### Gray- and White-Matter Changes 1 Year after First Clinical Episode of Multiple Sclerosis: MR Imaging

**Purpose:**
To assess, by means of magnetic resonance (MR) imaging, the longitudinal changes in white matter (WM) and gray matter (GM) in a cohort of patients with clinically isolated syndrome (CIS) who were followed up for 1 year.

**Materials and Methods:**
This prospective, HIPAA-compliant study was approved by the institutional review board. Written informed consent was obtained from all the participants. Changes in GM and WM integrity were respectively investigated by using three-dimensional T1-weighted and diffusion-tensor (DT) imaging sequences and by applying voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS) analyses. Thirty-four consecutive patients (21 women, 13 men; mean age, 32.8 years ± 7.7 [standard deviation]) who had CIS were recruited. All the patients underwent a neurologic and an MR examination at baseline and 12 months later; the MR examination consisted of three-dimensional T1-weighted dual-echo turbo spin-echo DT imaging. VBM and TBSS were used to analyze GM volume and WM fractional anisotropy, respectively.

**Results:**
After 1 year, multiple sclerosis (MS) was diagnosed in 33 (97%) of 34 patients with CIS. Longitudinal volumetric analysis revealed a significant (*P* < .001) reduction in global GM volume. The VBM analysis showed the development of regional GM atrophy involving several cortical and subcortical regions in both hemispheres (*P* < .05). No significant longitudinal change in global or regional WM fractional anisotropy was otherwise observed.

**Conclusion:**
WM damage was detectable early and involved most fiber tracts in patients with MS, but it did not worsen significantly during the 1st year after clinical onset. In contrast, GM damage was not detectable at the time of clinical onset, but a significant decrease in cortical and deep GM volume was observed at 1 year.
Multiple sclerosis (MS) is an inflammatory, demyelinating disease that predominantly affects young adults and in most cases leads to chronic disability (1). Although the paramount importance of magnetic resonance (MR) imaging in diagnosis, monitoring of treatment efficacy, and follow-up in patients with MS is widely acknowledged, the correlation between clinical signs and radiologic evidence of pathologic abnormalities associated with MS is not clear. This lack of clarity is hypothesized to be due, at least partially, to the involvement of gray matter (GM), which is not readily detectable with conventional MR techniques (2,3). New MR techniques have been aimed at finding more reliable markers of MS, including the evaluation of GM. Numerous MR approaches have shown GM involvement in MS (4–6).

One important question that warrants further study is whether white-matter (WM) damage determines abnormalities in GM regions through a disconnection mechanism, or whether GM and WM tissues are affected independently. The results of pathologic studies (7,8) that have addressed this question are controversial, primarily because of the relative lack of specimens from patients in the earliest stages of disease. Moreover, previous MR imaging studies suggest that pathologic GM abnormalities in MS may be secondary to WM damage (9) or independent, as recently shown in patients who responded to the criteria for “truly benign” MS (10).

Investigators of a recent cross-sectional study (11) in patients with clinically isolated syndrome (CIS) (the earliest clinical stage of MS) reported a widespread pattern of WM damage in the absence of any detectable GM atrophy. Those findings suggest that the macroscopic and microscopic involvement of WM represents the first pathophysiologic event of MS, whereas GM damage is likely to appear or to become evident at a more advanced stage of the disease. It is conceivable that individual variability in the accumulation of GM damage might account for the different clinical outcomes observed in patients with relapsing–remitting MS. Against this background, a longitudinal study of patients with a first clinical episode of MS at baseline may shed light on the complex relationship between GM and WM damage.

The aim of this study was to use MR imaging to assess the longitudinal changes in WM and GM in a cohort of patients with CIS who were followed up for 1 year. Changes in GM and WM integrity were respectively investigated by using three-dimensional T1-weighted and diffusion-tensor (DT) imaging sequences and by applying voxel-based morphometry (VBM) and tract-based spatial statistic (TBSS) analyses.

