Brief report

R2* magnetic resonance imaging of the liver in patients with iron overload

Jane S. Hankins,[1] M. Beth McCarville,[2] Ralf B. Loeffler,[2] Matthew P. Smeltzer,[3] Mihaela Onciu,[4] Fredric A. Hoffer,[2] Chin-Shang Li,[3] Winfred C. Wang,[1] Russell E. Ware,[1] and Claudia M. Hillenbrand[2]

Departments of [1]Hematology, [2]Radiological Sciences, [3]Biostatistics, and [4]Pathology, St Jude Children's Research Hospital, Memphis, TN

R2* magnetic resonance imaging (R2*-MRI) can quantify hepatic iron content (HIC) by noninvasive 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, n = 32; β-thalassemia major, n = 6; and bone marrow failure, n = 5) were analyzed: median age, 14 years, median transfusion duration, 15 months, average (±SD) serum ferritin 2718 plus or minus 1994 ng/mL, and average HIC 10.9 plus or minus 6.8 mg Fe/g dry weight liver. Regions of interest were drawn and analyzed by 3 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 $P < .001$). This high correlation confirms prior reports, calibrates R2*-MRI measurements, and suggests its clinical utility for predicting HIC using R2*-MRI. This study was registered at www.clinicaltrials.gov as #NCT00675038. (Blood. 2009;113:4853-4855)

Introduction

Monitoring body iron content is critical for clinical management of patients with iron overload. Prior reports of iron measurements by R2 magnetic resonance imaging (MRI) and R2*-MRI relaxometry in the liver have shown good correlations with hepatic iron content (HIC).[1-3] However, MRI calibration varies according to instrumentation and technique. To calibrate the R2*-MRI technique for noninvasive HIC assessment, we conducted a study to estimate the correlation of R2*-MRI with liver biopsy-proven HIC determination in patients with iron overload.

Methods

Patients

Patients 7 years of age and older with iron overload (ferritin > 1000 ng/mL within 3 months of enrollment or ≥ 18 erythrocyte transfusions) were eligible. All participants underwent nonsedated liver MRI examination and ferritin measurement, followed within 30 days by liver biopsy with HIC determination. The St Jude Children's Research Hospital Institutional Review Board 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).[4]

MRI technique

The single breath-hold R2*-MRI used a 1.5T MRI scanner (Siemens Symphony, Siemens; Malvern, PA) using a multiecho 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 (MathWorks, 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.[1] Regions of interest (ROIs) 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 more than 25% of total available pixels.

Statistical analyses

To attain a positive linear relationship with HIC, T2* was transformed into reciprocal R2*: R2*[Hz] = 1000/T2*[ms]. Agreement among R2*-MRI measurements by the 3 reviewers was assessed using the intraclass correlation coefficient (ICC). Spearman rank order correlation coefficient (r_s) was used to investigate the relationship between R2*-MRI and fibrosis, and R2*-MRI and HIC. The relationship between R2*-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^2 values than least squares regression.[5] For comparability with other published MRI techniques, the limits of agreement (95%) between R2*-MRI and HIC were calculated using both the Bland-Altman method[6,7] and its percentage difference version.[8]

Submitted December 15, 2008; accepted February 26, 2009. Prepublished online as *Blood* First Edition paper, March 4, 2009; DOI 10.1182/blood-2008-12-191643.

This work was presented in part at the 49th meeting of the American Society of Hematology, Atlanta, GA, December 9, 2007.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Results and discussion

Forty-seven patients had both liver biopsy and R2*-MRI examinations performed, but one biopsy was inadequate and 3 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, 0-327 months). R2*-MRI examinations averaged 15 to 20 minutes; no liver biopsy complications occurred.

Mean HIC was 10.9 plus or minus 6.8 mg Fe/g of dry weight liver (median, 10.3 mg Fe/g; range, 0.6-27.6 mg Fe/g). Mean liver R2* ranged from 394 plus or minus 234 to 412 plus or minus 239 Hz (median range, 345-385 Hz; coefficient of variation, 17.0-20.9 Hz) among the reviewers. There was robust interobserver agreement (ICC = 0.98), and R2* values were strongly associated with HIC (r_s = 0.96-0.98; all $P < .001$). Regression models for R2*-MRI versus HIC had R^2 values ranging from 0.68 to 0.72, and slopes of 28.02 to 28.15 (all $P < .001$, example in Figure 1A,B).

