fat [5,9,10]. In addition the

ultrasound threshold for a detectable fat infiltration within liver tissue is 30%, and not sensitive enough to detect mild steatosis [11]. Computed tomography allows diagnosis of hepatic steatosis but it is unreliable for identification of a small fraction of fatty infiltration and it is associated with radiation exposure [5]. New imaging modalities, primarily magnetic resonance (MR) imaging and proton MR spectroscopy are promising methods that are being used for the detection and quantification of fat content in the liver, as they offer relatively high accuracy even for low percentages of fatty infiltration. These tools hold great promise to provide cost-effective, accessible and accurate evaluation of diffuse liver disease [5,9].

In recent years, significant attention has been focused on FLD prevalence and risk factors in HIV-infected patients. The estimated prevalence of FLD in HIV-infected patients ranges from 30% to 72% respectively in patients with non-alcoholic fatty liver disease (NAFLD) and in HIV-HCV-coinfected patients [12–15]. In this setting, FLD results from a complex interplay of host and viral factors such as gender, alcohol abuse, metabolic syndrome, and HBV and HCV coinfections. We also know that HIV medications can promote insulin resistance and have been associated with fatty liver infiltration; moreover, some authors found cumulative exposure to nucleoside reverse transcriptase inhibitors (NRTIs) was independently associated with hepatic steatosis, presumably due to direct mitochondrial toxicity [12,14,16].

The need for FLD detection and quantification by liver biopsy is suggested by expert opinion in special populations at risk for the development of steatohepatitis [2], nevertheless this procedure is seldom performed in the clinical practice of HIV-infected patients without viral coinfection. Only a few studies have examined steatosis using MR in HIV-infected patients and none have simultaneously compared different MR techniques with liver biopsy [17–20]. The aim of this study was to compare sensitivity and specificity of different MR techniques in the quantitative assessment of liver steatosis, using liver biopsy as the reference standard, in a heterogeneous group of patients with and without HIV infection.

Methods

This was a cross-sectional observational pilot study of consecutively evaluated patients having an indication for liver biopsy assessment for steatosis seen at two tertiary level referral centers (Metabolic Clinic, Clinic of Infectious Diseases and Hepatology Outpatient Clinic of University of Modena and Reggio Emilia School of Medicine, Italy) between December 2009 and March 2011. Included patients underwent liver biopsy and MR. Exclusion criteria were contraindications to percutaneous liver biopsy (international normalized ratio

>1.5, platelet count <50,000 cells/ μ l, ongoing anticoagulant therapies, haemoglobin level <9 g/dl and severe comorbidities) and contraindications to execution of MR (severe obesity, pregnancy, pace-maker, metallic implants and claustrophobia). Written informed consent was obtained from all participants. Approval was obtained from the local ethics committee of the province of Modena. This study had no external funding sources.

Clinical data

Demographic characteristics and clinical data, including duration of HIV infection, prior opportunistic diseases (CDC classification) and lifestyle were obtained from medical files. Alcohol use was defined as heavy when >20 g of ethanol per day was consumed. HIV-specific biomarkers collected were CD4⁺ T-cell counts (most recent value), viral load and duration of HIV infection. HBV and HCV serology, as well as relevant comorbidity conditions were recorded. Lipodystrophy was defined using the Multicenter AIDS Cohort Study definition, with anthropometric categorizations of lipoatrophy, fat accumulation and mixed form [21].

MR imaging technique

MR imaging was performed with a 1.5-T superconducting magnet (Achieva, Philips Medical System, Best, the Netherlands) and a Phased Array Sinergy-Body coil (Philips Medical Systems, Best, the Netherlands).

The imaging protocol (total acquisition time was 20 minutes) included the following: T2-weighted fast spin echo with and without fat suppression (repetition time/echo time, 1,931/120 ms, flip angle 90°, section thickness 5 mm, gap 1 mm and matrix 288×256; fat suppression was applied by using selective partial inversion recovery [SPIR] sequence); T1-weighted dual phase gradient-echo (250/2.3 out-of-phase [OP], 250/4.6 in-phase [IP], flip angle 75°, section thickness 6 mm, gap 1 mm and matrix 256×256); multiecho gradient-echo (repetition time 360 ms, 19 serial OP and IP echo times with a time gap of 2.3 ms, flip angle 75°, section thickness 6 mm, gap 1 mm and matrix 256×256); and ¹H MR spectroscopy, single voxel-PRESS (2,000/47 ms, flip angle 90°; voxel dimension 15×15×15 mm, sampling bandwidth 1,000 Hz and 512 data points).

