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Quantitative susceptibility mapping (QSM) of white matter multiple sclerosis lesions: interpreting positive susceptibility and the presence of iron

Cynthia Wisnieff^{a,b}, Sriram Ramanan^c, John Olesik^d, Susan Gauthier^e, Yi Wang^{a,b,f}, and David Pitt^c

^aDepartment of Biomedical Engineering, Cornell University, Ithaca, New York, USA

^bDepartment of Radiology, Weill Cornell Medical College, New York, New York, USA

^cDepartment of Neurology, Yale School of Medicine, New Haven, Connecticut, USA

^dSchool of Earth Sciences, Ohio State University, Columbus, Ohio, USA

^eDepartment of Neurology, Weill Cornell Medical College, New York, New York, USA

^fDepartment of Biomedical Engineering, Kyung Hee University, Seoul, South Korea

Abstract

Purpose—Within multiple sclerosis (MS) lesions iron is present in chronically activated microglia. Thus, iron detection with MRI might provide a biomarker for chronic inflammation within lesions. Here, we examine contributions of iron and myelin to magnetic susceptibility of lesions on quantitative susceptibility mapping (QSM).

Methods—Fixed MS brain tissue was assessed with MRI including gradient echo data, which was processed to generate field (phase), R2* and QSM. Five lesions were sectioned and evaluated by immunohistochemistry for presence of myelin, iron and microglia/macrophages. Two of the lesions had an elemental analysis for iron concentration mapping, and their phospholipid content was estimated from the difference in the iron and QSM data.

Results—Three of the five lesions had substantial iron deposition that was associated with microglia and positive susceptibility values. For the two lesions with elemental analysis, the QSM derived phospholipid content maps were consistent with myelin labeled histology.

Conclusion—Positive susceptibility values with respect to water indicate the presence of iron in MS lesions, though both demyelination and iron deposition contribute to QSM.

Keywords

MS lesion; Quantitative susceptibility mapping; QSM; MRI; LA-ICP-MS iron quantification

Introduction

Recent work on multiple sclerosis (MS) with MRI has detected dynamic changes in magnetic susceptibility (1) and MRI phase (2) that reflect active demyelination and iron deposition in lesions. Iron retained by activated microglia/macrophages in MS lesions (3-5) may serve as a marker of innate immune system activation. Activated microglia are typically present at the vicinity of chronic active MS lesions and are the predominant source of iron within lesions (4,6,7). MRI and histological studies of iron report significant changes of iron content during lesion development from active demyelination to chronic inflammation and chronic inactivity (3,4,7,8).

Phase imaging and $R2^*$ mapping in MRI have been explored to assess lesion iron content qualitatively (3,4,6) and quantitatively (9,10). However, there are many confounding factors associated with $R2^*$ and phase imaging including dependence on orientation, field strength and distribution of susceptibility sources (11,12). These factors influence the heterogeneity of the field and subsequently the $R2^*$ (13,14). Particularly for MS lesion studies, magnetic susceptibility sources outside a lesion can also generate fields inside the lesion and hence contribute to $R2^*$ and phase of the lesion. These effects must be considered as well when interpreting the $R2^*$ or phase in order to investigate iron specific to MS lesions.

The tissue field derived from phase data can be deconvolved to generate quantitative susceptibility mapping (QSM) that reflects local tissue property (15-17), offering an alternative means to process gradient echo data. QSM can measure magnetic sources in tissue including iron, myelin and calcification that are present in normal and diseased brain tissue (1,18-22). A susceptibility tensor (23,24) can be used to model susceptibility anisotropy observed in ordered myelin that induces orientation dependence of $R2^*$ (25), phase (26,27) and apparent susceptibility (23,28-31). Both myelin and iron contribute significantly to the QSM value in white matter. The connection between QSM and iron content remains to be established for MS lesions.

The susceptibility of myelin is negative with respect to water. Therefore, severe or complete demyelination can increase susceptibility up to zero; when water is the reference. Positive susceptibility values must include contributions from paramagnetic material, which is iron in MS lesion. Here we propose to use an elemental measurement, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), to quantify the iron content of MS lesions in brain specimens. Histology and standard T2 weighted imaging (T2w) are compared with QSM to inform the interpretation of iron content within MS lesions.

Methods

MS brain section preparation

Human CNS tissue was obtained at autopsy of 1 MS subject (male, age 68, chronic MS, not treated with any disease-modifying treatment; cause of death: cardiac arrhythmia) according to an institutional Review Board-approved protocol. Seven 1 cm thick formalin fixed coronal MS brain specimens were imbedded in 1% agar for stabilization during MRI imaging.