Average serum ferritin was 2718 plus or minus 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.[9,10]

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 percentage bias, percentage SD, and limits of agreement with other R2*-MRI and R2-MRI techniques (Table 1). The closeness of our results to similar previously reported work[2,3] indicates an advantage over techniques that may have significant instrument-to-instrument variability, such as those that use signal intensity ratio.[11,12] Our calibration equation was in excellent agreement with the data of Wood et al[3] but showed a substantially different slope compared with the data from Anderson et al,[1] most probably representing systematic differences in liver biopsy and MRI acquisition and processing.

In clinical practice, different examiners will perform ROI measurements, which may add to measurement error from interexaminer 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 that this technique is robust and can be used by different reviewers and centers, allowing multicenter studies with R2*-MRI.

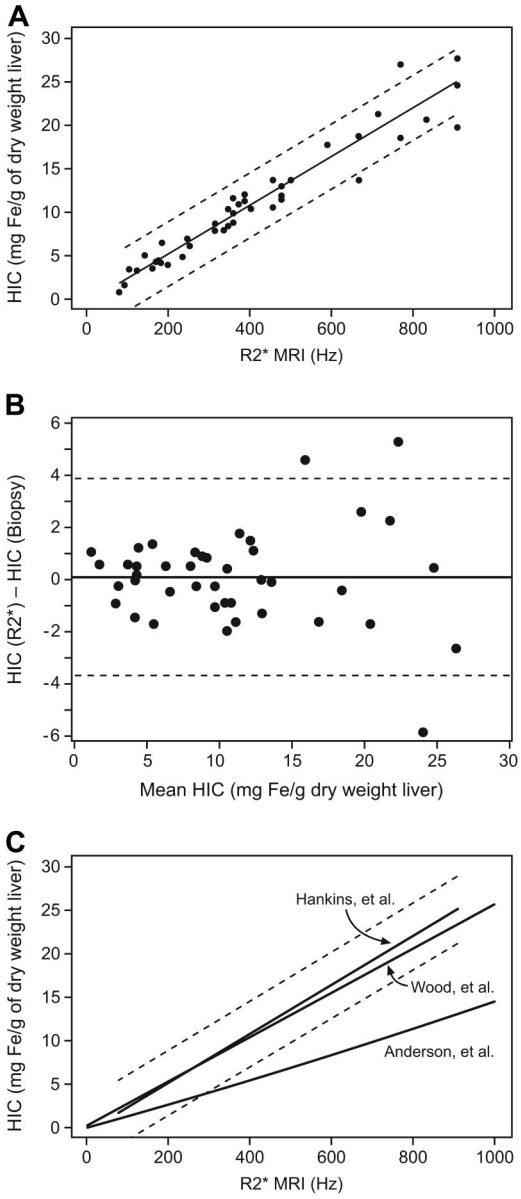

Figure 1. R2*-MRI versus HIC. Agreement among the 3 reviewers was very high (ICC = 0.98); therefore, only data for reviewer 1 are illustrated. (A) Plot of R2*-MRI measurements versus HIC values obtained by liver biopsy with linear regression lines and 95% prediction limits. The intercept was −454.85 ($P = .31$), the slope was 28.02 ($P < .001$), and R^2 was 0.72. The correlation coefficient for R2*-MRI and HIC was 0.98 ($P < .001$). (B) Standard Bland-Altman plot[6,7] shows the difference versus the average of HIC (R2*) and HIC (biopsy), that is, (HIC [R2*] − HIC [biopsy]) versus (HIC [R2*] + HIC [biopsy])/2. The solid line represents the mean difference between HIC (R2*) and HIC (biopsy); dashed lines, upper and lower 95% limits of agreement between the 2 measurements. (C) R2*-MRI versus HIC regression line overlaid with regression lines from 2 other published methods of R2*-MRI showing that the Wood et al[3] regression line falls well within our 95% predicted interval across the entire range of values, but the regression line from Anderson et al[1] (extrapolated from a published log-transformed plot) has a substantially lower slope, probably reflecting differences in instrumentation and biopsy iron quantification technique.

