Magnetization Transfer Ratio in Gray Matter
A Potential Surrogate Marker for Progression in Early Primary Progressive Multiple Sclerosis
Zhaleh Khaleeli, MRCP; Daniel R. Altmann, DPhil; Mara Cercignani, PhD; Olga Ciccarelli, PhD; David H. Miller, FRCP; Alan J. Thompson, FRCP

Background: Magnetization transfer imaging has the potential to provide a surrogate marker for progression in primary progressive multiple sclerosis (PPMS).

Objectives: To investigate whether brain magnetization transfer imaging, T2 lesion load, and atrophy changes over 3 years reflect concurrent clinical changes, and which baseline imaging measure best predicts progression over 3 years in early PPMS.

Design: Prospective study.


Patients: Forty-seven patients with PPMS (of whom 43 completed the study) and 18 control subjects.

Interventions: Brain magnetization transfer imaging (including T2-weighted images) and volume sequences every 6 months for 3 years.

Main Outcome Measures: Changes in Expanded Disability Status Scale (EDSS) score and associations with rate of change in imaging variables.

Results: More rapid decline in gray matter mean and peak location magnetization transfer ratio and T2 lesion load increase were associated with greater rates of progression on the EDSS. Baseline gray matter peak height magnetization transfer ratio best predicted progression over 3 years.

Conclusion: Gray matter magnetization transfer ratio meets many of the criteria for a surrogate marker of progression in early PPMS.

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Wide variations in the evolution of disability in primary progressive multiple sclerosis (PPMS) pose challenges for clinical management. The absence of predictive markers for clinical outcome has affected clinical trials, such as the glatiramer acetate study in PPMS, which was terminated early owing to a lack of the anticipated clinical progression. In addition, robust surrogate markers are needed to detect treatment effects. The potential of magnetic resonance (MR) imaging for this purpose was explored by a working group in 1999. The group adopted the criteria described by Prentice, stating that a surrogate marker should predict future clinical disability. In establishing these criteria, the requirement that the marker should change concurrently with clinical status was not discussed. However, this attribute is clearly advantageous because it allows monitoring of contemporaneous, as well as future, treatment effects. In addition, the criteria state that any intervention must alter both the surrogate marker and clinical outcome by the same mechanism.

Having examined several MR imaging measures in early PPMS, we achieved the best clinicoradiologic correlations by using magnetization transfer imaging. A reduction in magnetization transfer ratio (MTR) is thought to reflect demyelination and axonal loss. The MTR is an indirect measure of macromolecular density, derived from the reduction in magnetization exchange between free water protons and those bound to macromolecules when the bound protons are selectively saturated with an off-resonance pulse. In early PPMS, we found that MTR and volume reduction in both gray matter and normal-appearing white matter (NAWM) correlated with disability in cross-sectional studies and that baseline NAWM MTR predicted short-term clinical progression over 1 year.

In this study, we examined the potential of MTR as a surrogate marker to moni-
tor progression in early PPMS. First, we assessed whether changes over 3 years in MTR, brain volume, or T2 lesion load reflected concurrent clinical changes. Second, in a medium-term study suited to clinical trial design, we investigated whether baseline MTR was a better predictor of clinical progression than the other 2 MR measures.

METHODS

SUBJECTS

Forty-seven patients with definite or probable PPMS,11 within 5 years of symptom onset, were invited for radiologic assessment at baseline and every 6 months for 3 years (Table 1). Patients were scored on Kurtzke's Expanded Disability Status Scale (EDSS)12 at each time point. Patients who missed either the baseline (1 patient, who had to leave the assessment early) or the 3-year (4 patients) assessment were retained in the study because they attended multiple intermediate time points. The reasons for nonattendance at 3 years were death (2 patients, causes unrelated to multiple sclerosis [MS]), illness (1 patient, unrelated to MS), and withdrawal from the study (1 patient; Table 2). Clinical data were obtained in person or by telephone13 for patients who became too disabled to undergo imaging during the study. None of the patients was taking disease-modifying medications. One patient had received a single course of intravenous corticosteroids for a deterioration of symptoms, and 2 patients were taking oral corticosteroids every 3 months (the exact interval between corticosteroid administration and each time point was unknown).