Figure 1 shows examples of analyses used in the four different MR techniques depicting the selected regions of interest (ROIs).

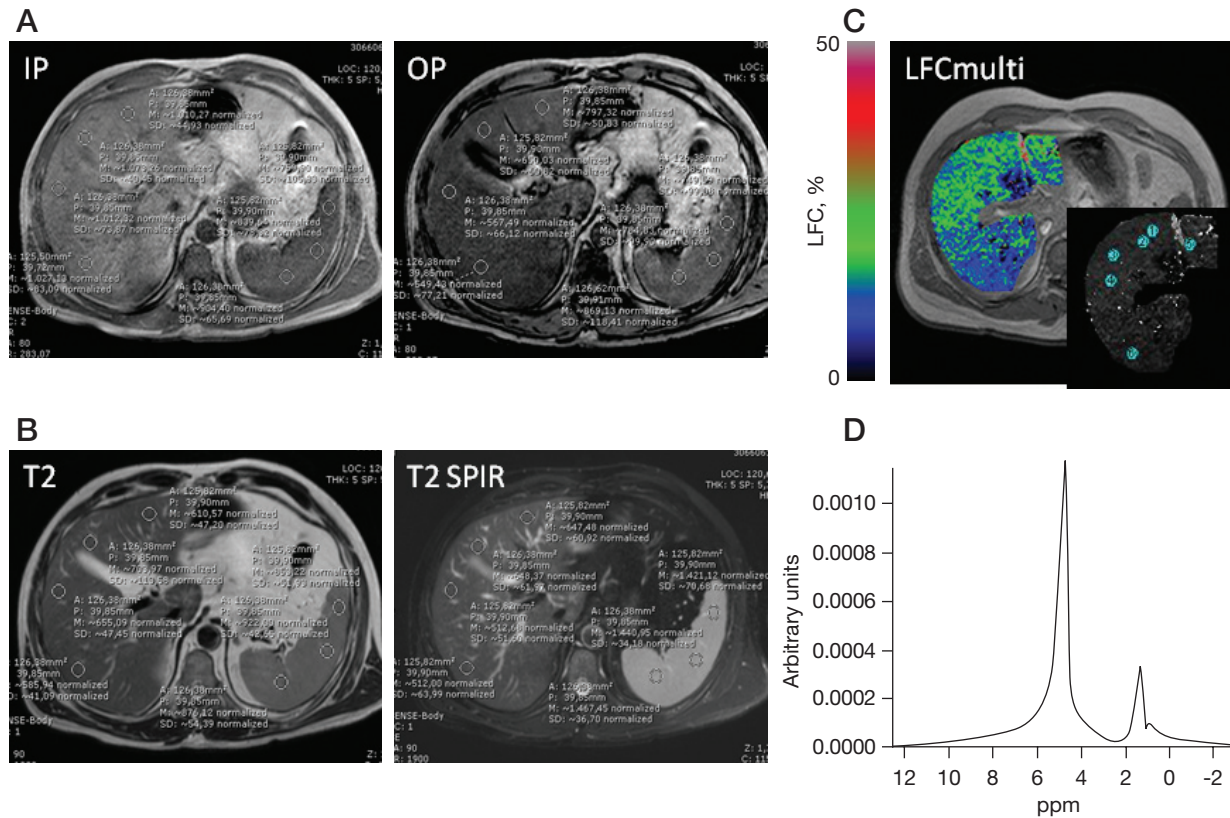
Image interpretation

T2/fat suppressed T2 and dual-phase

Images were reviewed with Synapse (Fujifilm USA Medical Systems, Fujifilm, Tokyo, Japan). The signal intensity (SI) values of liver and spleen were recorded on IP and OP T1-weighted MR images and T2-weighted MR

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Figure 1. Image interpretation



Example of analysis of (A) in-phase (IP) and out-of-phase (OP) images and of (B) T2-weighted with (T2 selective partial inversion recovery [SPIR]) and without (T2) fat suppression images. Four regions of interest in the liver and three in the spleen were selected in each image at the same level in order to calculate mean signal intensity. (C) Example of a multiecho gradient-echo colorimetric map showing the distribution of the liver fat content (LFC) and selection of six regions of interest to calculate a mean value. (D) Example of protonic spectrum of a fatty liver with water and fat peaks.

