Diffusion-Tensor Magnetic Resonance Imaging Detects Normal-Appearing White Matter Damage Unrelated to Short-term Disease Activity in Patients at the Earliest Clinical Stage of Multiple Sclerosis

Antonio Gallo, MD; Marco Rovaris, MD; Roberto Riva, MD; Angelo Ghezzi, MD; Beatrice Benedetti, MD; Vittorio Martinelli, MD; Andrea Falini, MD; Giancarlo Comi, MD; Massimo Filippi, MD

**Background:** Diffusion-tensor (DT) magnetic resonance imaging (MRI) has the potential to elucidate some characteristics of tissue microstructure inaccessible to other MRI techniques.

**Objective:** To investigate whether normal-appearing brain tissue abnormalities occur in patients with multiple sclerosis at the earliest clinical stage and whether their severity is predictive of a short-term disease evolution by using DT MRI.

**Design:** Forty-five patients and 22 healthy control subjects were studied. All patients had had a clinically isolated syndrome within the 3 months preceding study enrollment and paraclinical evidence of disease dissemination in space. During a single session, dual-echo, pulsed-gradient spin-echo echo-planar, and post-gadolinium T1-weighted images of the brain were obtained from each subject. In patients, dual-echo and enhanced images were obtained after 3 and 12 months, to detect MRI signs of disease dissemination in time. An on-study neurological examination was also conducted to ascertain the occurrence of clinical relapses. Mean diffusivity and fractional anisotropy maps were derived from DT images. Normal-appearing white matter (NAWM) and normal-appearing gray matter mean diffusivity and fractional anisotropy histograms were produced and analyzed.

**Results:** During the study period, 29 patients showed MRI evidence of disease dissemination in time. When compared with healthy controls, patients showed higher average NAWM mean diffusivity ($P = .01$), lower average NAWM mean diffusivity peak height ($P < .001$), and fractional anisotropy ($P < .001$). The DT MRI characteristics of patients did not differ between those with and those without disease dissemination in time at follow-up.

**Conclusions:** In patients with multiple sclerosis at the earliest clinical stage, the severity of NAWM damage does not predict new lesion formation in the short term, suggesting that the “diffuse” component of tissue damage is, at least partially, independent of the “discrete,” predominantly inflammatory aspects of the disease since its clinical onset.

Arch Neurol. 2005;62:803-808

Most patients with clinically isolated syndromes (CISs) suggestive of multiple sclerosis (MS) show multiple asymptomatic white matter (WM) abnormalities on T2-weighted magnetic resonance images (MRIs). The characteristics of these lesions are indistinguishable from those seen in established MS patients, and their presence and extent at CIS onset are significant predictors of the subsequent development of MS.

In patients with MS, postmortem studies reveal the presence of diffuse damage in brain tissues that seem normal on gross pathological examination and conventional MRIs. The in vivo detection and quantification of these abnormalities might be helpful for an accurate monitoring of MS evolution, because it has been shown that they may have an impact on patients’ disability. However, it remains to be established when normal-appearing WM (NAWM) and normal-appearing gray matter (NAGM) abnormalities develop during the disease, because conflicting results have been obtained in preliminary studies of patients with CIS suggestive of MS.

Diffusion-tensor (DT) MRI is sensitive to the microscopic random motion of water molecules, which is the result of the interactions with other molecules and barriers that can restrict it. By using DT MRI,
the brain tissue microstructure can be determined by quantitative indexes, such as mean diffusivity (MD), which is affected by cellular size and integrity, and fractional anisotropy (FA), which reflects the degree of alignment of cellular structures within fiber tracts and their structural integrity. In MS patients, several studies have found significant increases of MD and reductions of FA in the NAWM \(^{17}\) and NAGM.\(^ {18,19}\)

The present study was performed to evaluate whether DT MRI discloses the presence of occult tissue abnormalities in the NAWM and NAGM of patients with MS at the earliest clinical stage and to investigate whether the severity of these abnormalities predicts the short-term occurrence of clinical or MRI-detectable disease activity.

### SUBJECTS

To be included, patients had to have a CIS within the 3 months preceding study initiation and paraclinical evidence of disease dissemination in space (DIS).\(^ {20}\) Appropriate investigations were performed as necessary to exclude alternative diagnoses. Corticosteroid treatment, if any, had to be concluded at least 3 weeks before the first scanning session. The clinical examination comprised neurological visits with an Expanded Disability Status Scale\(^ {21}\) rating, within 3 days from the acquisition of baseline and follow-up MRIs. All patients were examined by a single neurologist, unaware of the MRI results. In case of symptoms suggestive of a clinical relapse during the study period, patients were instructed to contact the same neurologist for additional visits. Twenty-two sex- and age-matched healthy volunteers (8 men and 14 women; mean age, 31.9 years; SD, 8.6 years) with a normal neurological examination result served as control subjects. All subjects signed a written informed consent before study enrollment, and the study design was approved by the local ethical committee.

