Magnetization Transfer Magnetic Resonance Imaging and Clinical Changes in Patients With Relapsing-Remitting Multiple Sclerosis

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Background: Magnetization transfer (MT) magnetic resonance imaging (MRI) can provide in vivo quantitative estimates of microscopic tissue damage in normal-appearing white matter (NAWM) and gray matter (GM) from patients with multiple sclerosis (MS).

Objective: To determine whether a onetime MT MRI can provide markers of short-term disease evolution in patients with relapsing-remitting MS.

Design: Eighteen-month observational study.

Setting: Neuroimaging Research Unit, Scientific Institute and University Ospedale San Raffaele.

Patients: Twenty-two patients with untreated relapsing-remitting MS.

Main Outcome Measures: Relapse rate; disability according to the Expanded Disability Status Scale (EDSS); dual-echo, 2-dimensional gradient echo with and without a saturation MT pulse and T1-weighted MRIs of the brain; and MT ratio (MTR) histograms for NAWM and GM.

Results: During the study period, 13 patients (59%) experienced 25 relapses. The median EDSS score was 1.25 (range, 0-3.5) at study entry and 1.75 (range, 0-3) at study exit. Significant, although moderate, correlations were found between average GM MTR values at baseline and EDSS changes during the study period ($r = -0.44; P = .04$). A trend was observed for the correlation between NAWM MTR values at baseline and the EDSS changes throughout 18 months ($r = -0.42; P = .05$). For the correlation between EDSS changes and baseline GM MTR, the slope of the regression line was $-0.5$ (95% confidence interval, $-1.0$ to $0.0$), indicating that a decrease in the baseline GM MTR of 1% predicted an increase in the EDSS score of 0.5 point throughout the 18 months.

Conclusion: This study indicates that a “snapshot” MT MRI assessment detects subtle brain tissue changes that are associated with short-term disability accumulation in patients with relapsing-remitting MS.

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system that has a highly unpredictable clinical course. Although conventional magnetic resonance imaging (cMRI) is sensitive in detecting MS lesions, it makes reliable predictions about subsequent evolution of the disease only in patients with the clinically isolated syndrome suggestive of MS. On the contrary, the correlation between MRI findings and development of disability remains modest, if any, in patients with definite MS. This is likely the result, on the one hand, of the inherent weaknesses of the clinical scales used to rate disability and, on the other hand, of the limitations of cMRI. These limitations include the lack of histopathologic specificity to the various substrates of MS, the failure to reflect functional compensatory mechanisms of the cortex, and perhaps most important, its inability to detect microscopic pathologic changes that occur in the normal-appearing brain tissue.

The possibility of overcoming some of these limitations has come with the advent and clinical application of nonconventional MRI techniques that have the potential to provide a more complete picture of the complex pathologic changes of MS. Among these techniques, magnetization transfer (MT) MRI is one of the most promising. The present MT ratio (MTR) histogram-based study was designed to determine whether a onetime MT MRI evaluation can provide prognostic markers of the short-term disease evolution in patients with relapsing-remitting (RR) MS.
Patients

To be included in the study, patients had to have clinically definite MS for at least 1 year and an RR course. To avoid the potential confounding effect of disease-modifying treatments, additional inclusion criteria were the absence of previous immunosuppressive or immunomodulating treatments and the absence of disease-modifying treatments during the study period. Patients were fully informed about available disease-modifying treatments but did not receive any of them for either medical or personal reasons. Relapse rate and Expanded Disability Status Scale (EDSS) scores were assessed at regular visits at baseline and then every 3 months for 18 months. All patients were evaluated by a single neurologist (R.C.) who was blinded to the MRI findings. In case of symptoms suggestive of a clinical relapse, patients were instructed to contact the same neurologist for additional visits and treatment decisions. Clinical relapses were always treated with intravenous methylprednisolone, 1 g/d, for 3 to 5 consecutive days (with no subsequent tapering). In cases of recent relapses, EDSS score deteri- oration had to be confirmed by an additional visit after 1 month. In the experimental procedures were approved by the local ethics committee, and written informed consent was obtained from each patient before study inclusion.