images with and without fat suppression, according to a method previously described in the literature [22,23] (Figure 1A and 1B). A mean liver SI was calculated on each image from 12 circular (diameter 1–2 cm) ROIs obtained in the liver (three ROIs at each of three levels: above, below and at the level of the porta hepatis) excluding major vessels and artefacts. A mean spleen SI was similarly obtained on each image from nine ROIs. Liver fat content (LFC) was estimated by using fat suppression (LFCsuppr) and was calculated using Equation 1:

$$LFC_{suppr} = \left(\frac{(SI_{liver_{T2}} / SI_{spleen_{T2}}) - (SI_{liver_{T2suppr}} / SI_{spleen_{T2suppr}})}{(SI_{liver_{T2}} / SI_{spleen_{T2}})} \right) \times 100 \quad (1)$$

LFC estimated by using dual phase images (LFCdual) was similarly calculated using Equation 2:

$$LFC_{dual} = \left(\frac{(SI_{liver_{IP}} / SI_{spleen_{IP}}) - (SI_{liver_{OP}} / SI_{spleen_{OP}})}{(SI_{liver_{IP}} / SI_{spleen_{IP}})} \right) \times 100 \quad (2)$$

Multiecho gradient-echo

Images were reviewed with a bespoke application based on the MatLab 7.9 environment (The MathWorks, Inc., Natick, MA, USA). As previously described in the literature [24,25], the SI of the liver in each pixel of the images may be modelled according to Equation 3:

$$|SI| = |SI_w e^{-t/T2^*w} + SI_f e^{-t/T2^*f + i\Delta\omega t}| \quad (3)$$

where SI_w and SI_f are the components of the signal from water and fat compartments, respectively, and $T2^*w$ and $T2^*f$ are their respective decay constants, while t is the time after excitation and $\Delta\omega$ is the difference in frequency between fat and water (3.4 ppm). e is the Neper number and i is the imaginary unit. SI and $T2^*$ of water and fat were calculated with a curve-fitting technique, and LFC was estimated in each pixel using Equation 4:

$$LFC_{multipixel} = (SI_f / [SI_w + SI_f]) \times 100 \quad (4)$$

A colorimetric map was obtained to show LFC distribution in the liver and six ROIs were selected to calculate a mean LFC_{multi} value (Figure 1C).

¹H MR spectroscopy

Images were reviewed with a bespoke application based on the IDL (Exelis Visual Information Solutions, Boulder, CO, USA) environment. LFC estimated by using spectroscopy (LFC_{spectro}) was calculated with the previously described [26] formula shown in Equation 5:

$$\text{LFC}_{\text{spectro}} = (\text{SI}_f / [\text{SI}_w + \text{SI}_f]) \times 100 \quad (5)$$

where SI_f and SI_w indicate the amplitudes of fat and water peaks in the spectrum, respectively (Figure 1D).

Histopathological analysis

Liver tissue was obtained by using percutaneous core biopsy for histopathological analysis and reviewed by a single pathologist unaware of the imaging findings. Liver steatosis severity was determined by estimating the percentage of fat-containing hepatocytes on hematoxylin-eosin-stained specimens, according to standard protocol [27,28].

Statistical analysis

Demographic and HIV-related variables were described as median values.

Study end points were histological steatosis percentage assessed by liver biopsy, which estimates the percentage of hepatocytes showing visible fat droplets in the microscopic view [5] and LFC assessed by dual phase, fat suppression, multiecho and spectroscopy; MR techniques determine the volume fractions of lipids in the liver tissue [5].

These two end points are not directly comparable and quantitative results are not equivalent; therefore, correlations were only explored using univariate linear regression analyses. Pearson's *r* coefficients were calculated. Statistical significance was considered for a *P*-value <0.05.

Areas under receiver operating characteristic (ROC) curves were used to compare specificity and sensitivity of different MR methods. Cutoff values for diagnosis and quantitative evaluation of severity (from mild to moderate steatosis) were respectively 5% and 33% fat at histology, according to previous literature reference values [29].