Histology and LA-ICP-MS

Fixed tissue was transferred through sucrose, frozen in dry ice and *N*-methylbutane, and cut at 10 and 50µm thicknesses (6). For immunohistochemical labeling, 10 µm sections were incubated with primary antibody against myelin basic protein (polyclonal rabbit) and CD68 (monoclonal mouse IgG; both Dako) and processed with the appropriate biotinylated secondary antibody and avidin-biotin staining kit with diaminobenzidine as chromogen (Vector Laboratories Inc., CA), for Perl's stain sections, were treated in 10% formalin and then hydrated in distilled water, incubated in 5% potassium ferrocyanide with 10% Hydrochloric Acid solution in room temperature for 1.5hr and then microwaved. Sections were counterstained with hematoxylin.

LA-ICP-MS was performed on two lesions to generate iron concentration, [Fe], maps at $106 \times (110 - 250) \times 50 \mu\text{m}^3$ resolution from 50-micron sections (please see supplement for details). The LA-ICP-MS system was calibrated with gelatin, with known concentrations of Fe, before and after each line.

MRI

Sixteen orientations evenly distributed over a sphere on a GE 3T scanner were acquired using a 3D gradient echo (GRE) sequence in oblique planes with the following imaging parameters: 53.8ms TR, first echo 4.05ms, 4.15ms echo spacing, 12 acquired echoes at a resolution of $0.7 \times 0.7 \times 0.7 \text{mm}^3$, flip angle of 20° and bandwidth of 62.5kHz. A 32 channel receive coil was used to image the slab containing Lesion 1 and a 12 channel head coil was used to image all other slabs. For Lesion 1, T2w images were acquired with a 3D fast spin echo sequence with parameters: resolution, $0.332 \times 0.332 \times 2 \text{mm}^3$, TE 84.34ms, TR 1.067s, echo train length 48, field of view 17cm, acceleration factor 2 and a bandwidth of 100kHz. For all other lesions, T2w images were acquired with the same sequence, but parameters; acquired resolution $0.75 \times 0.75 \times 1.2 \text{mm}^3$, Echo time 103.3ms, Echo train length 140, repetition time 2.5s, field of view 24cm, bandwidth 125kHz.

Data Analysis

MRI to Histology Registration—2D LA-ICP-MS [Fe] maps and histological images were manually aligned to form a 3D volume; slices were distributed in the volume according to their relative location cut from the surface of the slab. A non-rigid 3D affine registration of the histology and COSMOS QSM images was calculated from corresponding fiducial markers placed within and around the lesion. MRI images were linearly interpolated to the resolution of the LA-ICP-MS [Fe] maps.

MRI Data Analysis—The total field was estimated from a fit of the complex signal evolution (32). Background field subtraction was performed using projection of dipole fields, PDF(33). Mono-exponential $R2^*$ was estimated with linear least squares estimation of the logarithm transformed multi-echo GRE data. Rigid linear registration of GRE orientations was performed with FLIRT and tri-linear interpolation (34-36). Multiple orientation reconstruction of QSM (COSMOS-QSM) from the tissue fields was calculated with COSMOS (15), Single-orientation morphology enabled dipole inversion QSM, (MEDI-QSM) (32) was reconstructed for each of the 16 orientations. The reference susceptibility in

all the QSMs was set to agar. Susceptibility tensor imaging, STI, reconstruction was performed with a conjugate gradient solver of the tissue field to source inverse problem (23). The fractional anisotropy, FA, of the STI of the slabs was estimated from a least squares fit of the definition of FA from diffusion tensor imaging(37),

$$\sqrt{\sum_i \lambda_i^2} FA = \sqrt{3/2} \sqrt{\sum_i (\lambda_i - \bar{\lambda})^2} \quad [1]$$

Where λ_i , $i=1,2,3$, are the eigenvalues of the tensor ordered from greatest to least in signed value and $\bar{\lambda} = (\lambda_1 + \lambda_2 + \lambda_3)/3$ is the normalized tensor trace, STI-Trace. R2* was estimated from a linear fit of the signal evolution in log space.

Segmentations of lesions were generated with a grayscale threshold chosen by selecting the half-maximum of the lesion edge along 20 profiles radially through the lesion edge on QSM and T2w images interpolated to eight times the GRE resolution to better define the edge; the mean volume detected from all of the half maximum thresholds is reported.

