Measuring Demyelination and Remyelination in Acute Multiple Sclerosis Lesion Voxels

Paul S. Giacomini, MD, FRCPC; Ives R. Levesque, MSc; Luciana Ribeiro, MD; Sridar Narayanan, PhD; Simon J. Francis, MSc; G. Bruce Pike, PhD; Douglas L. Arnold, MD, FRCPC

Objective: To validate the use of the magnetization transfer ratio (MTR) as a practical imaging marker of demyelination and remyelination in acute multiple sclerosis lesions.

Design: Case study.

Setting: University hospital multiple sclerosis clinic.

Patients: Six patients with relapsing-remitting multiple sclerosis and acute gadolinium-enhancing lesions were studied serially using a quantitative magnetization transfer examination.

Main Outcome Measures: Changes in the water content and macromolecular content, a marker of myelin content that, unlike MTR, is not affected by changes in water content (edema) associated with acute inflammation, and changes in MTR of lesions.

Results: Both the macromolecular content and MTR were lower than normal in acute lesions and recovered over several months. The decrease in macromolecular content relative to contralateral normal-appearing white matter was greater than the decrease in MTR (0.46 vs 0.75 at the time of gadolinium enhancement), likely because edema in the acute lesion increased the T1 relaxation time of water and attenuated the decrease in MTR. Nevertheless, there was still a strong correlation between changes in the relative MTR and macromolecular content ($R^2=0.70; P<.001$).

Conclusion: Our data support the use of MTR as a practical marker of demyelination and remyelination, even in acute lesions where decreases in MTR are attenuated because of the effects of edema.

Arch Neurol. 2009;66(3):375-381
romolecules and free water protons to enable MRI of semisolid components of tissue such as myelin membranes, whose T2 relaxation times are too short for them to be imaged directly. By generating voxel-by-voxel maps of the percentage of decrease in signal caused by an MT saturation pulse, the MT effect can be quantified to generate an MTR image. In white matter, the MT effect is dominated by the constituents of myelin, and changes in the MTR values observed in MS correlate with changes in myelin content in the white matter. The MTR is a relatively stable measure in adult white matter and chronic lesions. In animal models, MTR decreases with acute demyelination and increases with remyelination. Imaging studies on postmortem, fixed brains of patients with MS have supported this relationship by demonstrating a strong correlation between measured myelin content on pathologic samples and MTR. The MTR decreases acutely in acute gadolinium-enhancing lesions, consistent with demyelination, after which variable recovery can follow over the subsequent months. However, the MTR change can also be affected by changes not specific to myelin, such as increased water content and changes in tissue relaxation times, potentially complicating the interpretation of MTR changes in the context of acute gadolinium-enhancing lesions. Magnetization transfer studies using models of acute inflammation and demyelination have raised questions about the specificity of MTR vs other MRI-derived markers of myelin content. Changes in MTR in models of inflammation with limited demyelination also demonstrate a decrease in the MTR, raising concerns about the degree to which MTR changes reflect changes in myelin content in acute lesions.

A comprehensive quantitative interpretation of MT characteristics provides a more complete description of the MT phenomenon and overcomes some of the limitations of MTR. This approach was first adapted for human in vivo quantitative MT imaging (qMTI) studies by Sled and Pike, with other groups since introducing related techniques. This approach enables the generation of quantitative maps of all observable parameters of the binary spin-bath model for MT, particularly the fractional size of the restricted pool, as well as relaxation time constants. The ratio of restricted to free pool sizes, F, quantifies macromolecular over liquid content within an individual voxel, and in white matter, F is expected to provide a more accurate estimate of myelin content. Given that F represents a ratio of macromolecular content (MMC) over liquid content, in this study we implemented a method to correct for local increases in tissue water that would dilute the macromolecular proton fraction by measuring the water content relative to the contralateral, homologous NAWM. The resultant measurement, the relative MMC, represents the proton density of the MMC and should be relatively insensitive to local edema. A recently published postmortem study investigated the relationship of quantitative MT (qMT) parameters to histological measures and found strong associations between the measure of macromolecular proton fraction and myelin density as well as to axonal count.