**Materials and Methods**

**Patients**

This prospective study was approved by the institutional review board of Sapienza University and complied with the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all the participants at the beginning of the study and at the 1-year follow-up evaluation. For the current longitudinal investigation, we included 34 patients with CIS who had previously been enrolled in a cross-sectional study (11). Thirty-nine consecutive patients who came to the MS center at our institution between April 2006 and March 2008 were consecutively enrolled in the study if they met the following inclusion criteria: single clinical episode indicating MS (optic neuritis, brainstem syndrome, spinal cord syndrome) and age 18 to 50 years. The exclusion criteria were as follows: any diagnoses other than MS, the presence of other relevant diseases, contraindications to MR imaging, and withdrawal from the study. Five patients were excluded on the basis of the exclusion criteria: One received a diagnosis other than MS; one had other relevant diseases, and three had contraindications to MR imaging. The remaining 34 patients were included in the study. The patients’ clinical characteristics have been described in detail elsewhere (11). At 1-year follow-up (mean duration, 375 days; range, 342–393 days), the patients had a mean age of 32.8 years ± 7.7 (standard deviation). Of these patients, 21 were women (mean age, 32.3 years ± 7.7) and 13 were men (mean age, 33.8 years ± 8.0).

All the patients repeated the neurologic examination (performed by C.P., a

**Implication for Patient Care**

- Longitudinal evaluation of brain damage in patients with CIS suggestive of MS revealed that GM damage may play an important role in the early phase of the disease.

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**Abbreviations:**

- CIS = clinically isolated syndrome
- CSF = cerebrospinal fluid
- DT = diffusion tensor
- EDSS = Expanded Disability Status Scale
- GM = gray matter
- MS = multiple sclerosis
- TBSS = tract-based spatial statistics
- VBM = voxel-based morphometry
- WM = white matter

**Author contributions:**

 Guarantors of integrity of entire study, E.R., P.T., P.P.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, E.R., C.P.; clinical studies, E.R., E.S., C.P., M.B., P.P.; experimental studies, E.S.; statistical analysis, M.C., P.T., M.B.; and manuscript editing, E.R., M.C., M.B., P.P.

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board-certified neurologist with 26 years of experience, and E.S., a 5th-year resident in neurology) and were reclassified as patients with CIS or MS; the latter diagnosis was based on the occurrence of a second MS relapse during follow-up (12) or fulfillment of the McDonald revised MR imaging criteria for the diagnosis of MS (dissemination of lesions in space and time) (13). The Expanded Disability Status Scale (EDSS) score (14) and use of corticosteroid or disease-modifying treatment between the baseline and the follow-up examinations were recorded. Within 5 days of the 1-year clinical follow-up examination, and not less than 3 weeks after a clinical relapse, MR imaging was performed in all patients by using the same MR imager and acquisition protocol as those used at baseline.

**MR Imaging Acquisition**

At both time points, imaging was performed in all patients with a 1.5-T MR imager (Gyroscan NT 15; Philips Medical Systems, Best, the Netherlands). The body coil was used for signal transmission, and the manufacturer 16-section head coil designed for sensitivity encoding (SENSE) was used for signal reception. Section orientation parallel to the subcallosal line was assured by acquiring a multiplanar T1-weighted localizer at the beginning of each study (15). The following sequences were performed: (a) axial dual-echo turbo spin-echo sequence on 48 contiguous 3-mm-thick sections (repetition time msec/echo time msec, 2150/30, 120; echo train length, 12; matrix, 256 × 256; field of view, 250 mm²; SENSE reduction factor, 1.4; acquisition time, 5 minutes 48 seconds); (b) three-dimensional T1-weighted turbo field-echo sequence on 150 contiguous axial 1-mm-thick sections (40/4; flip angle, 30°; matrix, 512 × 512; field of view, 240 mm²; SENSE reduction factor, 1; acquisition time, 7 minutes 18 seconds); and (c) DT imaging sequence with single-shot echo-planar pulse sequence on 25 contiguous 5-mm-thick sections (3894/95; matrix, 128 × 128; field of view, 250 mm²; SENSE reduction factor, 2.5; acquisition time, 8 minutes 51 seconds). Diffusion-sensitized gradients were applied along six noncollinear directions by using two b values (0 and 1000 sec/mm²), and amplitude images were averaged from 10 measurements. A battery of quality assurance tests on phantoms was carried out monthly to ensure the stability of measurements throughout the study. No major hardware upgrades on the imager were performed during the study.