All statistical analyses and graphics were performed using the statistical software package Stata 10.1 (Intercooled Version for Mac; StataCorp, College Station, TX, USA).

Results

A total of 28 patients with indication for liver biopsy assessment of steatosis were screened. Among them, 12

Table 1. Demographic and HIV-related characteristics

Characteristic	Value
Demographic characteristics	
Patients, <i>n</i>	16
Sex	
Male, <i>n</i> (%)	12 (75)
Female, <i>n</i> (%)	4 (25)
Median age, years (range)	50 (39–63)
HIV-positive, <i>n</i> (%)	11 (69)
HBV-positive, <i>n</i> (%)	2 (12.5)
Anti-HCV antibody-positive, <i>n</i> (%)	6 (37.5)
HCV-RNA-positive, <i>n</i> (%)	5 (31)
<u>HIV-HCV-coinfected</u> , <i>n</i> (%)	5 (31)
Alcohol consumption, <i>n</i> (%)	3 (19)
Diabetes, <i>n</i> (%)	3 (19)
Subclinical diabetes, <i>n</i> (%)	3 (19)
HIV-related characteristics	
Patients, <i>n</i>	11
Suppressed HIV viral load, <i>n</i> (%)	11 (100)
Median CD4 ⁺ T-cell count, cells/μl (range)	750 (114–1,385)
CDC group C, <i>n</i> (%)	2 (18)
Median HIV duration, years (range)	13 (3–23)
Risk group for HIV	
Intravenous drug use, <i>n</i> (%)	4 (36)
Homosexual, <i>n</i> (%)	4 (36)
Heterosexual/other, <i>n</i> (%)	3 (27)
Lipodystrophy	
No lipodystrophy, <i>n</i> (%)	5 (45)
Lipoatrophy, <i>n</i> (%)	1 (9)
Fat accumulation, <i>n</i> (%)	1 (9)
Mixed forms, <i>n</i> (%)	4 (36)

[AU: Should HIV-HCV be changed to reflect that it includes HBV, e.g. HIV-HBV/HCV?]

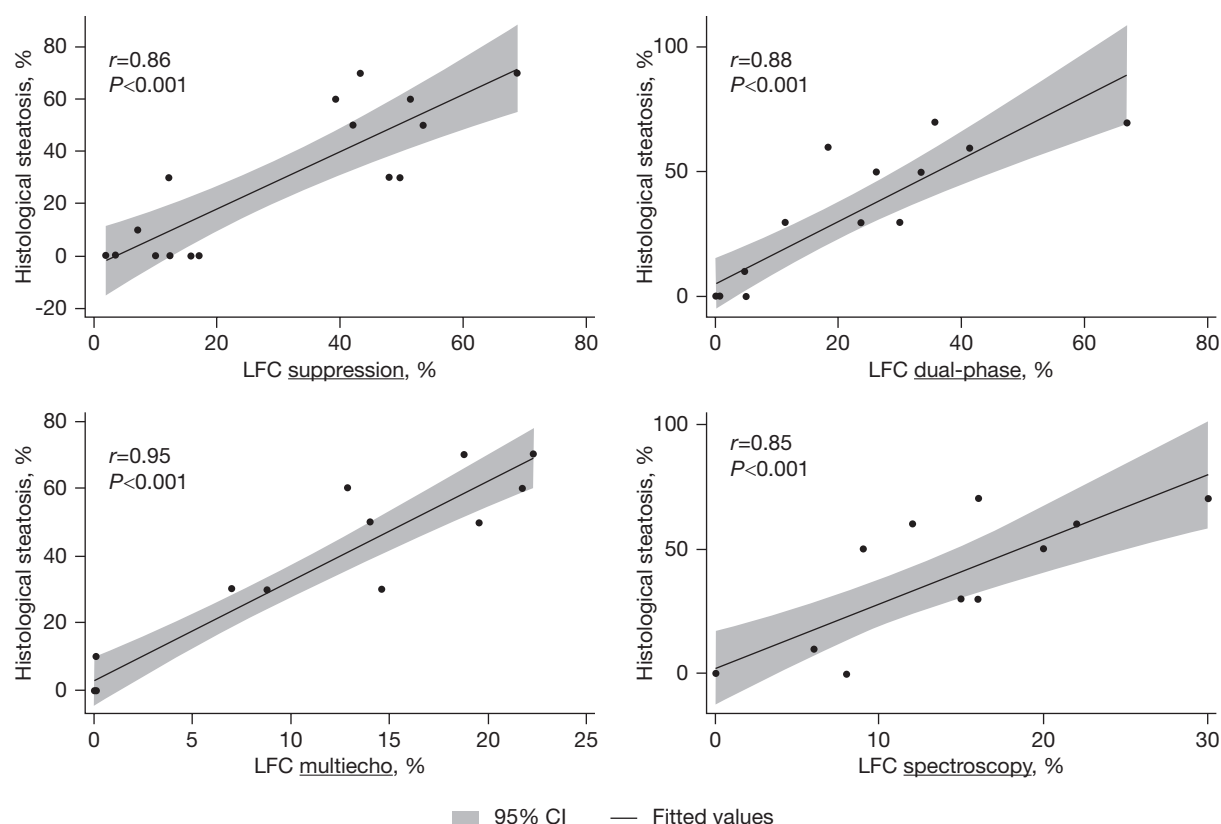
were excluded per protocol: 3 had a contraindication to perform the liver biopsy, 1 had a contraindication to execute an MR procedure and 8 refused consent to liver biopsy. In total, 16 patients were evaluated: 12 (75%) were men and 4 (25%) were women. Median patient age was 50 years (range 39–63).