Eighteen healthy control subjects also underwent imaging (Table 1). All participants gave written informed consent. The study was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology, London, England.

MR IMAGE ACQUISITION AND PROCESSING

The imager (1.5 T; Signa; General Electric Co, Milwaukee, Wisconsin) was upgraded during the study, and the gradient amplifiers, but not the gradient coils, were changed. Maximum gradient strength increased from 22 milli-Tesla per meter (mTm⁻¹) to 33 mTm⁻¹. The imager software was upgraded from version 5x to version 11x. The voxel MTR was calculated and the segment probability maps and location (PL) were extracted. Mean MTR was measured for the lesion segment. and volumes were calculated in SPM2. We avoid the term normal-appearing gray matter because gray matter lesions are not visible on T2-weighted images at 1.5 T. Gray matter and NAWM volumes were normalized by dividing the total intracranial volume (sum of the gray matter, NAWM, lesion, and cerebrospinal fluid volumes) and multiplying by 100 to produce percentage gray matter and NAWM fractions (PGMF and NAWM MTR).

STATISTICAL ANALYSIS

Analysis was carried out with Stata 9.2 statistical software (Stata-Corp, College Station, Texas).

Clinical Data

Raw EDSS scores at baseline and 3 years were compared by means of the Wilcoxon matched-pairs signed rank test. Changes in EDSS were converted into steps. For predictors of clinical outcome, 3 step change categories were created: stable EDSS, mild progression (deterioration of 0.5-1.5 steps), and marked progression (deterioration of ≥2 steps).

Rates of Change in Brain MTR, Volume, and T2 Lesion Load

For the following analyses, except those predicting EDSS outcome, piecewise mixed-effect linear regression models were fitted. The models used random intercept and random time coefficient. Such models are designed to estimate gradients of change in repeated measures within subjects, taking account of both intrasubject and intersubject variability. To adjust for the upgrade, 2 separate trajectories, with common gradient, were fitted before and after the upgrade but estimated simultaneously.

Table 1. Baseline Clinical and Imaging Characteristics of Patients and Control Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Controls</th>
<th>P Valueab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range), y</td>
<td>45.1 (19-65)</td>
<td>34.6 (27-52)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sex, No. M/F</td>
<td>28/19</td>
<td>8/10</td>
<td>.08</td>
</tr>
<tr>
<td>Median EDSS score (range)</td>
<td>4.75 (1.5-7)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Mean T2 lesion load, mL</td>
<td>30.3</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Mean gray matter volume (SD)</td>
<td>710.4 (78.6)</td>
<td>726.4 (70.6)</td>
<td>.001</td>
</tr>
<tr>
<td>Mean NAWM volume (SD)</td>
<td>369.2 (50.5)</td>
<td>395.5 (58.3)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS, Expanded Disability Status Scale; MTR, magnetization transfer ratio; NA, not applicable; NAWM, normal-appearing white matter; PGMF, percentage gray matter fraction; PH, peak height; PL, peak location; PNAWMF, percentage normal-appearing white matter fraction; PU, percentage units.

a Adjustments for age and sex differences between the patient and control group were made at each stage of the analysis, as described in the "Methods" section.

b P values were derived from unpaired, 2-tailed t tests for patient vs control variables.

PNNAWMF). The voxel MTR was calculated and the segment probability maps were applied to the MTR images as previously described. Normalized MTR histograms were generated for gray matter and NAWM (bin width, 0.1 percentage unit [PU]; smoothing window, 0.3 PU), and the mean, peak height (PH), and peak location (PL) were extracted. Mean MTR was measured for the lesion segment.

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within a single model. The assumption of common gradient was tested, and if the gradient was unchanged, the model gave the rate of change, adjusting out the discontinuity due to upgrade. The model assumes linearity; however, nonlinearity was tested for by adding a quadratic term in time, and none was found.

