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Integrated Quantitative Susceptibility and R_2^* Mapping for Evaluation of Liver Fibrosis – An Ex Vivo Feasibility Study

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Abstract

To develop a method for noninvasive evaluation of liver fibrosis, we investigated the differential sensitivities of QSM and R_2^* , using corrections for the effects of liver iron. Liver fibrosis is characterized by excessive accumulation of collagen and other extracellular matrix proteins. While collagen increases R_2^* relaxation, measures of R_2^* for fibrosis are confounded by liver iron, which may be present in the liver over a wide range of concentrations. The diamagnetic collagen contribution to susceptibility values measured on quantitative susceptibility mapping (QSM) is much less than the contribution of highly paramagnetic iron. In nineteen ex vivo liver explants with and without fibrosis, QSM (χ), R_2^* and proton density fat fraction (PDFF) maps were constructed from multiecho gradient-recalled echo (mGRE) sequence acquisition at 3T. Median parameter values were recorded and differences between the MRI parameters in non-fibrotic vs. advanced fibrotic/cirrhotic samples were evaluated using Mann-Whitney U tests and ROC analyses. Logistic regression with stepwise feature selection was employed to evaluate the utility of combined MRI measurements for detection of fibrosis. Median R_2^* increased in fibrotic vs. non-fibrotic liver samples ($p=0.041$), while differences in χ and PDFF were non-significant ($p=0.545$ and $p=0.395$, respectively). Logistic regression identified the combination of χ and R_2^* significant for fibrosis detection ($\text{logit}(\text{prediction}) = -8.45 + 0.23 R_2^* - 28.8 \chi$). For this classifier, a highly significant difference between non-fibrotic vs. advanced fibrotic/cirrhotic samples was observed ($p=0.002$). The model exhibited an AUC of 0.909 ($p=0.003$) for detection of advanced fibrosis/cirrhosis, which was substantially higher compared to AUC's of the individual parameters (AUC 0.591 – 0.784). An integrated QSM and R_2^* analysis of mGRE 3T imaging data is promising for noninvasive diagnostic assessment of liver fibrosis.

Keywords

quantitative susceptibility mapping; liver fibrosis; magnetic resonance imaging

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Introduction

Liver fibrosis develops in patients with chronic liver disease (CLD), and, if untreated, fibrosis may progress into cirrhosis. Significant risks are associated with liver cirrhosis, including portal hypertension, liver failure, and hepatocellular carcinoma ¹. It is therefore important to accurately diagnose and stage liver fibrosis in CLD patients to intervene in time to prevent progression to advanced fibrosis or cirrhosis ². Liver biopsy is the reference standard for liver fibrosis assessment, but is invasive, costly and prone to sampling errors ³. Therefore, development of noninvasive methods to detect and stage liver fibrosis is urgently needed.

Noninvasive MRI methods have increasingly been studied as tools for evaluation of liver fibrosis including T_1 ⁴ and $T_1\rho$ ⁵ mapping, diffusion-weighted imaging⁶, perfusion imaging⁷ and MR elastography (MRE)⁸. MRE has shown the best performance for diagnosing fibrosis and cirrhosis in CLD patients, with area-under-the curve (AUC) ranging between 0.78-0.998 ⁸. Still, MRE requires external hardware, is available only in a few specialized centers, and is prone to failure in certain patients, including in subjects with severe liver iron deposition and ascites ⁸.

Liver fibrosis is characterized by excessive accumulation of collagen and other extracellular matrix proteins ⁹. Collagen deposition induces diamagnetic susceptibility changes in liver tissue ¹⁰, which potentially could be measured by MRI. A recent study showed promise with susceptibility tensor imaging (STI) for imaging of liver fibrosis ¹¹. Nonetheless, STI is hampered in the clinical setting by unacceptably long acquisition times. Quantitative susceptibility mapping (QSM) ¹², which measures susceptibility in a single orientation, can be performed in a single breath-hold and may thus be a more clinically useful method for liver fibrosis imaging ¹³⁻¹⁵. Recently, a linear negative correlation of susceptibility measurements from QSM with collagen concentration was reported in a phantom study, while R_2^* values were positively associated with collagen ¹⁵. R_2^* and QSM were both also positively associated with iron concentration, which may affect the utility of these MRI parameters for assessment of liver fibrosis, given that iron deposition is often present in the liver. In addition to iron deposition, there are several other histological features of chronic liver disease that may also affect R_2^* measurements for the purpose of fibrosis detection, including edema, inflammation and ballooning¹⁵⁻¹⁷. We hypothesize that an integrated analysis of QSM and R_2^* data may minimize the effects of confounders to provide a suitable tool for liver fibrosis evaluation.