In this study, we explored the relationships between changes of MMC, an estimate of myelin content that should be insensitive to edema, and changes of MTR in contrast-enhancing voxels of acute MS lesions, to determine whether MTR can be used as a marker of myelin content change in acute MS lesions.

**METHODS**

To evaluate the reliability of MTR within acute MS lesions, we studied a single acute gadolinium-enhancing lesion in 6 patients serially over time using qMTI to assess MMC and compared these estimates with MTR measures. The qMT parameters, F, has been shown to be a reliable marker of the MMC in previous work. The fractional size of the restricted pool, F, is directly estimated as part of the binary spin-bath model, and it represents the ratio of the size (or proton density) of the restricted pool (macromolecules) to the proton density of the free pool (water). As a result, F is sensitive to changes in both the restricted and free proton pool sizes. To estimate the size of the restricted pool, it is necessary to obtain an independent measure of the free pool. One can measure water content independently by computing the total observable signal in a spin-echo experiment, correcting for incomplete recovery of longitudinal magnetization due to the finite repetition time of the spin-echo sequence, and, finally, normalizing by a signal of known proton density.

In the absence of an external standard, the water content can be computed relative to a specific region of interest within the brain that, although not an absolute indicator of water content, can provide an index of relative changes in liquid content. In this study, we computed the proton density of the free pool in lesions relative to a region of homologous NAWM contralateral to the lesion. Thus, the contralateral region has a water content of 1, by definition. Water increases in the lesion compared with NAWM should be reflected by values greater than 1. By extension, we can define the relative MMC as the product F × relative water content. In principle, MMC more accurately reflects changes in the restricted pool size and should not be influenced by changes in lesion water content associated with inflammatory edema. In essence, MMC decreases provide a more accurate estimate of demyelination that is less influenced by dilution from the increased water content that is present in acute lesions.

**SUBJECT COHORT**

This study was conducted on a cohort of 6 patients with definite MS who had clinical evidence of an acute relapse and an acute gadolinium-enhancing lesion at the time of the first scan. Based on unpublished results from our group that showed an average observed longitudinal variability in F of 6% in controls, we estimated that 6 patients would be enough to observe a 25% change in MMC with a power of 0.95 (1-tailed t test). Research ethics board approval was obtained and each participant provided written informed consent. Inclusion criteria included a diagnosis of relapsing-remitting MS, an acute gadolinium-enhancing hemispheric lesion, and age older than 18 years. Patients were excluded if they were pregnant or unable to receive gadolinium or if they had received steroids prior to the baseline scan. All patients underwent clinical evaluation by a neurologist prior to the baseline scan and at subsequent intervals of 3 months. Patients’ baseline demographics are shown in the Table. Each patient was scanned serially on a monthly basis for at least 6 months and at least quarterly thereafter. Steroid use was permitted after the baseline scan. Two of the patients received steroids (intravenous methylprednisolone, 1 g for 5 days, no taper) following their baseline scan and 2 other patients received corticosteroids during the observational pe-
iod for new symptoms due to relapses that occurred but were unrelated to the lesion being studied. The remaining 2 patients did not receive any corticosteroid treatment during the study period. Only 1 patient showed a lesion with gadolinium enhancement that persisted beyond the baseline scan. The enhancement in that lesion subsequently resolved the following month without treatment. The intended observational period was to be 12 months, but several patients were not able to attend or were awaiting their final scan at the time of article submission. Three patients missed one of their monthly scans during the first 6-month interval (points 0-5). Thus, a total of 46 patient points were analyzed.

### DATA ACQUISITION

Imaging was performed on a 1.5-T Siemens Sonata whole-body scanner (Siemens Medical Systems, Erlangen, Germany). Each patient was scanned using our quantitative MRI protocol, which consists of high-resolution structural scans, a qMTI protocol, and T1 and T2 relaxometry sequences. Magnevist (Bayer HealthCare Pharmaceuticals Inc, Wayne, New Jersey) gadolinium contrast was given at a dose of 0.2 mL/kg. Total scan time was approximately 70 minutes per subject. High-resolution volumetric T1-weighted data were acquired using a 3-dimensional spoiled gradient echo sequence. Proton density- and T2-weighted images were acquired using turbo spin echo sequences. Quantitative MT, T1, and T2 data were acquired in a single 7-mm slice, as previously described,19,20,30 including field maps of the static and radio frequency fields.11 The quantitative imaging slice was centered on the gadolinium-enhancing lesion on images from the initial examination and repositioned as accurately as possible on all subsequent examinations.