All included patients had a chronic viral infection. A total of 11 patients were HIV-infected (4 were HIV-HCV-coinfected and 1 HIV-HBV-coinfected), 2 patients were HCV-monoinfected and 1 was HBV-monoinfected. Exotoxic liver damage was present in three patients, and diabetes or impaired fasting glucose in six patients. Demographic and HIV-related characteristics are shown in Table 1.

Median histological steatosis was 30%, ranging from 0% to 70%. Median MR LFC estimated by fat-suppression, dual-phase, multiecho and spectroscopy was, respectively, 28.15% (range 2.0–68.8), 14.8% (range 0–66.9), 7.9% (range 0–22.3) and 10.5% (range 0–30).

Figure 2 depicts univariate linear regressions of the associations between LFC and histological steatosis percentage. A high association was found between histology and the four studied MR techniques:

Figure 2. Univariate linear regressions between magnetic resonance and histology



[AU: x-axis labels changed, ok?]

The regressions show associations between magnetic resonance liver fat content (LFC) calculated with the four different techniques and histological steatosis percentage.

fat-suppression $r=0.86$ ($P<0.001$), dual-phase $r=0.88$ ($P<0.001$), multiecho $r=0.95$ ($P<0.001$) and spectroscopy $r=0.85$ ($P<0.001$).

Sensitivity and specificity of MR techniques in the diagnosis of hepatic steatosis were evaluated using a histological steatosis cutoff $>5\%$. Areas under the ROC curve (AUC) were 0.93 for fat-suppression, 0.97 for multiecho and 0.98 for dual phase and spectroscopy. No statistically significant difference was found between the four techniques ($P=0.70$; Table 2). The specificity and sensitivity of MR techniques in the differentiation between mild and moderate steatosis were evaluated using the histological steatosis cutoff $>33\%$. The AUC ranged from 0.88 (fat-suppression) to 0.96 (multiecho), without a significant difference between the four methods ($P=0.70$; Table 2). The most sensitive MR technique in the quantitative assessment of steatosis, in particular in the differentiation between mild and moderate steatosis, was multiecho; however, the difference between the four methods was not significant ($P=0.70$).

Discussion

This pilot study explored and compared four different MR techniques used for the assessment of FLD. Given the high burden of liver steatosis in HIV-infected patients, the studied population was mainly chosen from HIV-infected patients.

The first result was the detection of a strong association between LFC and histological steatosis percentage. Although these two measures are obtained by tools that do not capture the same biological entity (MR measures the volume fraction of lipids in the liver tissue and histology the percentage of hepatocytes showing visible fat droplets), they appear to be strongly associated.