Susceptibility Contribution from Iron—Perl's staining is sensitive to Fe3+ in ferritin. LA-ICP-MS measures all iron forms including those in ferritin, oxyhemoglobin and deoxyhemoglobin. QSM is predominantly affected by paramagnetic iron, Fe3+ in ferritin and Fe2+ in deoxyhemoglobin. In the MS lesions, in vitro experiments show mostly ferritin in microglia (4). Therefore, [Fe] maps were converted to susceptibility with an estimate of the molar susceptibility of iron at room temperature like ferritin; 3.78 effective Bohr magnetons (38,39) and a brain tissue density of 1.04g/cm³(40). Using the Langevin equation, iron contributes, $\chi_{[Fe]} = 1.4\text{ppb} * [\text{Fe}]$, where [Fe] is in mg/kg. The myelin related susceptibility was calculated as $\chi_{\text{Myelin}} = \text{QSM} - 1.4\text{ppb} * [\text{Fe}]$, considering that myelin is a major susceptibility component in white matter (20,41,42).

Myelin Quantification from χ_{Myelin} —The mean molar susceptibility contribution of phospholipids, a major tissue component of myelin(43), was estimated to be $\chi_{PL} = -449\text{ppb}$ (L/mol) (see supplementary materials for details). The mean susceptibility was used to assess the myelin content independent of molecular order and thus applicable in both the lesion and NAWM. The mean susceptibility observed, χ_{obs} , is related to the phospholipid concentration [PL], $\chi_{obs} = \chi_{PL} [\text{PL}]$. The commonly used average molecular weight, 775g/mol (44), density, 0.25g/cm³(45), and χ_{PL} was used to estimate the phospholipid fraction, PF, within a voxel from the observed χ_{Myelin} . Regions of interest, the center and border of the lesions, were drawn on the T2*w image of the lesions registered to the histological data; the border was the hyper-intense rim surrounding the center of the lesion and normal appearing white matter (NAWM) was identified nearby within the [Fe] map. Susceptibility from iron and χ_{Myelin} were observed along profiles through the border of the lesion.

Results

MRI signal Properties of MS Lesions

Orientation dependence of the MEDI-QSM and $R2^*$ is apparent in the NAWM, Fig. 1 row 2 and 4, particularly in the corpus callosum (fig. 1 row 4, arrows). The images in the right column for the lesions, Fig. 1, were registered to the orientation in the left column, there was slight blurring of bright susceptibility values within the lesion apparent in the left column after registration. Less dependence with orientation was observed within the lesions in MEDI-QSM. The nonlocal field extended from the lesion and changed in appearance between orientations in phase images (Fig. 1a row 3, circle). The COSMOS-QSM is very similar to the normalized tensor trace, STI-Trace, which is expected to estimate the same mean susceptibility under uniform measurements of the sample, see supplemental materials for the mathematical verification.

The measured phase switched sign when the tissue slab orientation changed in the magnet (Fig. 1a row 3, white arrows). The 2nd row of fig. 1b also shows changes in the estimated FA from STI showing a decrease in anisotropy within the lesion compared to NAWM particularly for lesion 1. The trace of the STI and COSMOS-QSM appeared the same with only small noise differences (Fig. 1b Column 1 for lesions 1 and 2), which was also confirmed theoretically in the supplemental materials.

In Fig. 3, Perls stain shows varying degrees of iron deposition with the lesions in the right column and relatively little in the lesion in the left column. The QSM+ row shows locations of positive susceptibility where the lesion is completely demyelinated on MBP, which correlates to the centers of the lesion. In the lesions on the right both Perls and CD68 slides showed the most staining at the rim of the lesions.