### IMAGE ACQUISITION

Magnetic resonance images of the brain were obtained using a scanner operating at 1.5 T (Vision; Siemens, Erlangen, Germany). During a single session, the following sequences were acquired in all subjects: (1) dual-echo turbo spin-echo (SE) sequence (repetition time/echo time: 3300 milliseconds/16-98 milliseconds/1; echo train length = 5; geometry, 24 contiguous, 5-mm-thick, axial sections with a 256 × 256 matrix and a 250 × 250-mm field of view); (2) pulsed-gradient SE single-shot echo-planar pulse sequence (interecho spacing = 0.8; echo time = 123 milliseconds; geometry, 10 contiguous, 5-mm-thick, axial sections with a 128 × 128 matrix and a 250 × 250-mm field of view), with diffusion gradients applied in 8 noncollinear directions; the duration and maximum amplitude of the diffusion gradients were 25 milliseconds and 21 mT/m, giving a maximum b factor in each direction of 1044 s/mm\(^2\); and (3) postcontrast (0.1 mmol/kg of gadolinium–diethylenetriamine pentaacetic acid; acquisition delay, 5 minutes) T1-weighted SE sequence (repetition time/echo time: 5000 milliseconds/100 milliseconds; geometry, same as for dual-echo images) (contrast medium was not administered to healthy controls). Sections were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum.\(^ {22}\) For DT MRIs, the second last caudal section was positioned to match exactly the central sections of the dual-echo images. This brain portion was chosen because the periventricular area is a common location for MS lesions. In addition, these central sections are less affected by distortions due to B\(_0\) field inhomogeneity, which can affect image coregistration. In patients, dual-echo and postcontrast T1-weighted images were also obtained 3 and 12 months after the first scanning session, to assess the presence of MRI signs of disease dissemination in time (DIT).\(^ {20}\) For follow-up images, patients were repositioned using ad hoc guidelines.\(^ {24}\)

### IMAGE ANALYSIS

Magnetic resonance imaging hard copies were revised in a random order by 2 observers (M.R. and A. Gallo) unaware of subjects’ identity. Lesions were identified and marked by consensus on the proton-density and postcontrast T1-weighted images. T2-weighted images were always used to increase the confidence in lesion identification. The number and location of T2-hyperintense and enhanced lesions were evaluated, and the fulfillment of MRI criteria for disease DIS and DIT was assessed.\(^ {20}\) Digital images were then transferred to a workstation (SUN Sparcstation; Sun Microsystems, Mountain View, Calif) for lesion volume measurements. These were performed by a single observer, unaware of subjects’ identity, using a semiautomated segmentation technique based on local thresholding and keeping the marked hard copies as a reference.\(^ {23}\)

On T1-weighted images, normalized volumes of the whole of the brain parenchyma were measured using a highly automated method, the cross-sectional version of the structural image evaluation of normalized atrophy software.\(^ {24}\) First, structural image evaluation of normalized atrophy software uses a brain extraction tool method to extract the brain and skull from MRIs. A tissue segmentation program is then used to segment the extracted brain image into brain tissue, cerebrospinal fluid (CSF), and background, yielding an estimate of total brain tissue volume. Original MRIs are subsequently registered to a canonical image in a standardized space (using the skull image to provide the scaling cue), a procedure that provides a spatial normalization scaling factor for each subject. The estimated tissue volume for a subject is then multiplied by the normalization factor to yield the normalized brain parenchymal volume.

Pulsed-gradient SE images were first corrected for geometrical distortion induced by eddy currents using an algorithm that maximizes mutual information between the diffusion unweighted and weighted images. Then, the DT was calculated and MD and FA were derived for each voxel, as previously described\(^ {25}\) (Figure 1). The diffusion images were interpolated to the same image matrix size as the dual-echo image, and then the b = 0 step of the pulsed-gradient SE images (T2 weighted, but not diffusion weighted) was coregistered with the dual-echo T2-weighted images using a 3-dimensional rigid body coregistration algorithm based on mutual information.\(^ {26}\) The subsequent step consisted of automatic transfer of lesion outlines onto the MD and FA maps and calculation of average lesion MD and FA.\(^ {23}\) By using statistical software (SPM99) and maximum image inhomogeneity correction,\(^ {27}\) brain GM, WM, and CSF were then automatically segmented from proton density– and T2-weighted images. Each pixel was classified as GM, WM, or CSF, dependent on which mask had the greatest probability (maximum likelihood) at that location. This pixel classification generated mutually exclusive masks for each tissue. The resulting masks were superimposed onto the MD and FA maps, on which hyperintense lesions were masked out previously, and the corresponding MD and FA histograms of the NAWM and NAGM were produced. For all the histograms, the average MD and FA values were calculated, as were the heights.
and locations of their peaks. Because histogram-derived metrics are strongly interrelated, only the values of average MD, average FA, and their respective peak heights were chosen a priori to enter the statistical analysis, to minimize the number of comparisons and, therefore, reduce the risk of type I errors.