We also compared sensitivity and specificity of MR techniques in both the diagnosis and evaluation of the severity of liver steatosis. To our knowledge, this is the first study which simultaneously investigated four MR methods (T2-weighted and fat-suppressed T2-weighted spin-echo, dual phase T1-weighted gradient-echo and

Table 2. Areas under the receiver operating characteristic curves for histological steatosis with cutoffs of 5% and 33%

Steatosis cutoff	AUC	P-value
Histological steatosis >5%	–	–
T2/fat-suppressed T2	0.93	0.70
Dual-phase	0.98	–
Multiecho	0.97	–
¹ H MR spectroscopy	0.98	–
Histological steatosis >33%	–	–
T2/fat-suppressed T2	0.88	0.70
Dual-phase	0.94	–
Multiecho	0.96	–
¹ H MR spectroscopy	0.91	–

Areas under the receiver operating characteristic curve (AUC) of different magnetic resonance techniques and respective P-value of the receiver operating characteristic curve with a histological steatosis percentage cutoff of 5% (diagnosis of steatosis) and 33% (differentiation between mild and moderate steatosis) are shown. MR, magnetic resonance.

multiecho gradient-echo and ¹H MR spectroscopy) in a comparative fashion with histology as the reference standard, and the first time in which multiecho was used to assess FLD in HIV-infected patients.

The HIV literature in this field is quite limited. Recently, Ghotb *et al.* [17] demonstrated a strong association between MR spectroscopy and histology in the quantification of steatosis in HIV–HCV-coinfected patients. This methodology still harbours some technical limitations including the calculation of hepatic fat content from a single voxel and the long acquisition time [5,9].

We believe that multiecho gradient-echo MR imaging is the most promising MR technique in the quantitative assessment of steatosis. Not only did this technique show higher sensitivity and specificity when FLD was >33% (although without a significant difference), but multiecho offers additional advantages: it allows for the study of the whole liver parenchyma in a short acquisition time and it performs better than other MR imaging techniques because multiple echo times permit it to estimate T2* effects [25].

A large number of patients with an indication for liver biopsy assessment of steatosis were screening failures (43%) and could not be included. Screening failure was mainly due to contraindication to perform liver biopsy or refusal to undergo invasive procedure. This rate is close to what is observed in the clinics where FLD is seldom definitively diagnosed and quantified and only rarely monitored over time with liver histology. This of course is due to the real or perceived risks associated with this invasive and potentially harmful procedure. The availability of a non-invasive technique may overcome this aversion and allow for a better description of the natural history of NAFLD. The validation of MR

as an alternative to liver biopsy will need appropriate pharmacoeconomic evaluation to justify its use in the clinical practice.

Hepatic fat is considered a source of inflammatory mediators and a receptacle of inflammatory cells, and has been linked with atherosclerosis and complications inherent with this disease [30,31]. Since liver steatosis is now considered a novel component of the metabolic syndrome [32], it is conceivable that hepatic fat content may turn out to be an innovative biological marker for the monitoring of metabolic diseases and prediction of clinical events, such as diabetes and cardiovascular diseases. This is consistent with the finding that carotid artery wall thickness is strongly associated with the histological degree of hepatic steatosis [33].

More than that, the availability of a non-invasive measurement for FLD quantification offers, in HIV-infected patients in particular, a new paradigm in the monitoring of metabolic alterations and drug toxicities. These patients in fact harbour an increased risk of ectopic fat distribution due to lipodystrophy syndrome and a chronic inflammatory status potentially linked to the evolution of steatosis towards steatohepatitis [34,35].

Some limitations of our study should be noted. This is a pilot study with a relatively small number of patients and there is no control group. Given the ethical concerns surrounding liver biopsies, even in patients with a strong indication, it is unlikely that healthy individuals will be undergoing liver biopsy for study purposes.

Second, histological steatosis percentage and LFC measures are not directly comparable for clinical use, therefore mathematical algorithm needs to be developed. At present, the main limitation of MR techniques with respect to liver biopsy evaluation is the lack of information regarding liver fibrosis by non-invasive methods [5,36]. Future advances in MR elastography hopefully will overcome this drawback allowing a new era in prospective evaluation of NAFLD and non-alcoholic steatohepatitis with these techniques [36–38].

In conclusion, our results confirm that MR may be a valid non-invasive alternative to biopsy for the diagnosis and quantification of steatosis that can be used in monitoring FLD. These new diagnostic tools need to be evaluated with greater *casuistry* in HIV patients to better interpret the peculiar metabolic derangements observed in this special population, and investigated as potential end points to assess antiretroviral drug related toxicities.

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AU: Is this correct?

Disclosure statement

The authors declare no competing interests.

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