Image Acquisition

This study was part of a larger research protocol that assessed longitudinal brain diffusion tensor MRI changes in patients with RRMS. Because of the duration of the overall MRI acquisition protocol, only 22 of the 26 patients enrolled in the diffusion tensor MRI study consented to undergo the additional studies needed to acquire MTR images. Using a 1.5-T scanner on a regular course of maintenance, the following sequences were obtained at baseline from all patients: (1) dual-echo turbo spin-echo (repetition time [TR]/echo time 1 [TE1]/echo time 2 [TE2] = 3300/16/98 milliseconds; echo train length = 5); (2) T1-weighted conventional spin-echo (TR/TE = 768/15 milliseconds); and (3) 2-dimensional gradient echo (TR/TE = 600/12 milliseconds, flip angle = 20°) with and without an off-resonance radiofrequency saturation pulse. The radiofrequency saturation pulse was 1.5 kHz below the water frequency, with a gaussian envelope of duration of 16.4 milliseconds, a bandwidth of 250 Hz, and an amplitude of 3.4 × 10^-6 T. For dual-echo and T1-weighted images, 24 contiguous axial sections were obtained with 3-mm thickness, 256 × 256-mm matrix size, and 250 × 250-mm field of view. The sections were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum. The MT MRIs were obtained with the same acquisition parameters except for the number of sections, which was 20. The set of sections for the MT images was positioned to obtain the same central 20 sections as for the dual-echo and T1-weighted images.

Image Analysis

Two experienced observers (C.O.-G. and A.C.), without knowing to whom the images belonged, identified by consensus the hyperintense lesions on the proton density–weighted studies and the hypointense lesions on the T1-weighted images. The T2-weighted images were always used to increase confidence in lesion identification. Total T2-hyperintense and T1-hypointense lesion volumes were measured by a single observer (C.O.-G.), using a local thresholding technique for lesion segmentation.

Table. Conventional and Magnetization Transfer Magnetic Resonance Imaging Baseline Characteristics of 22 Patients With Relapsing-Remitting Multiple Sclerosis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) T2 LV, mL</td>
<td>13.0 (9.0)</td>
</tr>
<tr>
<td>Mean (SD) T1 LV, mL</td>
<td>2.7 (2.8)</td>
</tr>
<tr>
<td>Mean (SD) WM volume, mL</td>
<td>823.1 (70.1)</td>
</tr>
<tr>
<td>Mean (SD) GM volume, mL</td>
<td>695.7 (64.3)</td>
</tr>
<tr>
<td>Average (SE) GM MTR, %</td>
<td>37.6 (0.2)</td>
</tr>
<tr>
<td>Average (SE) NAWM MTR, %</td>
<td>40.8 (0.3)</td>
</tr>
</tbody>
</table>

Abbreviations: GM, gray matter; LV, lesion volume; MTR, magnetization transfer ratio; NAWM, normal-appearing WM; WM, white matter.

Using T1-weighted images and SIENAx (the cross-sectional version of the software Structural Imaging Evaluation of Normalized Atrophy),16 normalized volumes of white matter (WM) and gray matter (GM) were estimated at baseline.

After coregistration of the 2 gradient echo images using a surface-matching technique based on mutual information, MTR maps were derived pixel by pixel.16 Extracerebral tissue was removed from MTR maps using a local thresholding segmentation technique, and the resulting images were coregistered with the T2-weighted images.16 Average lesion MTR was measured as previously reported.17 The GM, WM, and cerebrospinal fluid were automatically segmented from dual-echo images using SPM99 and maximum image inhomogeneity correction,18 and whenever present, T2-hyperintense lesions were masked out from the segmented tissues. The resulting masks were superimposed onto the coregistered MTR maps, and the corresponding MTR histograms of the normal-appearing WM (NAWM) and GM were produced.19 For each histogram, the average MTR was calculated. Given the strong correlation between average MTR and the other histogram metrics,20 only this quantity was a priori included in the analysis to minimize the number of comparisons and therefore reduce the risk of type I errors.