The objective of this study was to explore the differential sensitivities of QSM and R_2^* for noninvasive evaluation of liver fibrosis in ex vivo human liver explant samples.

Materials and Methods

Liver samples

This prospective study was approved by our Institutional Review Board, with waiver of the requirement for informed consent. Between August 2017 and January 2020, samples from liver explants were retrieved by a liver pathologist at our Medical Center. Exclusion criteria

were (1) insufficient liver parenchyma tissue for sampling and (2) cases with positive tumor margins. Sections cut from the whole liver were $\sim 7 \times 5 \times 1.5$ cm with a volume of ~ 50 cm³ and ~ 40 grams of weight. The samples were fixed in formalin, and, after blinding of samples, sent for MRI acquisition and analysis.

MRI acquisition

For MRI acquisition, the formalin-fixed liver explants were embedded together with a water balloon into a cylindrical agarose phantom. Phantoms were scanned at one of the two scanners (3T) depending on availability using a head coil. QSM and R_2^* data were obtained using a 3D multi-echo gradient-recalled echo (mGRE) sequence. Samples with very high iron content ($n=3$) were scanned using an ultrashort echo time gradient echo (UTE) sequence.

The 3D mGRE sequences on two scanners (Magnetom Prisma, Siemens Healthcare, Erlangen, Germany & Discovery MR750, GE Healthcare, Waukesha, WI) were performed with acquisition parameters: number of echoes = 8 & 7, flip angle = 15° & 12° , TE1 = 3.6 & 3.1 msec, TE = 5.7 & 5.4 msec, TR = 49 & 41 msec, reconstructed voxel size = $0.54 \times 0.54 \times 0.8$ & $0.4 \times 0.4 \times 0.7$ mm³, bandwidth = 315 & 244 Hz/pixel, reconstructed matrix = 512×512 . Typical image acquisition times were approximately 25 minutes. UTE 3D GRE sequence parameters on the GE scanner were: number of echoes = 6, flip angle = 10° , TE1 = 0.03 msec, TE = 1 msec, TR = 12 msec, reconstructed voxel size = $1 \times 1 \times 1$ mm³. Typical image acquisition times were approximately 15 minutes.

MRI analysis

MRI analysis was performed in MATLAB (version R2019b, MathWorks, Natick, MA, USA). An initial estimate for field f was generated from the complex mGRE signal $S(t)$ with N echoes by Simultaneous Phase Unwrapping and Removal of Chemical Shift (SPURS)¹⁸. The T_2^* -IDEAL algorithm¹⁹, was solved to generate water (W), fat (F), R_2^* and field maps f in a single-peak fat (ν_F , fat chemical shift) model:

$$E(W, F, f, R_2^*) = \underset{W, F, f, R_2^*}{\operatorname{argmin}} \sum_{j=1}^N \|S(t_j) - e^{-R_2^* t_j} e^{-i2\pi f t_j} (W + F e^{-i2\pi \nu_F t_j})\|_2^2. \quad [1]$$

Background field removal was performed using the projection-onto-dipole-field (PDF) method²⁰. The local field map was processed to generate a susceptibility map (χ) using the morphology enable dipole inversion algorithm (MEDI)²¹⁻²³. Susceptibility values were normalized to the average susceptibility value in the water ($\chi=0$ ppm) balloon²². Proton density fat fraction (PDFF) images were created from the calculated fat and water images²⁴. The susceptibility maps were corrected for fat using the PDFF maps and assuming pure fat susceptibility = 0.65 ppm²⁵, as described previously¹⁵.

Volumes of interest (VOIs) in the liver samples were automatically generated using a combination of threshold-based segmentation and connected component analysis. Specifically, a binary mask was created by using a threshold of $R_2^* > 15$ s⁻¹, which removes

the background of agarose gel and air. Next, a connected component analysis was employed to select the largest component in the resulting mask, corresponding to the liver sample. The VOI mask was eroded around large blood vessels to avoid false-positive fibrosis findings due to collagen present in blood vessel walls²⁶. Median R_2^* , PDFF and χ values in the liver samples were recorded.

Histopathological analysis

A sample adjacent to the scanned liver sample was embedded for histopathological analysis. Hematoxylin and eosin (H&E), Masson's trichrome and Prussian Blue stains were performed for histology, fibrosis and iron evaluation, respectively. A liver pathologist evaluated the sections and assigned scores for fibrosis, iron and fat using standard clinical scoring systems²⁷⁻²⁹.