To determine mean annual rates of MTR change in patients vs controls, mean, PH, and PL values for gray matter and NAWM MTR were modeled in turn as response variables. The covariates were as follows: binary upgrade indicator (0, before the upgrade; 1, after the upgrade), time (centered on the upgrade date to adjust for an imager upgrade effect), patient/control indicator, patient × time interaction, age, and sex. The mean annual rate of lesion MTR change in patients was calculated in the same way. No upgrade-induced change in gradient was found. For brain volume changes, age was significant, so an age × time interaction was added. The model was repeated with the use of PGMF and PNAWMF. In patients, lesion load changes were similarly modeled, with an age × time interaction. To assess the relationship with change in EDSS in patients, the same MTR and volume variables were modeled in turn as response variables, and clinical change and clinical change × time were additional covariates.

Baseline MR Imaging Predictors of EDSS Change

Multiple proportional odds ordinal logistic regression was used. Ordinal categories of EDSS step change were the response variables, and baseline MTR and volume measures, age, and sex were covariates. Predictors were modeled individually, then the most significant predictor from each modality was selected for each segment (gray matter, NAWM, and lesions) and modeled together to identify the best overall predictor. Baseline imaging was performed before the upgrade, so no adjustment was necessary.

RESULTS

All models were adjusted for age and sex.

CHANGES IN EDSS SCORES AND MR IMAGING VARIABLES OVER 3 YEARS

Median raw EDSS scores increased from 4.5 to 6 (P < .001). Fourteen patients remained stable, and 14 demonstrated mild and 15 marked progression. In patients, mean gray matter MTR declined by 0.60 PU and mean NAWM MTR by 0.26 PU (P < .001 in both cases; Table 3). Lesion MTR decreased by 0.77 PU, at a rate of 0.26 PU per year (P = .002; 95% confidence interval [CI], 0.09-0.42).

In patients, mean gray matter volume decreased by 12.0 mL, at an annual rate of −3.98 mL, 0.26% of the original gray matter volume (P < .001; 95% CI, −5.82 to −2.15).

Table 2. Number of Patients Assessed at Each Time Point and Reasons for Nonattendance

<table>
<thead>
<tr>
<th>Time Point, mo</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
<th>30</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Patients Assessed</strong></td>
<td>47</td>
<td>37</td>
<td>36</td>
<td>33</td>
<td>34</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>Imaging performed</td>
<td>46</td>
<td>34</td>
<td>33</td>
<td>33</td>
<td>30</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>Clinical assessment only, done in person</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Clinical assessment only, done by telephone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Patients Who Did Not Attend</strong></td>
<td>0</td>
<td>10</td>
<td>11</td>
<td>14</td>
<td>13</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Withdraw from study</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Personal commitments</td>
<td>0</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Non–multiple sclerosis–related illness</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>1</td>
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<tr>
<td>Upgrade</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
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</table>

Table 3. Mean Annual Rates of Change in Gray Matter and NAWM MTR in Patients and Controls

<table>
<thead>
<tr>
<th>MTR Histogram</th>
<th>Yearly Rate (P Value)</th>
<th>95% CI</th>
<th>Yearly Rate (P Value)</th>
<th>95% CI</th>
<th>P Value, Patients vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, PU</td>
<td>−0.20 (&lt;.001)</td>
<td>−0.25 to −0.15</td>
<td>0.02 (.62)</td>
<td>−0.05 to 0.08</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PH, PV</td>
<td>−0.12 (.03)</td>
<td>−0.23 to −0.01</td>
<td>0.06 (.31)</td>
<td>−0.19 to 0.56</td>
<td>.41</td>
</tr>
<tr>
<td>PL, PU</td>
<td>−0.10 (.007)</td>
<td>−0.17 to −0.03</td>
<td>0.02 (.58)</td>
<td>−0.06 to 0.10</td>
<td>.01</td>
</tr>
<tr>
<td>NAWM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, PU</td>
<td>−0.09 (&lt;.001)</td>
<td>−0.12 to −0.05</td>
<td>0.002 (.30)</td>
<td>−0.34 to 0.40</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PH, PV</td>
<td>−0.17 (.14)</td>
<td>−0.40 to 0.05</td>
<td>0.06 (.63)</td>
<td>−0.29 to 0.18</td>
<td>.42</td>
</tr>
<tr>
<td>PL, PU</td>
<td>−0.07 (.003)</td>
<td>−0.13 to −0.03</td>
<td>−0.0006 (.98)</td>
<td>−0.56 to 0.55</td>
<td>.02</td>
</tr>
</tbody>
</table>

| **Controls**  |                       |       |                       |       |                             |
| Mean, PU      | −0.18 (<.001)         | −0.25 to −0.15 | 0.02 (.62) | −0.05 to 0.08 | <.001 |
| PH, PV        | −0.12 (.03)           | −0.23 to −0.01 | 0.06 (.31) | −0.19 to 0.56 | .41  |
| PL, PU        | −0.10 (.007)          | −0.17 to −0.03 | 0.02 (.58) | −0.06 to 0.10 | .01  |

