R2* MAGNETIC RESONANCE IMAGING OF THE LIVER IN PATIENTS WITH IRON OVERLOAD

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ABSTRACT

R2*-MRI can quantify hepatic iron content (HIC) by non-invasive means but is not fully investigated. Patients with iron overload completed 1.5T R2*-MRI examination and liver biopsy within 30 days. Forty-three patients [sickle cell anemia (32), β-thalassemia major (6), and bone marrow failure (5)] were analyzed: median age 14 years, median transfusion duration 15 months, average (± 1SD) serum ferritin 2718 ± 1994 ng/mL, and average HIC 10.9 ± 6.8 mg Fe/g dry weight liver. Regions of interest were drawn and analyzed by three independent reviewers with excellent agreement of their measurements (intraclass correlation coefficient = 0.98). Ferritin and R2*-MRI were weakly but significantly associated (range of correlation coefficients among the 3 reviewers = 0.41-0.48, all P<.01). R2*-MRI was strongly associated with HIC for all 3 reviewers (correlation coefficients 0.96-0.98, all P<.0001). This high correlation confirms prior reports, calibrates R2*-MRI measurements, and suggests its clinical utility for predicting HIC using R2*-MRI. This study is registered at www.clinicaltrials.gov under #NCT00675038.
INTRODUCTION

Monitoring body iron content is critical for clinical management of patients with iron overload. Prior reports of iron measurements by R2-MRI and R2*-MRI in the liver have shown good correlations with hepatic iron content (HIC).\textsuperscript{1-3} However, MRI calibration varies according to instrumentation and technique. To calibrate the R2*-MRI technique for non-invasive HIC assessment, we conducted a study to estimate the correlation of R2*-MRI with liver biopsy-proven HIC determination in patients with iron overload (www.clinicaltrials.gov # NCT00675038).

METHODS

Patients

Patients $\geq 7$ years of age with iron overload (ferritin $>1,000$ ng/mL within 3 months of enrollment or $\geq 18$ erythrocyte transfusions) were eligible. All participants underwent non-sedated liver MRI examination and ferritin measurement, followed within 30 days by liver biopsy with HIC determination. The St. Jude Children's Research Hospital IRB provided continuing approval and all participants or legal guardians signed informed consent in accordance with the Declaration of Helsinki.

Liver biopsies

Two liver specimens were obtained: the first for liver iron quantitation (Mayo Laboratories, Rochester MN), and the second for pathology review. All histology was
reviewed by a single pathologist blinded to clinical status and HIC values. Liver fibrosis was scored from zero (absent fibrosis) to 6 (cirrhosis).

**MRI Technique**

The single breath-hold R2*-MRI used a 1.5T MRI scanner (Siemens Symphony, Siemens, Malvern, PA) using a multi-echo gradient echo sequence to acquire 20 images with increasing echo times (range 1.1-17.3 ms). Liver images were obtained in transversal slice orientation through the center at the main portal vein origin. Slice thickness measured 10 mm with in-plane resolution of 3.125 mm. Quantitative T2* maps were calculated offline using custom-written MATLAB software (The MathWorks, Inc., Natick, MA) and signal intensity drop was fitted on a pixel-by-pixel basis to a monoexponential decay using a least-squares fit method. Truncated exponential fitting was used to reduce bias introduced by low iron-containing tissues, as previously performed. Regions of Interest (ROI) were drawn either on source images or T2* maps in a homogeneous area of the right hepatic lobe, avoiding blood vessels and obvious bile ducts. Three independent reviewers, blinded to patients’ clinical status and the other reviewers’ interpretations, performed ROI analysis. Pixels with failed fit in an ROI were tracked, but R2* measurements were reported only when the percentage of fitted pixels within the ROI was >25% of total available pixels.