Statistical analysis

Differences in median R_2^* , PDFF and χ between non-fibrotic and fibrotic/cirrhotic liver samples were evaluated using Mann-Whitney U tests. Demographic and pathologic characteristics were also compared between the groups using Mann-Whitney U or chi-squared tests dependent on the variable type. Correlation analysis of the MRI measurements with age was performed using Spearman tests. Logistic regression analysis with stepwise feature selection (input variables R_2^* , χ and PDFF) was performed to evaluate the potential value of combined parameters for fibrosis detection. Logistic regression analysis with other combinations of parameters that were not selected by the step-wise regression was also performed to evaluate the performance against other models. Receiver operating characteristic (ROC) analysis was employed to assess the diagnostic performance of individual parameters and the logistic regression classifier for detection of fibrosis. AUC values were compared using DeLong tests. The optimal threshold was selected by the Youden index, and sensitivity and specificity values at that threshold value were calculated. For all tests, the significance level was set at 0.05.

Results

Demographics and histopathological characteristics.

In total, 19 samples from 19 patients were analyzed. Eight samples (42%) exhibited liver cirrhosis (stage 4) and 3 samples showed advanced fibrosis (stage 3-4, 16%), leading to a total of 11 samples with advanced fibrosis or cirrhosis. The remaining 8 samples (42%) showed no evidence of fibrosis. Demographics and histopathological characteristics of the entire cohort and the different groups (non-fibrotic vs advanced fibrotic/cirrhotic) are shown in Table 1. Age was significantly higher in the fibrotic group ($p < 0.001$), while sex was not significantly different ($P = 0.26$). Iron deposition (either in hepatocytes or Kupffer cells) was observed in 7 (37%) of samples with liver iron deposition of which 6 were fibrotic and 1 was non-fibrotic. There was a trend to significantly higher prevalence of iron deposition in the fibrotic/cirrhotic group ($p = 0.064$). Steatosis was seen in 4 samples, of which 3 were fibrotic. There were no significant differences in steatosis stage between the groups ($p = 0.436$).

R₂* QSM and PDFF measurements

Figure 1 a-c shows boxplots of R₂*, χ and PDFF for the samples without fibrosis and samples with advanced fibrosis or cirrhosis. Only median R₂* showed a significant difference between fibrotic and non-fibrotic samples (p=0.041), with higher R₂* found in fibrotic samples [median (interquartile range) R₂* non-fibrotic 40.2 s⁻¹ (18.5 s⁻¹), fibrotic 63.8 s⁻¹ (68.1 s⁻¹)].

None of the MRI parameters showed a significant correlation with age in the entire cohort of samples (p-value range 0.307 – 0.811), in the non-fibrotic group (p-value range 0.501 – 0.582) and in the fibrotic group (p-value range 0.271 – 0.816).

Logistic regression modeling

Logistic regression with stepwise feature selection identified the combination of R₂* and χ as the optimal classifier for differentiating between no fibrosis and advanced fibrosis/cirrhosis (p-value of model 0.002). The following model was generated:

$$\text{logit}(\text{prediction}) = -8.45 + 0.23 R_2^* - 28.8 \chi$$

Figure 1d shows a boxplot of the model prediction, exhibiting a strongly significant differentiation between the samples without fibrosis vs. advanced fibrosis/cirrhosis (p=0.002).

Logistic regression models using other combinations of parameters yielded a significant model for the combination of all three parameters (R₂*, χ and PDFF) with a p-value of 0.002. The differentiation between the groups was similar for this model as for the optimal model (p-value fibrosis vs. advanced fibrosis/cirrhosis 0.002), indicating no additional value of PDFF to the model. Other combinations of parameters (R₂* + PDFF and χ + PDFF) yielded non-significant models (p=0.075 and 0.411, respectively). Figure 2 provides a graphical representation of the optimal model as function of R₂* and χ , showing high likelihood of advanced fibrosis/cirrhosis for samples with low χ and high R₂*, whereas samples with high χ and lower R₂* have higher likelihood to be non-fibrotic. The data points of the samples are also shown in Figure 2, showing poor differentiation between the two groups using χ or R₂* individually, but improved differentiation with the model combining these parameters. Figure 3 provides representative examples of R₂*, χ and PDFF maps as well as model prediction maps of a non-fibrotic and a cirrhotic liver sample. The R₂* maps showed a slight increase in the cirrhotic vs. non-fibrotic sample, while the χ maps exhibited a mild decrease in the cirrhotic liver sample. The PDFF maps indicated low fat deposition in both samples. The model prediction maps provided much clearer differentiation between the non-fibrotic and cirrhotic sample compared to the maps of the individual MRI parameters.