### DATA ANALYSIS

Images underwent several steps of preprocessing and analysis, including linear image registration of the subsequent scans to the first scan to enable us to study evolution of individual voxels in a longitudinal manner. The gadolinium-enhancing voxels were manually segmented on the high-resolution anatomical scans, and labels corresponding to the initial gadolinium-enhancing lesion voxels were propagated to subsequent points using software developed in our laboratory (Figure 1). The qMTI parameter maps were generated as previously reported.19,27 The labels generated on the structural scans were resampled to the resolution of the qMTI scans (2 × 2 × 7 mm³ voxel size) (Figure 2). Because of the resampling process, the larger qMTI voxels could contain combinations of lesion and surrounding normal-appearing tissues. To minimize this partial-volume effect, our analysis included only qMTI voxels that had at least 80% of their volume filled by gadolinium-enhancing voxels. The quantitative values for the label volumes were extracted from the parameter maps. Subsequent data analysis was performed using JMP IN 5.1 (SAS Institute JMP, Cary, North Carolina). Statistical significance between baseline and subsequent qMTI values was calculated using the t test.

As noted earlier, measures of MTR, F, and MMC from the lesions were normalized to contralateral, homologous NAWM to yield relative measures. The values presented in the “Results” section therefore represent a proportion of the values in NAWM.

### RESULTS

The baseline normalized MTR in lesion voxels was mean (SD) 0.75 (0.05) at the time of gadolinium enhancement. The value rose to 0.84 (0.09) after 1 month and to 0.86 (0.07) by the second month postenhancement. The difference in normalized MTR was statistically significant between baseline and the points from month 2 postenhancement onward (P < .001) (Figure 3).

The normalized MMC was mean (SD) 0.46 (0.10) at baseline. The normalized MMC increased to 0.74 (0.24) the following month and 0.80 (0.13) the second month postenhancement. The differences in normalized MMC from baseline to month 1 and month 2 onward were statistically significant (P = .007 and < .001, respectively) (Figure 3). Normalized F closely paralleled the normalized MMC and was similarly significant.

Conversely, the normalized water content values were proportionally greater than the water content in the contralateral homologous NAWM. The lesion to NAWM water content ratio was mean (SD) 1.14 (0.07) at baseline and decreased to 1.09 (0.07) the following month and 1.04 (0.04) by the second month post–gadolinium enhancement. These findings are consistent with increased fluid content in the acute gadolinium-enhancing voxels due to edema and subsequent resolution of this edema as the lesion evolved over time. The differences in normalized water content were statistically significant between baseline and points later than month 2 (P = .009) (Figure 3).

Figure 3 also illustrates the combined trends of normalized MTR, MMC, and water content and shows the similar trends in normalized MTR and MMC as well as the initial elevation of normalized water content that subsequently decreases with time.

We found the normalized MTR and MMC to be highly correlated (R²=0.70; P value < .001) (Figure 4). The MMC also correlated well with the observed T1 relaxation