Abbreviations: CI, confidence interval; MTR, magnetization transfer ratio; NAWM, normal-appearing white matter; PH, peak height; PL, peak location; PU, percentage units; PV, percentage volume.

*P* values were derived from piecewise mixed-effect linear regression models. The MTR changes in controls were not statistically significant.
The NAWM volume decreased by 0.77 mL per year, which was not significant. T2 lesion volume increased annually by 2.80 mL, or 9.25% ($P < .001$; 95% CI, 1.87-3.74). In controls, there were no significant changes. The rate of change in PGMF ($P = .005$) but not PNAWMF ($P = .47$) was significantly different between patients and controls.

**SURROGATE MR IMAGING MARKERS OF CLINICAL CHANGE**

**Markers of Concurrent Clinical Progression**

In the gray matter, more rapid mean MTR decrease was associated with greater rates of EDSS progression (there was a 0.04-PU greater annual MTR decline for each EDSS step deterioration; $P = .03$; 95% CI, −0.82 to −0.003; Figure 1), as was PL MTR (0.07-PU greater annual MTR decline for each EDSS step deterioration; $P = .008$; 95% CI, −0.01 to −0.2) but not PH MTR decrease. The NAWM and lesion MTR changes were not associated with progression rate.

Volume changes in gray matter and NAWM were not associated with the rate of EDSS change. Greater rate of T2 lesion load increase was associated with faster progression on EDSS (lesion volume increase of 0.70 mL for each EDSS step deterioration; $P = .02$; 95% CI, 0.09-1.31).

**Baseline Predictors of Clinical Progression**

The following baseline variables predicted worse EDSS outcome: lower baseline gray matter mean (odds ratio [OR], 2.34; $P = .02$; 95% CI, 1.18-4.70; Figure 2) and lower gray matter PH MTR (OR, 2.43; $P = .008$; 95% CI, 1.27-4.65; Figure 2), lower NAWM PL MTR (OR, 2.5; $P = .04$; 95% CI, 1.04-5.88; PH showed a trend $P = .09$; Figure 2), lower NAWM PL MTR (OR, 2.5; $P = .04$; 95% CI, 1.04-5.88; PH showed a trend $P = .09$), lower baseline PGMF (OR, 1.42; $P = .04$; 95% CI, 1.01-2.00) and PNAWMF (OR, 1.36; $P = .03$; 95% CI, 1.03-1.80), and greater T2 lesion load (OR, 1.03; $P = .02$; 95% CI, 1.00-1.06). When the most significant univariate pre-

dictors from each modality and segment were modeled together, only gray matter PH MTR remained significant (OR, 2.9; $P = .04$; 95% CI, 1.06-8.17).

**COMMENT**

We have reported MTR decline, particularly in the gray matter, gray matter atrophy, and increasing T2 lesion load over 3 years in early PPMS. The rate of change in gray matter MTR and T2 lesion load, but not gray matter volume, reflected the rate of clinical deterioration. Baseline MTR, brain volume, and T2 lesion load predicted clinical progression, and gray matter PH MTR emerged as the strongest predictor.

The disproportionate evolution of gray matter damage, measured by means of MTR and atrophy, was already evident at 1 year in a subgroup of this cohort and has been identified in other MS subtypes. Cortical lesions exhibit demyelination, contain apoptotic neurons, have been associated with axonal transection and loss, and may account for the majority of this injury. Wallerian degeneration secondary to axonal damage from white matter inflammation may also reduce gray matter MTR and tissue volume. Recent studies suggest that iron deposition may be a mechanism of deep gray matter damage in MS, but its effect on MTR is not established. Conversely, the sensitivity of our techniques to NAWM damage may be limited. For example, inflammatory processes can mask atrophy in the NAWM, whereas cortical lesions are less inflammatory. Notably, the relatively small decline in NAWM MTR is not explained by the impact of lesions, which showed an overall increase in MTR. This may be due to remyelination, as demonstrated in pathological studies in patients with PPMS.