ROC analysis

Figure 4 shows ROC curves for each of the individual parameters as well as for the model prediction. For individual parameters, the highest AUC for cirrhosis detection was found for

R_2^* (AUC=0.784, $p=0.039$). PDFF and χ exhibited non-significant AUCs of 0.625 ($p=0.364$) and 0.591 ($p=0.509$), respectively. The logistic regression model of combined R_2^* and χ showed a substantially improved AUC of 0.909 ($p=0.003$) compared to individual parameters. The AUC value of the model was significantly higher than the AUC of χ ($p=0.040$), but it was not significantly higher than the AUC values of R_2^* ($p=0.273$) and PDFF (0.087). Table 1 provides additional results of the ROC analysis, including sensitivity and specificity values. The logistic regression model combining R_2^* and χ exhibited improved sensitivity compared to R_2^* alone (81.8% vs 45.5%), while maintaining excellent specificity (100%).

Discussion

In this study, we first evaluated the feasibility of susceptibility imaging for noninvasive assessment of liver fibrosis in ex vivo liver explant samples. QSM alone did not provide significant diagnostic performance for detection of advanced fibrosis or cirrhosis, but we found that the combination of R_2^* and QSM susceptibility values differentiated between liver samples without fibrosis and those with advanced fibrosis or cirrhosis (AUC=0.91).

Collagen, like most biomolecules, has diamagnetic susceptibility originating from a change in orbital motion of electrons in the presence of an external magnetic field. Alterations in tissue susceptibility due to collagen deposition in liver fibrosis could potentially be measured by QSM. However, other accompanying tissue components, including liver fat and iron, also contribute to the susceptibility signal in the liver. Steatosis, or fat deposition, often co-exists with liver fibrosis, in particular in patients with nonalcoholic fatty liver disease (NAFLD) or steatohepatitis (NASH)³⁰. The dependency of the QSM signal on fat was reduced in this study by separating water and fat signals and performing fat correction¹⁵. Liver iron overload may develop in patients with hereditary hemochromatosis and in patients undergoing blood transfusion therapy. Liver iron deposition is also prevalent with variable amounts in CLD patients, especially in patients with viral hepatitis, NAFLD, NASH and alcoholic liver disease³¹. In our study, iron deposition (ferritin and hemosiderin), in either the hepatocytes or Kupffer cells, was observed in 37% of the samples. The paramagnetic properties of iron greatly influence liver tissue susceptibility, to a much greater extent and in an opposing direction compared to diamagnetic contributions from collagen³². QSM is therefore most useful for liver iron quantification^{14,15}. By contrast, in the application of QSM for fibrosis measurement, liver iron deposition is a strong interfering effect. Consequently, QSM in itself would seem to have restricted utility for assessment of liver fibrosis. This limitation became evident in the current study, in which we observed no significant difference in QSM susceptibility values between non-fibrotic and cirrhotic liver samples.

The magnetic susceptibility effects of iron deposition and collagen both lead to increased R_2^{*15} , as a result from more rapid dephasing of transverse magnetization due to dynamic changes in the local field experienced by water protons diffusing through the tissue³³. In addition, the presence of collagen may significantly increase the correlation time of water protons, leading to an elevation of R_2 and R_2^* relaxation rates. We indeed found significantly increased R_2^* in fibrotic vs. non-fibrotic samples. Nonetheless, ROC analysis

showed poor sensitivity of R_2^* for differentiation between the fibrotic and non-fibrotic samples. The complex interplay of iron deposition and collagen effects on the R_2^* relaxation rate likely accounts for the poor performance of R_2^* in evaluation of fibrosis. Edema and inflammation were not confounding factors in this ex-vivo study, but their effect on R_2^* should be included with in vivo models^{15,16}. Fat was minimal in these samples and fat-effects were modeled using a single peak chemical shift; Multipeak fat spectral analysis may be explored if needed for NAFLD samples^{17,34}.

We found significant additional value of QSM combined with R_2^* for evaluation of liver fibrosis. The potential complementary value of R_2^* and QSM for fibrosis evaluation is indicated in a recently published phantom study¹⁵. In that study, the effects of fat, gadolinium (as substitute for iron) and collagen on R_2^* and χ from QSM were modelled. As expected, significant positive associations of R_2^* with both collagen and gadolinium were observed, while χ was negatively associated with collagen and positively associated with gadolinium. These findings generated the idea that a regression model with a positive coefficient for R_2^* and a negative coefficient for χ would amplify the associations of each of R_2^* and χ with collagen, while partly cancelling out the associations of these MRI parameters with iron deposition. In our current study, the logistic regression model combining R_2^* and χ indeed provided enhanced detection of non-fibrotic from cirrhotic samples and yielded a substantially improved AUC for differentiation between the two groups compared to individual parameters. The model significantly outperformed χ , but not R_2^* and PDFF, in terms of diagnostic performance for evaluation of advanced fibrosis or cirrhosis. The lack of significant difference of the model in comparison to R_2^* and PDFF is potentially related to the small sample size in this feasibility study, leading to underpowered analysis of the differences in AUC. Larger studies are needed to further evaluate the additional value of the model combining the parameters vs. individual parameters for detection of fibrosis and cirrhosis. In addition, those larger studies may allow for analysis of subsets of samples (e.g. those without and with iron and those without and with fat) to provide a deeper understanding of the influence of the histological measurements on the MRI measurements.