### Table. Baseline Demographics of the Study Participants

<table>
<thead>
<tr>
<th>Sex/ Age, y</th>
<th>EDSS Score</th>
<th>Disease Duration, y</th>
<th>Disease-Modifying Therapy (Baseline)</th>
<th>Disease-Modifying Therapy During Observational Period (Point After Which Therapy Initiated)</th>
<th>Points Included</th>
<th>T2 Lesion Volume, mm³</th>
<th>Gadolinium Lesion Voxel Volume, mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/56</td>
<td>2.0</td>
<td>10</td>
<td>None</td>
<td>None</td>
<td>0, 1, 2, 3, 4, 5, 9</td>
<td>11,699 134</td>
<td></td>
</tr>
<tr>
<td>F/41</td>
<td>4.0</td>
<td>3</td>
<td>Glatiramer acetate</td>
<td>Natalizumab (point 9)</td>
<td>0, 1, 2, 3, 4, 5, 9</td>
<td>18,831 398</td>
<td></td>
</tr>
<tr>
<td>F/49</td>
<td>4.0</td>
<td>15</td>
<td>Glatiramer acetate (point 5)</td>
<td>Glatiramer acetate (point 1)</td>
<td>0, 1, 2, 3, 5, 9</td>
<td>15,490 429</td>
<td></td>
</tr>
<tr>
<td>F/42</td>
<td>2.0</td>
<td>2</td>
<td>Glatiramer acetate (point 1)</td>
<td>Glatiramer acetate (point 1)</td>
<td>0, 1, 2, 3, 4, 5, 9</td>
<td>32,939 186</td>
<td></td>
</tr>
<tr>
<td>F/25</td>
<td>2.5</td>
<td>1</td>
<td>None</td>
<td>Interferon beta-1B (point 4)</td>
<td>0, 1, 2, 4, 5, 6, 8, 10</td>
<td>28,993 83</td>
<td></td>
</tr>
<tr>
<td>F/49</td>
<td>1.0</td>
<td>1</td>
<td>Interferon beta-1A subcutaneous (point 6)</td>
<td>Interferon beta-1A subcutaneous (point 6)</td>
<td>0, 1, 2, 3, 4, 5, 6, 8, 9</td>
<td>6884 501</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: EDSS, Expanded Disability Status Scale.
(R² = 0.79; P value < .001). However, observed T1 also showed high correlation with water content (R² = 0.70; P value < .001), more so than MTR (R² = 0.50; P value < .001).

**COMMENT**

Magnetization transfer ratio imaging is available on most modern scanners and, with appropriate supervision, can be practically implemented in multicenter clinical trials. The MTR is sensitive to changes in the myelin content of cerebral white matter, and changes in MTR in postmortem imaging of fixed tissue are strongly correlated with changes in myelin content. However, MTR is also sensitive to changes in the T1 relaxation time of tissue water, and in the context of an acute MS lesion in which there is active inflammation and edema, it is reasonable to question how changes in free water might affect...
the relationship of MTR to myelin content. A previous study using a model of inflammation with limited demyelination suggested that MT imaging might become an unreliable measure of myelin content in the setting of inflammation because of the inflammation and associated pH changes. However, the decrease in F observed in that study could have been due to myelin pathology, which is clearly present in their model. To explore how MTR changes, which are known to be affected by changes in tissue T1 relaxation time, behave in acute MS lesions that are liable to have increases in their free water content due to edema, we determined the relation of MTR changes to changes in the MMC of tissue. We defined and calculated the relative MMC of cerebral white matter to provide a theoretically more accurate reflection of myelin content, since it is based on independent measures of the different tissue water components and relaxation times.

Water content in the gadolinium-enhancing lesions was increased by approximately 15%. Both the MTR and MMC were decreased in these lesions to an extent that could not be explained by dilution alone, and both partially recovered over the next 2 months. The MMC also closely mirrored the changes seen in F, suggesting that

Figure 2. Quantitative magnetization transfer imaging parameter map (A) and corresponding label on high-resolution conventional sequence (B). Labels are segmented on conventional images and then combined with parameter map to extract quantitative parametric information from region of interest label.

Figure 3. Mean (SEM) normalized magnetization transfer ratio (MTR), fractional size of the restricted pool (F), macromolecular content (MMC), and water content vs time. Gd indicates gadolinium; NAWM, normal-appearing white matter.

Figure 4. Correlation of mean normalized magnetization transfer ratio (MTR) and mean normalized macromolecular content (MMC). NAWM indicates normal-appearing white matter.
changes in F also reflect demyelination and remyelination, even in acute lesions, despite the fact that F is also sensitive to changes in water content.

The normalized MMC value in acute lesions was substantially lower at the time of enhancement than the normalized MTR (0.46 vs 0.75). This difference likely reflects the fact that lengthening of the T1 relaxation time of tissue water associated with edema in acute lesions attenuates the decrease in MTR. Thus, MTR may underestimate the decrease in myelin density in acute lesions.