**Image Analysis and Postprocessing**

Image data processing was performed by an MR imaging physicist (M.C., with 11 years of experience) and a radiology resident (E.R., with 5 years of experience) on a personal computer running Jim 4.0 software (Xinapse System, Leicester, England), the FMRIB Software Library 4.1 software package (FMRIB Image Analysis Group, Oxford, England) (16), MATLAB 7.0 software (MathWorks, Natick, Mass), and Statistical Parametric Mapping 5 software (Wellcome Department of Cognitive Neurology, London, England).

**Lesion Volume, TBSS, Mean Fractional Anisotropy, and Mean Diffusivity**

Lesion volumes and binary lesion masks were obtained by using proton density–weighted images with a semiautomated technique based on local thresholding (17); T2-weighted images were always used to increase the confidence level of lesion identification. DT imaging data were modulated by multiplying each voxel intensity by the Jacobian determinant of the nonrigid transformation used for normalization. The volumes of each tissue were recorded and then used to calculate intracranial volume as GM + WM + CSF and the brain parenchymal fraction as follows: (GM + WM)/intracranial volume. Voxelwise comparison of the GM volume between the baseline and the follow-up examinations was performed by using a paired t test. P values less than .05 were considered to indicate statistically significant differences at the voxel level after family-wise error correction for multiple comparisons.

**Statistical Analysis**

Statistical analysis was performed by one author (P.T., with 6 years of experience) using Predictive Analytics software (version 17.0, SPSS, Chicago, Ill); differences in clinical (EDSS) and radiologic (fractional anisotropy, mean diffusivity, lesion volume, GM volume, WM volume, CSF volume, and brain parenchymal fraction) measures between the baseline and the follow-up examinations were evaluated with a paired t test. The relationship between follow-up GM volume and baseline measures (fractional anisotropy, mean diffusivity, lesion volume, GM volume, and WM volume) was assessed by using multivariate analyses. These analyses were performed by entering
variables with a $P$ value less than .05 from previous univariate analyses into a linear regression analysis with backward elimination. $P$ values less than .05 were considered to represent statistically significant differences.

**Results**

At reclassification at the 1-year follow-up, 33 (97%) of the 34 patients fulfilled the diagnostic criteria for relapsing-remitting MS. Twelve patients had at least one new relapse during the previous year, and an additional 21 patients (who did not experience a second relapse) exhibited at least one new T2 lesion on images obtained at the follow-up examination compared with the baseline examination. Only one patient did not satisfy the clinical or the radiologic criteria for the diagnosis of MS. The median follow-up EDSS score for all the patients taken together was 1.5 (range, 0–3). The mean T2 lesion volume was 4.37 mL, which was statistically significantly higher than the baseline values (see Table 1).

The mean fractional anisotropic value at follow-up for all the voxels included in the skeleton was 0.40 ± 0.01; this value was not significantly different from that observed at the baseline examination (0.41 ± 0.01) ($P = .13$). The follow-up mean diffusivity value for all the voxels included in the skeleton was 0.83 mm$^2$/sec ± 0.02; this value was not significantly different from that observed at the baseline examination (0.83 mm$^2$/sec ± 0.02) ($P = .19$). A voxelwise comparison of the fractional anisotropic values between the follow-up and the baseline images did not reveal regions of significantly reduced fractional anisotropy ($P$ corrected $>.05$).

To check the reliability of our data and exclude the presence of potential systematic biases, we investigated the presence of clusters with a higher fractional anisotropic value at follow-up than at baseline. This analysis did not reveal any significant changes, even at a statistical threshold uncorrected for multiple comparisons ($P$ uncorrected $<.001$).

Global brain volumes of GM, WM, CSF, and brain parenchymal fraction obtained at the baseline and the 1-year follow-up examination are summarized in Table 1. Global GM volume was the only measure that significantly decreased; the volumes in the other tissues remained unchanged.

The VBM longitudinal analysis (checking for those clusters of reduced volume at follow-up) depicted 10 clusters of significantly reduced GM at the 1-year follow-up examination ($P < .05$, family-wise error–corrected) (Fig 1). These clusters involved cortical and subcortical regions in both cerebral hemispheres, including the thalamus, the head of the caudate nucleus, the paracentral lobule, the precentral gyrus, the cuneus, the insula, and the temporal lobe. Table 2 shows the coordinates of the maxima and the size for each region, along with the corresponding $P$ values.