Iron and Myelin Quantification

QSM correlated with the iron deposition [Fe] at the centers of Lesion 1 and Lesion 2 in Fig. 2. Lesion 1 had substantial iron content and correspondingly high paramagnetic susceptibility 29.6 ± 5.5 ppb (Table 1), but Lesion 2 had little iron and nearly zero diamagnetic susceptibility -1.6 ± 4.8 ppb (Table 1). There was no significant staining of iron containing CD68 labeled cells within Lesion 2. The PFs in Figs. 2 depicted loss of myelin in the lesion centers, consistent with the MBP labeled slides where PFs were close to 0 (Table 1). Row 5 shows decreased in $R2^*$ in both lesions correlating spatially with demyelination observed on MBP labeled slides, Fig. 2. CD68 labeling showed evidence of microglia associated with the iron detected on LA-ICP-MS at the border of Lesion 1. Fig. 2 showed iron uniformly distributed within and surrounding lesion 2, with few punctate iron sources, two lines were skipped in the LA-ICP-MS data acquisition (two green lines in $\chi_{[Fe]}$, Fig. 2). The lesion borders showed differing amounts of iron and myelin contributing to the total susceptibility; lesion 1 had a high iron contribution, 25.1 ± 6.1 ppb and a small myelin contribution -10.3 ± 12.5 ppb, whereas lesion 2 had a small iron contribution, 7.5 ± 2.1 ppb and a large myelin contribution -25.8 ± 6.9 ppb, table 1. Lesions with perls staining only, Fig. 3, show mean susceptibilities of -4.4 ± 8.7 ppb (left column), -1.2 ± 10.7 ppb and -2.8 ± 7.7 ppb (top

and bottom lesion in the right column of Fig. 4). The lesions in the right column showed highest peak susceptibility, 24.4 and 14.2ppb compared to 13.3ppb.

In Lesion 1 the contribution to the susceptibility near the border came from both iron and myelin, while the main contribution to the susceptibility at the edge of Lesion 2 came from myelin, Table 1. Fig. 4 shows a rapid increase in the myelin content at the immediate edge of Lesion 1 observed on T2w imaging. In this region, the iron content was high at the border and decreased to baseline NAWM within 2mm. The volumes of Lesion 1 were: COSMOS-QSM 312mm³ and T2w 245.4mm³, for Lesion 2: COSMOS-QSM 7.0mm³ and T2w 6.6mm³. In Lesion 1 where there is significant iron at the edge, the volume observed on QSM is 27% larger than the volume of the lesion on T2w, while in Lesion 2 there was only 5.6% difference in the volume of the lesion between T2w and QSM.

Discussion

While both iron and myelin can contribute to QSM, our results suggest the following two observations on interpreting iron from QSM of MS lesions in white matter. 1) When susceptibility values are above zero (relative to water), there is iron in lesion. This was corroborated on lesions with iron staining and can be explained by the physical limit that complete demyelination can only increase susceptibility values to zero. 2) Susceptibility values permit iron quantification in fully demyelinated locations, which was corroborated on lesions with iron quantification and myelin staining.

In general, the mixture of iron and myelin makes it difficult to identify iron specifically from gradient echo (GRE) MRI data using the three quantitative processing methods: tissue field (or phase), R2* and QSM. Deconvolution of the tissue field to generate QSM only removes contributions outside a voxel that affect both tissue field and R2*, but does not separate diamagnetic myelin from paramagnetic iron within that voxel. It may be possible to identify myelin based on the anisotropy of its magnetic susceptibility, which makes tissue field, R2* and QSM dependent on orientation as observed in Fig.1. Susceptibility tensor imaging fractional anisotropy (STI FA) decreases in a lesion compared to NAWM, but STI FA is very difficult to quantify and prone to noise and other artifacts and may be impossible to perform in routine clinical practice (28,30).

Previous results have been promising relating tissue iron content to measured R2* and QSM. In this data, iron deposition and myelin loss content effect QSM and R2* in opposite ways; R2* decreased within the center of both lesions though Lesion 1 showed significant iron deposition and correspondingly high susceptibility, Fig. 2. This decrease in R2* is consistent with an increase in the homogeneity of the tissue that occurs within MS lesions (46). Though R2* may not be directly related to iron content observed this data, together QSM and R2* reflect differences in the total magnetization and organization between NAWM and the lesion.

Most white matter lesions in MS brain specimens seem to be completely demyelinated (47), as observed in this study. For completely demyelinated lesion center, iron may be regarded as the main source for paramagnetic susceptibility, which was corroborated in Fig.4. Iron

deposition is associated predominantly with activated microglia/macrophages in the rim of demyelinating lesions (4), as observed in Fig. 3; iron is observed infrequently in the lesion center, as in Fig. 2 for lesion 1. The non-zero iron concentration varied in space in all lesions (Fig. 2&3). The huge difference in slice thickness between QSM and LA-ICP-MS makes it difficult to generate meaningful linear voxel-based linear regression, which would require multiple LA-ICP-MS samples through a QSM slice.