Three R2*-MRI measurements had insufficient available pixels in the T2* map because of 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 (eg, HIC > 25 mg Fe/mg), sensitive MRI techniques with ultrashort echo times need to be developed.[13]

Table 1. Comparison of Bland-Altman statistics by percentage difference and standard methods for different R2-MRI and R2*-MRI methods

Comparison method / MRI method	Bias	SD	95% confidence interval
Percentage difference method			
R2*-MRI (using robust regression analysis), %	3	21	−39 to 45
R2*-MRI (Wood et al[3]; using least squares regression analysis), %	1	23	−46 to 44
R2-MRI (St Pierre et al[2]; using nonlinear regression algorithms), %	−3	27	−56 to 50
Standard method			
R2*-MRI (Altman and Bland[6,7]; using robust regression analysis), mg Fe/g	0.0757	1.84	−3.60 to 3.76

The Bland-Altman percentage difference method[8] was calculated using the formula (HIC [R2*] − HIC [biopsy])/([HIC (R2*) + HIC (biopsy)]/2).

Our study represents good conditions for calibrating R2*-MRI with biopsy HIC because 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,[1,3] providing strong evidence for using this technique to predict HIC noninvasively in patients with iron overload.

Acknowledgments

The authors thank Eileen Hansbury, Martha Rieman, Melody Cunningham, and Nicole Mortier, for help with patient enrollment and outstanding clinical care to our patients; Ruitian Song for ROI analysis and helpful discussions regarding the analysis and interpretation of R2*-MRI data; Robert Gold for performance of some ultrasound-guided liver biopsies; Robert Kaufman for support with performance of MRI testing; Wei Zhao for support with data analysis; Betsy Williford for help with preparation of the figure; Paula Naidu, Laura Martino, Jamila Batts, and Ashish Pagare for help with data management; and James Boyett and Banu Aygun for constructive criticism of the manuscript.

The work was supported by National Heart, Lung, and Blood Institute (Bethesda, MD; grant 5 U54HL070590) and American Lebanese Syrian Associated Charities (Memphis, TN).

Authorship

Contribution: J.S.H. designed and conducted the study, analyzed and interpreted data, and wrote the manuscript; M.B.M. performed ROI analysis, designed the study, and wrote the manuscript; R.B.L. performed ROI analysis, developed R2*-MRI programming for HIC measurement, and wrote the manuscript; M.O. performed histopathology review of all liver biopsy samples; M.P.S. performed statistical analysis and wrote the manuscript; F.A.H. designed the study and performed most ultrasound-guided liver biopsies; C.-S.L. designed the study and performed statistical analysis; W.C.W. interpreted the data and wrote the manuscript; R.E.W. designed the study, interpreted the data, and wrote the manuscript; and C.M.H. designed the study, developed R2*-MRI programming for HIC measurement, performed ROI analysis, interpreted the data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Jane S. Hankins, Department of Hematology, St Jude Children's Research Hospital, 262 Danny Thomas Pl, MS #800, Memphis, TN 38105-3678; e-mail: jane.hankins@stjude.org.

References

1. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J. 2001;22:2171-2179.
2. St Pierre TG, Clark PR, Chua-Anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood. 2005;105:855-861.
3. Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. Blood. 2005;106:1460-1465.
4. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696-699.
5. Yohai VJ. High breakdown point and high efficiency robust estimates for regression. Ann Stat. 1987;15:642-656.
6. Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. Statistician. 1983;32:307-317.
7 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1:307-310.
8. Linnet K, Bruunshuus I. HPLC with enzymatic detection as a candidate reference method for serum creatinine. Clin Chem. 1991;37:1669-1675.
9. Brittenham GM, Cohen AR, McLaren CE, et al. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. Am J Hematol. 1993;42:81-85.
10. Karam LB, Disco D, Jackson SM, et al. Liver biopsy results in patients with sickle cell disease on chronic transfusions: poor correlation with ferritin levels. Pediatr Blood Cancer. 2008; 50:62-65.
11. Alustiza JM, Artetxe J, Castiella A, et al. MR quantification of hepatic iron concentration. Radiology. 2004;230:479-484.
12. Gandon Y, Olivie D, Guyader D, et al. Noninvasive assessment of hepatic iron stores by MRI. Lancet. 2004;363:357-362.
13. Robson MD, Gatehouse PD, Bydder M, Bydder GM. Magnetic resonance: an introduction to ultrashort TE (UTE) imaging. J Comput Assist Tomogr. 2003;27:825-846.