**Statistical Analyses**

To attain a positive linear relationship with HIC, T2* was transformed into reciprocal R2*: \( R2^*[^{Hz}] = \frac{1000}{T2^*[^{ms}]} \). Agreement among R2*-MRI measurements by the 3 reviewers was assessed using the intraclass correlation coefficient (ICC).
Spearman’s Rank Order Correlation coefficient ($r_s$) was used to investigate the relationship between $R_2^*$-MRI and fibrosis, and $R_2^*$-MRI and HIC. The relationship between $R_2^*$-MRI and HIC was also investigated through robust linear regression modeling, a method that does not minimize the sum of the squared residuals, and therefore yields lower R-squared ($R^2$) values than least squares regression. For comparability with other published MRI techniques, the limits of agreement (95%) between $R_2^*$-MRI and HIC were calculated using both the Bland-Altman method and its percent difference version.

**RESULTS AND DISCUSSION**

Forty–seven patients had both liver biopsy and $R_2^*$-MRI exams performed, but one biopsy was inadequate and three with very high HIC (range, 25.2-38.8 mg Fe/g) had insufficient available fitted pixels in the ROI. For the remaining 43 patients, median age was 14 years (range 7–35 years), and 22 (51.2%) were male. Thirty-two (74.4%) subjects had sickle cell anemia, 6 (14.0%) had β-thalassemia, and 5 (11.6%) had bone marrow failure syndromes. Median transfusion duration was 15 months (range, 7–425 months), however, in 3 patients the total number of transfusions was not available and may have been underestimated. Twenty-four patients (55.8%) used iron chelation (desferrioxamine or deferasirox) for a median of 3.5 months (range, 0 to 327 months). $R_2^*$-MRI examinations averaged 15 - 20 minutes; no liver biopsy complications occurred.

Mean HIC was $10.9 \pm 6.8$ mg Fe/g of dry weight liver (median 10.3, range 0.6–27.6 mg Fe/g). Mean liver $R_2^*$ ranged from $394 \pm 234$ to $412 \pm 239$ Hz (median 345-385 Hz, coefficient of variation 17.0 to 20.9 Hz) among the reviewers. There was robust inter-
observer agreement (ICC = 0.98), and R2* values were strongly associated with HIC ($r_s = 0.96-0.98$, all $P < .0001$). Regression models for R2*-MRI versus HIC had $R^2$ values ranging from 0.68-0.72, and slopes of 28.02-28.15 (all $P < .0001$, example in Figures 1A and 1B).

Average serum ferritin was $2718 ± 1994$ ng/mL (median 2127 ng/mL, range 351–9845 ng/mL). Ferritin and R2*-MRI liver values showed positive but weak associations with correlation coefficients among the 3 reviewers ($r_s = 0.41-0.48$, all $P < .01$). These weak associations, although generally lower than previously reported, support the widely-held view that ferritin cannot accurately predict HIC. Ferritin is inappropriate as a sole measure of body iron burden and should not be used alone in clinical decisions.10;11

Forty-one biopsies were suitable for fibrosis analysis: 9 showed none and 19 had grade 1 fibrosis. Fibrosis scores of 2, 3, 4, 5, and 6 were found in 3, 5, 1, 3 and 1 participants, respectively. No relationship was found between mild liver fibrosis and R2* measurements, suggesting that mild fibrosis does not interfere with R2*-MRI signal acquisition.

Our study provides the largest reported patient sample to date with paired liver R2*-MRI and biopsy measurements. Our R2*-MRI technique demonstrated comparable % bias, % standard deviation, and limits of agreement with other R2*-MRI and R2-MRI techniques (Table 1). The closeness of our results to similar previously reported work2;3 indicates an advantage to techniques that may have significant instrument to instrument variability, such as those that use signal intensity ratio.12;13 Our calibration equation was in excellent agreement with the data of Wood et al.,3 but showed a substantially different
slope when compared with the data from Anderson et al,\textsuperscript{1} most likely representing systematic differences in liver biopsy and MRI acquisition/processing.

In clinical practice, different examiners will perform ROI measurements, which may add to measurement error from inter-examiner variation. Our study documents that with appropriate training, qualified reviewers can obtain very similar ROI measurements for iron quantitation, thereby reducing measurement variability. This operator-independence suggests this technique is robust and can be utilized by different reviewers and centers, allowing multi-center studies with R2*-MRI.