The age of the patients in the fibrotic group was significantly higher than in the non-fibrotic group. This can be explained by the design of the study, using liver explant as a reference. Non-fibrotic liver explants are often from pediatric patients with diseases such as metabolic disorders or pediatric hepatoblastoma where liver fibrosis did not develop, whereas fibrotic liver explants are mostly from adult patients with CLD, naturally leading to a higher age in the fibrotic cohort³⁵. We performed a correlation analysis of the MRI measurements with age in the different groups, given the potential dependency of hepatic R_2^* and QSM measurements on age³⁶. No significant correlations were observed, suggesting limited effects of age on our analysis.

The ex vivo analysis on liver explant samples performed here for validating MRI has several advantages compared to liver biopsy for validating MRI in patients. The histopathological analysis was performed on a sample adjacent to the MRI-scanned sample, eliminating issues with intrahepatic heterogeneity of liver pathology, including fibrosis³⁷⁻³⁹. The spatial heterogeneity and resulting sampling variability is one of the major limitations of liver

biopsies, which only sample a small part of the liver⁴⁰. Histopathological analysis is easier and more reliable on large explants compared to small biopsy fragments. In addition to the semi-quantitative standard histopathological staging performed in our study, more advanced ex vivo analysis could also provide quantitative measurements of collagen and iron concentration^{41,42}, which may further the understanding of the combined effects of these tissue components on R_2^* and QSM measurements. On the other hand, formalin fixation has shown to cause increased R_2^* in brain⁴³, kidney⁴⁴ and cardiac tissues⁴⁵ and may have affected MRI evaluation of our liver explant specimens. QSM quantification is less affected by fixation, although the higher R_2^* values may cause noisier data and therefore increased QSM variance⁴³. Therefore, in vivo validation of these results in CLD patients is required to evaluate the potential clinical translatability of the presented methodology.

After further validation in independent clinical cohorts, the susceptibility-based method for liver fibrosis quantification presented here may provide an attractive alternative to current noninvasive liver fibrosis imaging methods or in combination with other methods may yield higher diagnostic accuracy. In subjects, R_2^* and QSM measurements can be obtained from a single breath-hold GRE acquisition¹³, facilitating clinical translation of this methodology for staging of liver fibrosis in CLD patients. We expect that the results observed here can be directly translated into studying liver fibrosis in patients with concomitant iron. However, the logistic regression model may need to be adjusted in studying liver fibrosis in patients who have concomitant inflammatory edema, in addition to iron¹⁶.

This study has limitations. First, the sample size was small in this feasibility study, although it was sufficient to show a high degree of statistical significance for our primary result. Second, the explant liver samples either exhibited no fibrosis or advanced fibrosis/cirrhosis, precluding analysis of the performance of the proposed method for more intermediate fibrosis stages. Third, while no short- T_2^* elevated signal was observed in UTE acquisition results, it is important to model and study its effect in fatty livers and in-vivo studies⁴⁶. Last, MRI acquisitions were performed on two different 3.0T systems with different acquisition parameters. Reproducibility of the phantom results at different scanners and acquisition parameters should be studied in the future⁴⁷⁻⁴⁹.

In conclusion, our study shows that an integrated QSM and R_2^* analysis of multiecho GRE 3T imaging data is promising for noninvasive diagnostic assessment of liver fibrosis. After in vivo validation, the presented methodology may provide an attractive alternative tool for noninvasive detection and staging of hepatic fibrosis in CLD patients.

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References

1. Friedman SL. Liver fibrosis -- from bench to bedside. *Journal of hepatology*. 2003;38 Suppl 1:S38–53. [PubMed: 12591185]