Of the various baseline measures (EDSS score, fractional anisotropy, mean diffusivity, lesion volume, GM volume, and WM volume) entered into the regression analysis, baseline fractional anisotropy was the only measure found to be significantly ($P = .01$) correlated with follow-up GM volume (Fig 2).

**Discussion**

By using DT imaging and VBM, we examined quantitative longitudinal changes in the WM and GM of individuals with CIS suggestive of MS.

The main finding was that at 1-year follow-up, patients with CIS presented a pattern of regional GM atrophy that was not detected at baseline (11). Of note, these prominent changes in GM were not accompanied by any marked changes in WM, which was already extensively damaged at the baseline examination (11).

GM changes were analyzed on both a global scale (by assessing total GM volume and brain parenchymal fraction) and a local scale (by means of VBM, which allowed damage to be localized more accurately).

Global GM volume at 1-year follow-up was significantly reduced compared with the baseline volume. Although control patients in our study, described elsewhere (11), underwent imaging just at baseline, we may infer from published data that the GM loss we observed in our patients is both significant and in keeping with the results of previous studies (22–24).

At the baseline analysis, GM volume in our cohort was similar to that of healthy control subjects, which is again consistent with previous studies conducted in patients with CIS or those in the early clinical stages of MS (25–29). Moreover, a progressive development of global atrophy of GM has been reported in patients with early stage MS and those with CIS in cross-sectional (9,22,30–32) and longitudinal (23,24) studies. To our knowledge, our study represents the first dynamic demonstration of the evolution of such damage within the 1st year of disease onset.

Our results, obtained in a cohort of patients with early disease who have stable EDSS scores, agree with those

<p>| Clinical and Radiologic Characteristics of Patients at Baseline and at 1-year Follow-up |
|----------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>$P$ Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median EDSS score†</td>
<td>1.5 (0–3)</td>
<td>1.5 (0–3)</td>
<td>.76</td>
</tr>
<tr>
<td>Mean lesion volume (mL)</td>
<td>3.22 ± 3.9</td>
<td>4.37 ± 4.2</td>
<td>.001</td>
</tr>
<tr>
<td>Mean GM volume (mL)</td>
<td>675.8 ± 70.8</td>
<td>663 ± 76.5</td>
<td>.001</td>
</tr>
<tr>
<td>Mean WM volume (mL)</td>
<td>440.2 ± 50.5</td>
<td>442.4 ± 51.2</td>
<td>.23</td>
</tr>
<tr>
<td>Mean CSF volume (mL)</td>
<td>253.2 ± 48.6</td>
<td>258.4 ± 43.4</td>
<td>.37</td>
</tr>
<tr>
<td>Mean brain parenchymal fraction</td>
<td>0.81 ± 0.02</td>
<td>0.81 ± 0.02</td>
<td>.14</td>
</tr>
<tr>
<td>Mean fractional anisotropy</td>
<td>0.41 ± 0.01</td>
<td>0.40 ± 0.01</td>
<td>.13</td>
</tr>
<tr>
<td>Mean diffusivity (mm$^2$/sec)</td>
<td>0.83 ± 0.02</td>
<td>0.83 ± 0.02</td>
<td>.19</td>
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Note.—WM, GM, CSF, and brain parenchymal fraction values are derived from mean normalized segmented volumes. Unless otherwise noted, values are means ± standard deviations.

* $P$ values derived by using paired t test.
† Numbers in parentheses are ranges.

References:

1. Clinical and Radiologic Characteristics of Patients at Baseline and at 1-year Follow-up

2. Results

3. Discussion
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Our findings also align with those of a longitudinal study (29) of patients with relapsing-remitting MS in which a correlation between the rate of thalamic atrophy and disability rate during follow-up was observed.

The pathologic substrate of GM atrophy in MS is still largely unknown. Postmortem studies suggest that cortical atrophy might be related to gliosis because it does not appear to be correlated with cortical demyelination (34). Atrophy is also known to occur in deep GM structures of patients with MS (25,29,33,35,36). However, the relationship between cortical and subcortical atrophy remains a matter of debate.