Clinically, gray matter MTR changes were most relevant. In a subgroup of this cohort studied at 1 year, the association with clinical change was stronger for NAWM MTR than for gray matter MTR, suggesting that the role...
of NAWM pathology in determining disease progression may be decreasing over time. Regarding gray matter MTR variables, an 8-year study in different MS subtypes also found that gray matter MTR PH predicted disability. However, the importance of a specific MTR measure should not be overemphasized: each one describes only a single point of the histogram. In this study, changes in mean and PL gray matter MTR were significantly correlated with the rate of clinical progression, whereas baseline mean and PH MTR predicted disability. This highlights the importance of viewing histogram measures as a group when evaluating pathological changes and their clinical significance.

Our findings advocate gray matter MTR as a possible surrogate marker of progression in PPMS. It was the strongest predictor of future disability and changed contemporaneously with clinical status. In addition, treatments shown to counteract MTR reduction could be applied to MS therapies on MTR are required. Finally, practical challenges must be addressed, such as the optimization of standardized MTR sequences across different scanners.

Our findings also suggest T2 lesion load as a potential surrogate, as identified in other MS subtypes. To reflect both focal white matter injury and diffuse neurodegenerative change, a combination of gray matter MTR and T2 lesion load may be optimal. However, our combined model suggests that T2 lesion load is a weaker predictor than gray matter MTR in this group, as it has been in other MS subtypes. Interestingly, developing brain atrophy, regarded as a potential surrogate marker in PPMS, did not reflect concurrent clinical change in this study. This may reflect a delay between demyelination and axonal loss, the development of associated atrophy, and the clinical consequences of the tissue loss. Indeed, clinical trials using brain volume as a surrogate have required extensive follow-up to demonstrate treatment effects, and in advanced PPMS brain atrophy predicted clinical outcome 5 years later. Furthermore, spinal cord presentations predominate in PPMS, and spinal cord atrophy may also contribute to clinical outcome.

The study was limited by incomplete attendance and by worsening disability that prevented imaging. However, our statistical model minimized bias due to dropout by using all available data at each time point, so that subjects who missed time points still contributed to the gradient. Another potential limitation was the imager upgrade. However, changes to the acquisition settings were minimized, and rigorous statistical correction was applied to account for any effect of the upgrade on our results. Imager upgrades are common during longitudinal MR studies and should be considered in analysis models, especially when measuring subtle quantitative MR abnormalities. Our study applied a statistical model to detect and, in effect, “edit out” discontinuity caused by the upgrade from the trajectory of change, making the longitudinal correlations more robust. In addition, all changes in patients are described with reference to controls imaged both before and after the upgrade.

In conclusion, changes in gray matter MTR mirrored concurrent clinical progression in early PPMS, and baseline gray matter MTR was a better predictor of medium-term disability than volume measures and T2 lesion load. Gray matter MTR may reflect the pathological processes underlying progression. Its evident potential to satisfy the criteria for a surrogate marker of progression in early PPMS could be investigated in larger studies including natural history observations and therapeutic trials.

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Correspondence: Alan J. Thompson, FRCP, Department of Brain Repair and Rehabilitation, Institute of Neurology, Queen Square, London WC1N 3BG, England.

Author Contributions: Dr Khaleeli had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Khaleeli, Altmann, Miller, and Thompson. Acquisition of data: Khaleeli and Cercignani. Analysis and interpretation of data: Khaleeli, Altmann, Cercignani, Ciccarelli, and Miller. Drafting of the manuscript: Khaleeli and Altmann. Critical revision of the manuscript for important intellectual content: Khaleeli, Altmann, Cercignani, Ciccarelli, and Miller. Obtained funding: Miller and Thompson. Administrative, technical, and material support: Khaleeli. Study supervision: Ciccarelli, Miller, and Thompson. Image analysis: Cercignani.

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Additional Contributions: Chris Benton and Ros Gordon performed the imaging. We thank all study participants for their enthusiasm and commitment.

REFERENCES