2. Schuppan D, Ashfaq-Khan M, Yang AT, Kim YO. Liver fibrosis: Direct antifibrotic agents and targeted therapies. *Matrix biology: journal of the International Society for Matrix Biology*. 2018;68-69:435–451. [PubMed: 29656147]
3. Sebastiani G, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol*. 2006;12(23):3682–3694. [PubMed: 16773685]
4. Everett RJ, Stirrat CG, Semple SI, Newby DE, Dweck MR, Mirsadraee S. Assessment of myocardial fibrosis with T1 mapping MRI. *Clin Radiol*. 2016;71 (8):768–778. [PubMed: 27005015]
5. Allkemper T, Sagmeister F, Cicinnati V, et al. Evaluation of fibrotic liver disease with whole-liver Tip MR imaging: a feasibility study at 1.5 T. *Radiology*. 2014;271(2):408–415. [PubMed: 24475807]
6. Bonekamp S, Torbenson MS, Kamel IR. Diffusion-weighted magnetic resonance imaging for the staging of liver fibrosis. *J Clin Gastroenterol*. 2011 ;45(10):885–892. [PubMed: 21716125]
7. Zhang W, Kong X, Wang ZJ, Luo S, Huang W, Zhang LJ. Dynamic Contrast-Enhanced Magnetic Resonance Imaging with Gd-EOB-DTPA for the Evaluation of Liver Fibrosis Induced by Carbon Tetrachloride in Rats. *PLoS One*. 2015; 10(6):e0129621. [PubMed: 26076199]
8. Kennedy P, Wagner M, Castera L, et al. Quantitative Elastography Methods in Liver Disease: Current Evidence and Future Directions. *Radiology*. 2018;286(3):738–763. [PubMed: 29461949]
9. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest*. 2005; 115(2):209–218. [PubMed: 15690074]
10. Luo J, He X, d'Avignon DA, Ackerman J J, Yablonskiy DA. Protein-induced water 1H MR frequency shifts: contributions from magnetic susceptibility and exchange effects. *J Magn Reson*. 2010;202(1):102–108. [PubMed: 19879785]
11. Wei H, Decker K, Nguyen H, et al. Imaging diamagnetic susceptibility of collagen in hepatic fibrosis using susceptibility tensor imaging. *Magn Reson Med*. 2020;83(4): 1322–1330. [PubMed: 31633237]
12. de Rochefort L, Liu T, Kressler B, et al. Quantitative susceptibility map reconstruction from MR phase data using bayesian regularization: validation and application to brain imaging. *Magn Reson Med*. 2010;63(1): 194–206. [PubMed: 19953507]
13. Jafari R, Sheth S, Spincemaille P, et al. Rapid automated liver quantitative susceptibility mapping. *J Magn Reson Imaging*. 2019;50(3):725–732. [PubMed: 30637892]
14. Sharma SD, Fischer R, Schoennagel BP, et al. MRI-based quantitative susceptibility mapping (QSM) and R_2^* mapping of liver iron overload: Comparison with SQUID-based biomagnetic liver susceptometry. *Magn Reson Med*. 2017;78(1):264–270. [PubMed: 27509836]
15. Li J, Lin H, Liu T, et al. Quantitative susceptibility mapping (QSM) minimizes interference from cellular pathology in R_2^* estimation of liver iron concentration. *J Magn Reson Imaging*. 2018;48(4):1069–1079. [PubMed: 29566449]
16. Bray TJP, Bainbridge A, Punwani S, Ioannou Y, Hall-Craggs MA. Simultaneous Quantification of Bone Edema/Adiposity and Structure in Inflamed Bone Using Chemical Shift-Encoded MRI in Spondyloarthritis. *Magn Reson Med*. 2018;79(2):1031–1042. [PubMed: 28589660]
17. Hernando D, Levin YS, Sirlin CB, Reeder SB. Quantification of liver iron with MRI: state of the art and remaining challenges. *J Magn Reson Imaging*. 2014;40(5):1003–1021. [PubMed: 24585403]
18. Dong J, Liu T, Chen F, et al. Simultaneous phase unwrapping and removal of chemical shift (SPURS) using graph cuts: application in quantitative susceptibility mapping. *IEEE Trans Med Imaging*. 2015;34(2):531–540. [PubMed: 25312917]
19. Reeder SB, McKenzie CA, Pineda AR, et al. Water-fat separation with IDEAL gradient-echo imaging. *J Magn Reson Imaging*. 2007;25(3):644–652. [PubMed: 17326087]
20. Liu T, Khalidov I, de Rochefort L, et al. A novel background field removal method for MRI using projection onto dipole fields (PDF). *NMR Biomed*. 2011 ;24(9):1129–1136. [PubMed: 21387445]
21. Liu J, Liu T, de Rochefort L, et al. Morphology enabled dipole inversion for quantitative susceptibility mapping using structural consistency between the magnitude image and the susceptibility map. *Neuroimage*. 2012;59(3):2560–2568. [PubMed: 21925276]
22. Liu Z, Spincemaille P, Yao Y, Zhang Y, Wang Y. MEDI+0: Morphology enabled dipole inversion with automatic uniform cerebrospinal fluid zero reference for quantitative susceptibility mapping. *Magn Reson Med*. 2018;79(5):2795–2803. [PubMed: 29023982]