Statistical Analysis

Correlations were performed with the Spearman rank correlation coefficient. Two regression lines relating changes in the EDSS (dependent variable) to the values of baseline GM MTR and NAWM MTR (independent variables) were fitted to estimate the extent of the EDSS score deterioration predicted by the reduction in each percentage change of baseline MTR. Because of the exploratory nature of this study, no correction for multiple testing was performed; as a consequence, the reported P values should be viewed with caution.

Results

We studied 22 patients with RRMS (15 women and 7 men; mean age, 36.6 years; age range, 25-50 years; mean disease duration, 10.4 years; disease duration range, 1-23 years). The median EDSS score was 1.25 (range, 0-3.5) at study entry and 1.75 (range, 0-3) at study exit. During the follow-up, 13 patients (59%) experienced 25 relapses. The Table reports baseline cMRI and MT MRI findings from the 22 patients with RRMS.

A significant, albeit moderate, correlation was found between average GM (r = -0.44; P = .04) MTR values at baseline and EDSS changes throughout the study period (Figure 1). A trend was observed (r = -0.42; P = .05)
for the correlation between NAWM MTR values at baseline and the EDSS changes throughout 18 months. The correlations between on-study relapses and either baseline GM MTR \((r = -0.16; P = .49)\) or baseline NAWM MTR \((r = -0.25; P = .26)\) were both weak and not significant. A trend was observed between EDSS changes and on-study relapses \((r = 0.40; P = .07)\). After adjusting for the number of on-study relapses, the partial correlation coefficients of the correlation between EDSS changes and baseline GM MTR \((r = -0.41; P = .06)\) remained virtually unchanged. The correlations of baseline EDSS with baseline average GM MTR \((r = 0.26; P = .24)\) and baseline average WM MTR \((r = 0.29; P = .20)\) were both weak and not statistically significant. No significant correlations were found between baseline GM and WM volumes or between T2 and T1 lesion volume and EDSS score changes at study end. No significant correlation was found between average lesion MTR and EDSS changes at study end. For the relation between EDSS changes and baseline GM MTR, the slope of the regression line was \(-0.5\) (95% confidence interval, \(-1.0\) to \(0.0\)), indicating that a decrease in the baseline GM MTR of \(1\%\) predicted an increase in the EDSS score of \(0.5\) point throughout the 18 months.

**COMMENT**

Despite its high sensitivity to detect the presence of MS lesions, cMRI fails to provide specific information about the nature of the pathologic substrates of MS lesions, which include edema, demyelination, remyelination, gliosis, and axonal loss.\(^{21}\) In addition, the occult damage to the normal-appearing brain tissue remains unaccounted for by cMRI, which, together with the limitations of the EDSS, is likely to contribute to the poor correlations that have been reported to date between clinical and MRI findings in patients with definite MS.\(^{10}\)

Histogram-based analysis of MT MRI has the potential to provide information about the microstructural damage that is invisible on cMRI and that reflects the global or tissue-specific degree of damage.\(^{25}\) The MTR reduction was found to be strongly correlated with the percentage of residual axons and the degree of demyelination.\(^{23,24}\) Preliminary studies have reported promising findings on the predictive value of MT MRI on the medium-term clinical evolution of the disease.\(^{25,26}\) However, the predictive value of a single MT MRI on the short-term clinical deterioration in MS is still unclear, despite the fact that the possibility of deriving prognostic information from a single baseline study is extremely appealing in terms of cost and labor saving, especially in the context of clinical trials for patient selection and stratification.

The present study shows a moderate correlation between average GM MTR at baseline and EDSS score changes during a follow-up of 18 months and a trend for the correlation between average WM MTR at baseline and EDSS score changes at study end. Interestingly, no correlation was observed between average GM MTR and on-study relapse rate, and the strength of the correlation between baseline GM MTR and EDSS score change during the study period remained unchanged when adjusting for on-study relapse. This finding confirms the notion that the inflammatory component of MS is not the only factor associated with accumulation of disability since the RR phase of the disease.\(^{4,27}\) No correlations were found between EDSS score changes and baseline T2 and T1 lesion volumes, as well as with GM and WM volumes. Additionally, no significant correlation was found between baseline average lesion MTR and EDSS score changes at study end. This finding suggests that in established MS, the extent of diffuse microstructural damage in the GM at baseline, rather than the morphologic characteristics of brain tissues, the lesion load as seen on cMRI, or even the microscopic damage occurring within lesions, is a reliable indicator of subsequent clinical deterioration in a time frame close to that typically used in the context of MS trials.