Our regional GM analysis showed volume loss in specific cortical (temporal...
and parietal) and subcortical (thalamus and caudate nucleus) regions, thus pointing to the early involvement of these GM areas in the pathologic process of MS.

No significant changes were observed in the WM of the same group of patients, as assessed with DT imaging and TBSS. DT imaging is particularly suitable for the evaluation of WM because it assesses the microstructural properties of tissues, which cannot be visualized with conventional MR imaging (3). The pathologic counterpart of reduced fractional anisotropy in the normal-appearing WM among patients with MS is likely to be demyelination and axonal loss (37,38). Cross-sectional TBSS analysis in a baseline study (11) revealed diffuse changes in fractional anisotropy of the WM of patients with CIS compared with healthy volunteers; those changes involved almost the entire skeletonized fractional anisotropic map. This finding indicates that in addition to the T2-visible lesions (39–43), there are other diffuse abnormalities in the brains of patients with MS from the earliest clinical phases. In contrast, the longitudinal analysis presented here did not show any change in the fractional anisotropy of the WM of patients with CIS at 1-year follow-up. This result supports the hypothesis that WM damage is present at MS onset but evolves slowly, as shown by the absence of significant DT image changes during the 1st year of disease. This finding is in accordance with the results of previous longitudinal DT imaging studies, in which no significant longitudinal changes in WM damage among patients with relapsing-remitting MS or those with CIS were found, even after 3 years of follow-up (41,44,45).

These data support the hypothesis that different pathophysiologic events occur in the WM and GM in different clinical stages of MS. In the early stages, the pathophysiologic features of MS appear to be dominated by WM inflammation, which is far more widespread than an assessment of macroscopic lesions reveals (11,46). In contrast, GM tissue, as assessed with VBM analysis, does not exhibit remarkable signs of tissue damage. These findings suggest that GM lesions (which are, as pathologic studies have shown, detectable from the early stages of MS [47]) probably play a less prominent role than axonal degeneration in determining GM atrophy. Previous studies assessing GM atrophy in patients with MS were cross-sectional and did not specifically focus on the earliest stages of the disease (9,30–32). Our current study was longitudinal and was specifically designed to investigate the temporal evolution of WM and GM in the same group of patients from the earliest clinical stages. A similar approach was previously used in one longitudinal study, but that study was based on only a subset of seven patients with CIS (23).

When a regression analysis with backward elimination was applied to our data, the only significant correlation we found was between GM volumes at the follow-up examination (independent variable) and fractional anisotropy of WM measured at baseline. There are several hypotheses regarding the evolution of GM damage in MS and its relationship with WM involvement (47). In keeping with the results of previous studies (22,24), our longitudinal analyses revealed a prominent involvement of WM at clinical onset, followed by a slower evolution during the subsequent year. In contrast, GM damage was undetectable at clinical onset but increased significantly in the same time interval.

We may speculate that varying degrees of combined GM and WM damage account for some of the critical changes that occur in the clinical course of patients with MS. Future studies, based on larger patient populations and longer durations of follow-up, are warranted to explore such an intriguing hypothesis. However, our data strongly support the idea that GM damage in MS is mainly due to anterograde neuronal degeneration as a consequence of axonal loss, a process called wallerian degeneration. Although the exact mechanisms underlying this degeneration have not yet been clarified (possibilities include virtual hypoxia and glutamate excitotoxicity [47]), it is beyond dispute that the correlation between the 1-year follow-up GM volumes and the baseline fractional anisotropy of WM in our patients is strong. Of note, no correlation was found between the follow-up GM volume and baseline lesion loads.

The main limitation of our study was the lack of longitudinal MR imaging data for the control group. Moreover, from a methodologic viewpoint, MS lesions can cause a confounding effect on the segmentation phase of the VBM analysis; lesion masking, which has been proposed as a solution to this problem (25,48), was adopted for our study.

GM atrophy increased significantly during the 1st year after the clinical onset of MS, whereas WM damage, which is present from the beginning, did not change significantly in the same time interval. This finding strongly supports the hypothesis that GM damage in MS is due to axonal degeneration as opposed to primary involvement.

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References