Three R2*-MRI measurements had insufficient available pixels in the T2* map due to high HIC. The inability to perform pixel-wise fitting indicates a decreased ability to accurately ascertain very fast R2* signal decay occurring at the highest HIC values. To allow iron quantitation among heavily iron overloaded patients (e.g., HIC > 25 mg Fe/mg), sensitive MRI techniques with ultra-short echo times need to be developed.\textsuperscript{14}

Our study represents good conditions for calibrating R2*-MRI with biopsy HIC, since most of our patients were young with a low degree of fibrosis and little chelation exposure. Our R2*-MRI technique is a robust method with great reproducibility when used by different trained examiners. The R2*-MRI association with HIC corroborates and extends previous findings,\textsuperscript{1,3} providing strong evidence for using this technique to predict HIC non-invasively in patients with iron overload.
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AUTHORSHIP

Jane S. Hankins, MD, MS: designed and conducted study, analyzed and interpreted data, and wrote manuscript

M. Beth McCarville: performed ROI analysis, designed study, and wrote manuscript

Ralf B. Loeffler, PhD: performed ROI analysis, developed R2*-MRI programming for HIC measurement, and wrote manuscript

Mihaela Onciu, MD: performed histopathology review of all liver biopsy samples

Matthew P. Smeltzer, MS: performed statistical analysis and wrote manuscript

Fredric A. Hoffer, MD: designed study and performed most US-guided liver biopsies
Chin-Shang Li, PhD: designed study and performed statistical analysis
Winfred C. Wang, MD: interpreted data and wrote manuscript
Russell E. Ware, MD, PhD: designed study, interpreted data, and wrote manuscript
Claudia Hillenbrand, PhD: designed study, developed R2*-MRI programming for HIC measurement, performed ROI analysis, interpreted data, and wrote manuscript
All authors have no conflicts of interest to disclose.

REFERENCES


<table>
<thead>
<tr>
<th>MRI Method</th>
<th>Bland Altman Statistics (Percent Difference Method)</th>
<th>Bland Altman Statistics (Standard Method)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bias (%)</td>
<td>Standard Deviation (%)</td>
</tr>
<tr>
<td>R2*-MRI (using robust regression analysis)</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>R2*-MRI Wood et al.³ (using least squares regression analysis)</td>
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<td>23</td>
</tr>
<tr>
<td>R2-MRI St. Pierre et al.² (using nonlinear regression algorithms)</td>
<td>-3</td>
<td>27</td>
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Notes: Bland Altman percent difference method was calculated using the formula: \( \frac{(HIC (R2^*) - HIC (Biopsy))}{\frac{(HIC (R2^*) + HIC (Biopsy))}{2}} \)
FIGURE LEGEND

Figure 1: R2* -MRI versus HIC. Agreement among the 3 reviewers was very high (ICC=0.98), therefore only data for Reviewer 1 are illustrated. Panel A shows the plot of R2*-MRI measurements versus HIC values obtained by liver biopsy with linear regression lines and 95% prediction limits. The intercept was -454.85 (P=.31), the slope was 28.02 (P<.0001), and R² was 0.72. The correlation coefficient for R2*-MRI and HIC was 0.98 (P<.001). Panel B presents a standard Bland and Altman plot and shows the difference versus the average of HIC (R2*) and HIC (Biopsy), i.e.: (HIC (R2*) – HIC (Biopsy)) vs. (HIC (R2*) + HIC (Biopsy)) / 2. The solid line shows the mean difference between HIC (R2*) and HIC (Biopsy), and the dashed lines indicate the upper and lower 95% limits of agreement between the two measurements. Panel C illustrates our R2*-MRI versus HIC regression line overlaid with regression lines from two other published methods of R2*-MRI showing that the Wood et al. regression line falls well within our 95% predicted interval across the entire range of values, but the regression line from Anderson et al. (extrapolated from a published log-transformed plot) has a substantially lower slope, likely reflecting differences in instrumentation and biopsy iron quantification technique.
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