23. Liu Z, Kee Y, Zhou D, Wang Y, Spincemaille P. Preconditioned total field inversion (TFI) method for quantitative susceptibility mapping. *Magn Reson Med*. 2016.
24. Reeder SB, Cruite I, Hamilton G, Sirlin CB. Quantitative Assessment of Liver Fat with Magnetic Resonance Imaging and Spectroscopy. *J Magn Reson Imaging*. 2011 ;34(4):729–749. [PubMed: 22025886]
25. de Rochefort L, Brown R, Prince MR, Wang Y. Quantitative MR susceptibility mapping using piece-wise constant regularized inversion of the magnetic field. *Magn Reson Med*. 2008;60(4):1003–1009. [PubMed: 18816834]
26. Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem*. 2009;78:929–958. [PubMed: 19344236]
27. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol*. 1995;19(12):1409–1417. [PubMed: 7503362]
28. Puri P, Sanyal AJ. Nonalcoholic fatty liver disease: Definitions, risk factors, and workup. *Clin Liver Dis (Hoboken)*. 2012;1(4):99–103. [PubMed: 31186860]
29. Turlin B, Deugnier Y. Evaluation and interpretation of iron in the liver. *Semin Diagn Pathol*. 1998;15(4):237–245. [PubMed: 9845425]
30. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. *N Engl J Med*. 2017;377(21):2063–2072. [PubMed: 29166236]
31. Mehta KJ, Farnaud SJ, Sharp PA. Iron and liver fibrosis: Mechanistic and clinical aspects. *World J Gastroenterol*. 2019;25(5):521–538. [PubMed: 30774269]
32. Wang Y, Spincemaille P, Liu Z, et al. Clinical quantitative susceptibility mapping (QSM): Biometal imaging and its emerging roles in patient care. *J Magn Reson Imaging*. 2017;46(4):951–971. [PubMed: 28295954]
33. Ghugre NR, Wood JC. Relaxivity-iron calibration in hepatic iron overload: probing underlying biophysical mechanisms using a Monte Carlo model. *Magn Reson Med*. 2011 ;65(3):837–847. [PubMed: 21337413]
34. Hernando D, Kramer JH, Reeder SB. Multipeak fat-corrected complex R_2^* relaxometry: theory, optimization, and clinical validation. *Magn Reson Med*. 2013;70(5):1319–1331. [PubMed: 23359327]
35. O'Leary JG, Lepe R, Davis GL. Indications for liver transplantation. *Gastroenterology*. 2008;134(6):1764–1776. [PubMed: 18471553]
36. Schwenzer NF, Machann J, Haap MM, et al. T2* relaxometry in liver, pancreas, and spleen in a healthy cohort of one hundred twenty-nine subjects-correlation with age, gender, and serum ferritin. *Invest Radiol*. 2008;43(12):854–860. [PubMed: 19002057]
37. Abdi W, Millan JC, Mezey E. Sampling variability on percutaneous liver biopsy. *Arch Intern Med*. 1979;139(6):667–669. [PubMed: 443970]
38. Maharaj B, Maharaj RJ, Leary WP, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet*. 1986;1 (8480):523–525. [PubMed: 2869260]
39. Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol*. 2002;97(10):2614–2618. [PubMed: 12385448]
40. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology*. 2003;38(6):1449–1457. [PubMed: 14647056]
41. Chang PE, Goh GBB, Leow WQ, Shen L, Lim KH, Tan CK. Second harmonic generation microscopy provides accurate automated staging of liver fibrosis in patients with non-alcoholic fatty liver disease. *PLoS One*. 2018;13(6):e0199166. [PubMed: 29924825]
42. St Pierre TG, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood*. 2005;105(2):855–861. [PubMed: 15256427]
43. Dusek P, Madai VI, Huelnhagen T, et al. The choice of embedding media affects image quality, tissue R_2^* , and susceptibility behaviors in post-mortem brain MR microscopy at 7.0T. *Magn Reson Med*. 2019;81(4):2688–2701. [PubMed: 30506939]

44. Daniel AJ, Buchanan CE, Kazmi I, et al. The Effects of Fixation and Age on Relaxometry Measurements of Ex-Vivo Kidneys. *Proc Intl Soc Mag Reson Med*. 2019;27.
45. Lohr D, Terekhov M, Veit F, Schreiber LM. Longitudinal assessment of tissue properties and cardiac diffusion metrics of the ex vivo porcine heart at 7 T: Impact of continuous tissue fixation using formalin. *NMR Biomed*. 2020:e4298. [PubMed: 32207190]
46. Zhu A, Hernando D, Johnson KM, Reeder SB. Characterizing a Short T₂ * Signal Component in the Liver Using Ultrashort TE Chemical Shift-Encoded MRI at 1.5T and 3.0T. *Magn Reson Med*. 2019;82(6):2032–2045. [PubMed: 31270858]
47. Bachtiar V, Kelly MD, Wilman HR, et al. Repeatability and reproducibility of multiparametric magnetic resonance imaging of the liver. *PLoS One*. 2019;14(4):e0214921. [PubMed: 30970039]
48. Deh K, Kawaji K, Bulk M, et al. Multicenter reproducibility of quantitative susceptibility mapping in a gadolinium phantom using MEDI+0 automatic zero referencing. *Magn Reson Med*. 2019;81(2):1229–1236. [PubMed: 30284727]
49. Deh K, Nguyen TD, Eskreis-Winkler S, et al. Reproducibility of quantitative susceptibility mapping in the brain at two field strengths from two vendors. *J Magn Reson Imaging*. 2015;42(6):1592–1600. [PubMed: 25960320]

