Research Article

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# Multiparametric magnetic resonance for the non-invasive diagnosis of liver disease

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**Background & Aims**: With the increasing prevalence of liver disease worldwide, there is an urgent clinical need for reliable methods to diagnose and stage liver pathology. Liver biopsy, the current gold standard, is invasive and limited by sampling and observer dependent variability. In this study, we aimed to assess the diagnostic accuracy of a novel magnetic resonance protocol for liver tissue characterisation.

**Methods**: We conducted a prospective study comparing our magnetic resonance technique against liver biopsy. The individual components of the scanning protocol were T1 mapping, proton spectroscopy and T2* mapping, which quantified liver fibrosis, steatosis and haemosiderosis, respectively. Unselected adult patients referred for liver biopsy as part of their routine care were recruited. Scans performed prior to liver biopsy were analysed by physicians blinded to the histology results. The associations between magnetic resonance and histology variables were assessed. Receiver-operating characteristic analyses were also carried out.

**Results**: Paired magnetic resonance and biopsy data were obtained in 79 patients. Magnetic resonance measures correlated strongly with histology ($r_s = 0.68$ $p < 0.0001$ for fibrosis; $r_s = 0.89$ $p < 0.001$ for steatosis; $r_s = -0.69$ $p < 0.0001$ for haemosiderosis). The area under the receiver operating characteristic curve was 0.94, 0.93, and 0.94 for the diagnosis of any degree of fibrosis, steatosis and haemosiderosis respectively.

**Conclusion**: The novel scanning method described here provides high diagnostic accuracy for the assessment of liver fibrosis, steatosis and haemosiderosis and could potentially replace liver biopsy for many indications. This is the first demonstration of a non-invasive test to differentiate early stages of fibrosis from normal liver.



## Introduction

Over 10% of adults in Western populations have some degree of liver disease [1,2]. The increasing prevalence of obesity, alcohol-related liver disease, and viral hepatitis has led to an epidemic of progressive liver disease and cirrhosis.

There is a pressing need for a reliable diagnostic tool to identify early stages of liver disease and to target therapies to those patients who may benefit from these (e.g., antiviral therapy in progressive hepatitis C). Liver biopsy, the current 'gold-standard', carries a significant risk of serious bleeding complications and is costly. Furthermore, a biopsy allows examination of only 0.002% of the liver, and there is great intra- and inter-observer variability in histological interpretation, such that many argue liver biopsy is not a true gold-standard [3]. Consequently, there is a real clinical need for non-invasive tools to evaluate and monitor liver disease.

Transient elastography, which in Europe is increasingly used in clinical practice, can only accurately diagnose cirrhosis and

Keywords: Magnetic resonance T1 mapping; Proton magnetic resonance spectroscopy; Magnetic resonance T2* mapping; Iron corrected T1; Liver fibrosis; Liver steatosis; Liver haemosiderosis.

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*Abbreviations:* MR, Magnetic Resonance; BMI, Body Mass Index; NAFLD, Non-Alcoholic Fatty Liver Disease; CPA, Collagen Proportionate Area; CoV, Coefficient of Variance; $^1$H MRS, Proton Magnetic Resonance Spectroscopy; shMOLLI, shortened Modified Look Locker Inversion; HLC, Hepatic Lipid Content; ROI, Region of interest; ANOVA, Analysis of Variance; AUROC, Area Under the Receiver Operating Characteristic Curve.

has limited utility in obesity [4]. Tests based on serum markers have been mainly studied in preselected populations – when applied to mixed cohorts they were found to lack sensitivity, particularly in differentiating early stages of disease [5].

We present a proof-of-principle study, describing a novel magnetic resonance (MR) protocol that can be performed without intra-venous contrast on existing scanners. MR methods are ideally suited for tissue characterisation as they can sample the entire liver quickly, and are safe, reproducible, and widely available. Using multiparametric MR, we were able to objectively quantify hepatic fibrosis, steatosis and haemosiderosis, an important step towards a safer alternative to liver biopsy.

## Patients and methods

### *Study design and population*

This was a prospective, comparative, non-randomised, study of a new diagnostic MR method to evaluate liver disease. The designated reference standard was histological assessment of liver fibrosis, steatosis and haemosiderosis. From March 2011 to May 2013, we invited all patients referred for liver biopsy at two UK study centres (Oxford and Reading), to take part, except for those with contraindications to MR scanning. 90 patients consented to participate. Two were unable to undergo MR investigation due to claustrophobia, and nine did not have liver biopsy within six months of consent, leaving 79 patients for the final analysis (baseline characteristics in Table 1). The study protocol is shown in Fig. 1. Reference MR data were also collected from seven healthy volunteers with no known liver disease and BMI <25 kg/m$^2$ (Supplementary Table 1).

MR operators were blinded to the indication for liver biopsy and to the patients' clinical details. MR data were analysed prior to histological reporting. The histopathologists were blinded to the MR data. Histological measures of steatosis, fibrosis and haemosiderosis were then compared to the non-invasive MR measures of the same parameters.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the institutional research departments and the National Research Ethics Service (11/H0504/2). The study was registered with clinicaltrials.gov (NCT01543646). All patients and volunteers gave written informed consent.