Interestingly, the lesion volume on QSM was observed to be bigger than that on T2w for lesions with iron (Fig. 4). QSM is sensitive to iron present outside lesion border and T2w is mostly sensitive to reduced cellular contents in lesion interior (48,49), resulting a larger volume on QSM. This QSM-T2w lesion volume difference could help identify the presence iron at the border of the lesion, where QSM alone cannot identify iron as both iron and myelin contribute to the measured magnetic susceptibility. Additional in vivo measurement of tissue composition, such as myelin water fraction data (50), may help to define the region of demyelination. It has been observed in vivo that T2w is highly sensitive to tissue inflammation increasing the observed lesion volume (1). Our T2w results support that MRI measurements of specific tissue content may potentially identify the spatial distribution of myelin and iron within MS lesions.

While this study focused on MS lesions, NAWM iron content was also measured. These NAWM iron values are ~4-5x smaller than previously observed in normal white matter within non-neurological controls (18). However, this iron content is consistent with observations of NAWM in autopsied MS brains where densitometric measurements showed NAWM 0.03 and normal white matter 0.13 (7).

One disadvantage of our study is that we examined MS lesions from one individual. Similar comparative work is usually also restricted to a small number of autopsy cases (3,4). Registration between MRI and histology sections is not trivial and may result in registration errors from differences in resolution and image contrast between techniques. The best registration results were found when using all histological data in the formation of a volume that was used to match multiple anatomical features present in both MR and histology. The focus of this work is in the assessment of sources of susceptibility in the lesions that contribute to the measured susceptibility observed with QSM. More pathology will be assessed in future work.

Conclusion

In this work, spatial resolution of iron distributed within and surrounding MS lesions identified iron contributions to the measured susceptibility with QSM across the anatomy of MS lesions. Positive susceptibility values in MS lesion are associated with iron presence in histology. In completely demyelinated lesion centers, susceptibility values are consistent with susceptibility contributions from ferritin measured by laser ablation inductively coupled plasma mass spectrometry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a, shows measurements of the GRE images within Lesion 1 and 2 between two different B_0 directions. From top to bottom, $T2^*$ weighted image, $R2^*$, the tissue field (phase), single orientation MEDI-QSMs. b, displays the COSMOS-QSM and the normalized STI-Trace, the second column shows a $T2w$ image and the FA estimated from the STI for the lesions. The circle corresponds to the lesion, and the arrows point to NAWM.

Figure 2.

MRI and histology data on Lesion 1 (left) and 2 (right) the top row shows the QSM of the lesion registered with the histological sections, the next the $\chi_{[Fe]}$ map, the phospholipid fraction (PF), the tissue field (TF), R2*, the CD68 and MBP labeled sections. Lesion 1 contains a significant amount of iron within the lesion in LA-ICP-MS which is nearly all accounted for in the QSM, consistent with the demyelination observed in the MBP labeled section and PF. Lesion 2 shows little iron deposition.

Figure 3.

MRI and histology data on three additional lesions, the lesion on the left column shows little iron on Perl's and two lesions on the right with varying iron staining. The 1st row (1) shows T2w, (2), only positive susceptibility w.r.t. agar, (3) QSM, (4) MBP, (5) CD68 and (6) Perl's.

Figure 4.

$\chi_{\text{Fe:ICP}}$, QSM and χ_{Myelin} across a profile through the lesions into NAWM; in lesions 1 the profile extents from within the lesion (location 0) to a location in NAWM. The gray bar indicates the location of the edge of the lesion as displayed on the registered T2w image.

Table 1

Measurements of [Fe], total susceptibility from QSM, $\chi_{\text{Fe:ICP}}$, χ_{Myelin} and phospholipid fraction within the lesions and surrounding NAWM.

	[Fe] mg/kg	QSM (ppb)	$\chi_{\text{Fe:ICP}}$ (ppb)	χ_{Myelin} (ppb)	Phospholipid fraction (PF)	
Lesion 1	Lesion	18.9±5.3	29.6±5.5	26.5±7.5	3.0±8.2	-0.02±0.06
	Border	17.9±4.3	14.8±12.9	25.1±6.1	-10.3±12.5	0.07±0.09
	NAWM	7.6±1.1	-23.2±1.4	10.7±1.5	-33.9±2.6	0.23±0.02
Lesion 2	Lesion	4.3±0.5	-1.6±4.8	6.0±0.6	-7.7±4.6	0.05±0.03
	Border	5.4±1.5	-18.3±6.6	7.5±2.1	-25.8±6.9	0.18±0.05
	NAWM	5.1±0.9	-42.5±1.2	7.1±1.2	-49.7±1.8	0.34±0.01