Figure 1. Boxplots of (a) R_2^* , (b) χ , (c) PDFF and (d) logistic regression model prediction values in samples with no fibrosis and samples with advanced fibrosis cirrhosis.

Note - The p-value of a Mann-Whitney U test comparing the groups is displayed on top of each plot.

Figure 2.

Heatmap of the likelihood of presence of advanced fibrosis or cirrhosis predicted by the logistic regression model as function of R_2^* and χ . The data points of the non-fibrotic (white) and advanced fibrotic/cirrhotic (black) samples are superimposed on the map.

Figure 3.

R_2^* , χ , PDFF and model prediction maps in a cross-sectional slice through a non-fibrotic liver sample (top) and a cirrhotic liver sample (bottom). The area around the vasculature was eroded from the masks used for the model prediction. Individual MRI parameters showed subtle changes between the two samples (non-fibrotic: median R_2^* 56 s⁻¹, median χ 0.29 ppm, median PDFF 3%; cirrhotic median R_2^* 64 s⁻¹, median χ 0.13 ppm, median PDFF 3%). The model prediction showed substantially higher likelihood of cirrhosis for the cirrhotic sample vs. non-fibrotic sample (prediction 0.86 vs. 0.02). Masson's trichrome staining confirmed lack of fibrosis in the top sample and cirrhosis in the bottom sample. Prussian Blue staining showed lack of iron deposition in the non-fibrotic sample and scattered Kupffer cell iron deposition in the cirrhotic sample.

Figure 4. ROC curves for differentiation between samples without liver fibrosis vs. samples with advanced fibrosis or cirrhosis for R_2^* , χ , PDFF and the logistic regression model combining R_2^* and χ .

Table 1.

Demographical and histopathological characteristics.

	All (n=19)	Non-fibrotic (n=8)	Advanced fibrosis/cirrhosis (n=11)	P
Age (y)	41 (0 – 69)	22 (0 – 50)	55 (16–69)	<0.001
Sex				0.260
- Male	10 (53%)	3 (38%)	7 (64%)	
- Female	9 (47%)	5 (62%)	4 (36%)	
Iron deposition				0.061 [#]
- Grade 0	14 [*] (74%)	8 [*] (100%)	6 [*] (55%)	
- Grade 1	2 (11%)	0 (0%)	2 (18%)	
- Grade 2	2 (11%)	0 (0%)	2 (18%)	
- Grade 3	1 (5%)	0 (0%)	1 (9%)	
- Grade 4	0 (0%)	0 (0%)	0 (0%)	
Steatosis				0.436
- Stage 0 (<5 %)	15 (79%)	7 (88%)	8 (73%)	
- Stage 1 (5-33 %)	4 (21%)	1 (13%)	3 (27%)	
- Stage 2 (34-66 %)	0 (0%)	0 (0%)	0 (0%)	
- Stage 3 (>66 %)	0 (0%)	0 (0%)	0 (0%)	

Note – p-values are from Mann-Whitney U tests for continuous variables and from chisquared tests for categorical variables.

* In two samples where no iron deposition was found in the hepatocytes, iron was observed in the Kupffer cells, including one non-fibrotic and one cirrhotic sample.

[#] p-value from chi-squared test comparing presence of iron deposition (either in Kupffer cells or hepatocytes) between the two groups.

Table 2.

Results of ROC analysis of individual MRI parameters and the logistic regression model combining R_2^* and χ measurements for differentiation between liver samples without liver fibrosis (n=8) and advanced fibrosis or cirrhosis (n=11).

Parameter	AUC (CI)	P	Cut-off	Sensitivity (%)	Specificity (%)
R_2^* (S^{-1})	0.784 (0.577 – 0.991)	0.039	87.2	45.5%	100%
PDFF (%)	0.625 (0.365 – 0.885)	0.364	2.4	54.5%	87.5%
χ (PPm)	0.591 (0.329 – 0.853)	0.509	0.37	27.3%	100%
Model prediction	0.909 (0.767 – 1)	0.003	-	81.8%	100%

AUC = area-under-the-curve; CI = confidence interval; PDFF = proton density fat fraction