Postmortem studies have shown that MS does not spare cerebral GM.\(^{28,31}\) In agreement with this, an increasing body of MRI evidence shows that GM damage is a consistent feature of MS in its RR phase.\(^{19,32-42}\) Proton magnetic resonance spectroscopy showed significantly reduced concentrations of the neuronal marker N-acetylaspartate in the cortical GM of mildly impaired patients with RRMS with short disease durations.\(^{32,35}\) Other investigators have revealed reduced MTR and abnormal diffusivity in the GM of patients with RRMS,\(^{19,34,35}\) which are correlated with the degree of cognitive impairment.\(^{36}\) Recent work has also shown that GM damage in RRMS accumulates over time.\(^{14,37,43}\) The development and accumulation of lesions in the GM are among the possible explanations for the observed changes in the GM of patients with RRMS. Frequently, GM lesions are present, and although they are usually undetected by cMRI, they represent a significant portion of the overall brain lesion burden in MS.\(^{28,31}\) Transected neuritis, transected axons, and apoptotic neurons have all been observed within cortical demyelinated lesions.\(^{44}\) Deep GM structures such as the thalamus are also affected by MS, as shown by a reduction in N-acetylaspartate concentrations, thalamic volume,\(^{38,39}\) and thalamic MTR\(^{11}\) and an increase in water diffusion.\(^{40}\) Therefore, the presence of lesions in the cortex and deep GM and the progressive
retrograde degeneration of neurons after fiber transection within WM lesions could likely explain the reduction of MTR values in the GM.

Although a significant correlation between baseline NAWM MTR values and changes in EDSS score over time has been reported previously, we found a trend that most likely would have reached statistical significance with increased degrees of freedom. This partial discrepancy with previous studies could be explained by differences in durations over which disability was evaluated, because our clinical evolution was measured during a relatively short period. Another reason might be that in addition to patients with RRMS, the previous study included patients with a secondary progressive disease course. The inclusion of a homogeneous group of mildly impaired patients with RRMS might be viewed as a strength of the present study; these are the patients who currently are considered to benefit most from treatment. In addition, the clinical, demographic, and cMRI characteristics of this patient sample were similar to those of the cohort enrolled in the diffusion tensor MRI study of the brain, which were in the range expected from previous natural history studies.

An explanation for the slightly better prognostic value of GM MTR over that of WM MTR might lie in the fact that GM abnormalities reflect direct and indirect (through axonal damage) neuronal abnormalities, which in turn are more likely to cause irreversible disability than less destructive changes that occur in the NAWM (such as inflammatory edema and demyelination), which can also cause MTR reductions.

One limitation of the present study may come from the use of the EDSS to rate clinical disability. The National Multiple Sclerosis Society’s Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis appointed a task force that developed the Multiple Sclerosis Functional Composite as an improved clinical outcome measure to overcome the limitations of the EDSS. The advantages of this measure are that it encompasses the major clinical dimensions of arm, leg, and cognitive function and that it is more sensitive to changes during short periods than the EDSS. Although this would suggest that the use of the Multiple Sclerosis Functional Composite as a clinical scale to rate the patients’ disability could yield better correlations between baseline MTR-derived measures and clinical disability changes throughout relatively short periods, the present study was designed at a time when the Multiple Sclerosis Functional Composite had not yet been fully validated and its use was still limited to a few centers. Nevertheless, most MS studies (including trials) are still conducted by rating disability with the EDSS (alone or in combination with other measures), which should make our results more easily interpretable against the background of existing literature on the topic. Additional studies with larger populations and better clinical outcome measures are now warranted.

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REFERENCES