Despite the effects of changes in T1 relaxation time and dilution on the MTR, there was still a strong correlation between the normalized MTR and MMC ($R^2=0.70$; $P<.001$) over the whole time course of lesion evolution. This correlation was comparable with previously published correlations between MTR and the qMT parameter F in chronic MS lesions, which showed an $R^2$ of 0.66. Correlations were also seen between the MMC and observed T1 relaxation time because of associated changes in water content; despite this correlation, the T1-weighted signal is inherently nonspecific, making it a less attractive marker of myelin content changes.

Our study was not designed or powered to elucidate the influence of corticosteroid use on these quantitative parameters, and we did not find any significant differences among any of the values.

As advantageous as the MMC seems as a marker of myelin content, it is not a practical technique for the purposes of clinical trials. It is not a standard pulse sequence on commercial scanners, it is time-consuming to acquire (at least 15 minutes for a single 7-mm slice), and it is very labor intensive to calculate. Conversely, the MTR is widely available, fast and easy to acquire, and has sufficient specificity to monitor changes in myelin content in the clinical trial setting. This study did have some limitations that could affect the generalizability of the results, such as the small sample size, a relatively narrow range of Expanded Disability Status Scale scores, and the fact that all our patients were female. Nevertheless, despite its limitations, this study establishes that MTR is a reliable marker of myelin content change even in the acute inflammatory context.

Fortuitously, since part of the decrease in myelin density in MS lesions was due to dilution from increased edema rather than demyelination per se, the attenuation of the decrease in MTR by edema served to partially compensate for the overestimation of demyelination that would occur if the decrease in our markers of myelin density were assumed to be entirely due to demyelination. Therefore, these data support the notion that the MTR is indeed a useful, practical marker of demyelination and remyelination, even in acute lesions, and support the use of MTR as a marker of myelin content changes in clinical trials.

Accepted for Publication: September 4, 2008.

Correspondence: Paul S. Giacomini, MD, FRCPC, McConnell Brain Imaging Centre, Montreal Neurological Institute, 3801 University, WB 327, Montreal, QC H3A-2B4, Canada (paul.giacomini@mail.mcgill.ca).

Author Contributions: Study concept and design: Giacomini, Ribeiro, Narayanan, Pike, and Arnold. Acquisition of data: Giacomini, Levesque, Ribeiro, Narayanan, Francis, and Pike. Analysis and interpretation of data: Giacomini, Levesque, Narayanan, and Pike. Drafting of the manuscript: Giacomini and Arnold. Critical revision of the manuscript for important intellectual content: Levesque, Ribeiro, Narayanan, Francis, Pike, and Arnold. Statistical analysis: Giacomini and Narayanan. Obtained funding: Narayanan, Pike, and Arnold. Administrative, technical, and material support: Giacomini, Ribeiro, Narayanan, Francis, Pike, and Arnold. Study supervision: Narayanan, Pike, and Arnold.

Financial Disclosure: None reported.

Funding/Support: This work was supported by a grant from the Multiple Sclerosis Society of Canada. Dr Giacomini's fellowship funding was supported by the Biogen Idec Canada MS Clinical Fellowship administered by the Canadian Network of MS Clinics.

REFERENCES

31. Sled JG, Pike GB. Correction for B(1) and B(0) variations in quantitative T(2) measurements using MRI. Magn Reson Med. 2000;43(4):589-593.

New Initiatives: Clinical Trials and Videos

We have embarked on 2 new initiatives: Clinical Trials and video presentations. We welcome manuscripts that describe double-blind, randomized, placebo-controlled clinical trials as our primary area of interest. Open-label studies will also receive our special attention. We plan on expediting the review process and time to publication and to include them online ahead of print as these studies are time sensitive and of direct benefit to our patients. We hope you will take advantage of this new initiative. Please refer to the Instructions for Authors when submitting a Clinical Trials paper, including the requirement to register the trial with an accepted clinical trials site.

We plan to utilize videos as part of published papers that highlight and provide convincing information about the observational and visual features of a patient’s neurologic findings. Please refer to Instructions for Authors for instructions on submitting video presentations.