### *Histological interpretation of liver biopsy samples*

The median biopsy length after processing was 20 mm (IQR 16-30) and each biopsy contained a median of 10 (IQR 8-15) portal tracts. All biopsies were included in the final analysis. As there is considerable inter-observer variation in reporting of liver biopsy [6,7], samples from 65 patients were assessed by three independent expert liver histopathologists (LMW, DWD, & KAF).

All samples were assessed for fibrosis by Ishak stage (F0–F6) [8]. For this study mild fibrosis was defined as Ishak F1–F2, moderate fibrosis as Ishak F3–F4, and severe fibrosis as Ishak ⩾F5. In cases where the biopsy showed steatohepatitis (n = 36), subgroup analysis was performed comparing MR data against the NAFLD Fibrosis Stage (F0–F4) [9]. In 54 biopsy samples available for this, collagen proportionate area (CPA) was also assessed by analysis of digital images using ImageJ (see Supplementary Methods for details).

Hepatic lipid content was measured by determining the percentage of hepatocytes with visible lipid vesicles. This was graded as 0 (<5%), 1 (5- <33%), 2 (33- <66%) and 3 (>66%), as described by Brunt [10].

Stainable iron was estimated using a Perls' histochemical stain and semi-quantified using a five tier grading system (0: no haemosiderosis to 4: severe haemosiderosis) [11].

Inter-observer variability between the three histology measurements for steatosis, fibrosis and haemosiderosis was determined using weighted kappa statistics. The final histology scores for all 79 patients were determined by consensus agreement.

### *Magnetic resonance protocol*

All MR scans were performed in Oxford with the patient lying supine in a 3 Tesla system (Tim Trio, Siemens Healthcare, Germany). Patients attended for their scans having fasted for at least 4 h. The average scan time for this protocol was 23 min. For the assessment of repeatability, T1 and T2* maps were acquired in ten volunteers on two occasions within a week. The mean coefficients of variance (CoV) for T1, T2*, and cT1 (see below) were calculated. $^1$H MRS using this protocol has previously been determined to have a CoV of 4.8% [12]. A more detailed description of the MRI methods and MR acquisition parameters is included in the Supplementary Methods.

### *Fibrosis (extracellular water) imaging*

A T1 relaxation time map was acquired using the shortened Modified Look Locker Inversion (shMOLLI) recovery sequence in a transverse plane of the liver [13]. This sequence samples the T1 recovery curve using single-shot balanced steady state free precession acquisitions. There is a quality assurance component – each acquisition generates an $R^2$ map for the fit of signal intensity to the exponential recovery curve [14]. For this study, an image was only considered for interpretation if the $R^2$ was ⩾99% which was the case in all patients.

### *Hepatic steatosis measurement with $^1$H MRS*

Hepatic lipid content (HLC) can be quantified using localised cardiac-triggered proton spectroscopy [15]. HLC as a percentage of the liver water content using $^1$H MRS was measured with water suppression in an 8 cm$^3$ voxel, avoiding vascular and biliary structures.

### *Iron content imaging*

A multi-gradient-echo acquisition was used to calculate a T2* map of the liver in a single plane with a slice thickness of 3 mm. Two patients had iron overload too severe to be accurately quantified using this protocol, with T2* <2 ms in each case.

### *MR image analysis*

MRI – Data were analysed by physicians blinded to the clinical information, using software tools available on the scanner console. For each patient, a single Region of Interest (ROI) was selected on the transverse T1 and T2* maps, corresponding to segment 8 of the liver, from where most percutaneous biopsies are taken. The tissue volume assessed in each ROI is between 25–30 ml (compared to 0.05–0.08 ml in a liver biopsy). Each ROI contained between 100–200 pixels each, returning a T1 and T2* value for the liver area corresponding to that pixel. Mean T1 and T2* values were recorded for each ROI and used in the final analysis (see also Supplementary Fig. 1).

MRS data were analysed offline, using AMARES in the jMRUI package and customised software running within MATLAB 2010b, as previously described.[15] HLC was expressed as % of water signal.

### *Correction for iron*

The T1 measurements in this work aim to detect the presence of elevated extracellular water, reflected in an increased T1 value. However, the presence of excess iron competes with this effect, reducing T1. Further, the shMOLLI method is affected by T2 and T2*, which are reduced with elevated iron levels. In an interim analysis of the data, we found that a substantial proportion of patients had elevated liver iron levels, and we therefore developed a method to correct for this confounder. Elevated iron concentrations can be determined accurately from the T2* maps. The T1 measurements provide an estimate of the extracellular water content, and thus it is necessary to remove the bias introduced by the elevated iron. To do this, the shMOLLI sequence was modelled using a Bloch simulation for varying extracellular fluid and iron concentrations, and a correction algorithm was generated [16]. This was then used to remove the effects of elevated iron from the T1 measurements, yielding an 'iron-corrected T1' (cT1; the T1 that would be measured using the shMOLLI sequence at a normal iron level; 1.3 mg/g) for the chosen ROI. Iron-corrected T1 could not be estimated in two patients with severe iron overload (T2* <2ms), leaving 77 patients with paired MR and histology data for fibrosis assessment.