**STATISTICAL ANALYSIS**

Group comparisons were analyzed using an unpaired t test. Univariate correlations were assessed using the Spearman rank correlation coefficient. A univariate logistic regression model was used to test whether there were conventional or DT MRI variables predicting the probability to show MRI or clinical evidence of disease DIT at follow-up.

**RESULTS**

Forty-five patients (13 men and 32 women; mean age, 29.4 years; SD, 5.9 years) were studied. Clinical presentations of CIS were optic neuritis in 14 patients (31%), hemispheric syndrome in 12 (27%), brainstem syndrome in 14 (31%), and spinal cord syndrome in 5 (11%). Oligoclonal bands were found in the CSF of 39 patients. One or more enhanced lesions were found on the baseline images of 13 (29%) of the patients (12 had 1 lesion and 1 had 6 lesions). By definition, all patients had paraclinical evidence of disease DIS: in 35 patients, this was demonstrated by MRI alone; and in the remaining 10, by the combination of MRI findings and the presence of oligoclonal bands in the CSF. At follow-up, 29 patients (64%) showed MRI evidence of disease DIT (12 at month 3 and 17 at month 12), thus fulfilling the criteria for a diagnosis of MS. Magnetic resonance imaging disease DIT was associated with evidence of clinical disease DIT during the study period in 12 of these patients.

The Table reports the MRI characteristics of the study subjects. When compared with healthy controls, patients showed a significant increase of average NAWM MD, a significant decrease of average NAWM MD peak height, and a significant decrease of average NAWM FA related with average NAWM MD (Spearman rank correlation coefficient, 0.53; P < .001), NAWM MD peak height (Spearman rank correlation coefficient, –0.46; P = .002), and average NAWM FA (Spearman rank correlation coefficient, –0.56, P < .001).

No significant differences for any of the DT MRI histogram-derived quantities were found between patients with and those without enhanced lesions at study enrollment, nor between patients with MRI and those with MRI plus CSF disease DIS (P value range, .19–.98). No conventional or DT MRI–derived variables were significant predictors of MRI or clinical disease DIT at follow-up (B value range, –4.06 to 5.99; P value range, .10–.96).

**Table. Conventional and DT MRI–Derived Measures at Study Enrollment in 45 Patients With a CIS and in 22 Control Subjects**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Patients With a CIS*</th>
<th>Healthy Controls*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 LV, mL</td>
<td>4.8 (4.8)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NBV, mL</td>
<td>1627.5 (53.8)</td>
<td>1630.5 (67.9)</td>
<td>.87</td>
</tr>
<tr>
<td>Average NAWM MD, (\text{mm}^2/\text{s} \times 10^{-3})</td>
<td>0.82 (0.03)</td>
<td>0.80 (0.02)</td>
<td>&lt;.01†</td>
</tr>
<tr>
<td>NAWM MD peak height</td>
<td>179.2 (20.3)</td>
<td>198.5 (19.3)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Average NAWM FA</td>
<td>0.28 (0.02)</td>
<td>0.30 (0.01)</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>NAWM FA peak height</td>
<td>33.3 (2.1)</td>
<td>32.5 (1.99)</td>
<td>.12</td>
</tr>
<tr>
<td>Average NAGM MD</td>
<td>0.99 (0.04)</td>
<td>0.98 (0.04)</td>
<td>.24</td>
</tr>
<tr>
<td>NAGM MD peak height</td>
<td>81.1 (11.9)</td>
<td>86.1 (17.5)</td>
<td>.18</td>
</tr>
<tr>
<td>Average NAGM FA</td>
<td>0.13 (0.007)</td>
<td>0.13 (0.006)</td>
<td>.71</td>
</tr>
<tr>
<td>NAGM FA peak height</td>
<td>84.3 (6.2)</td>
<td>84.1 (4.0)</td>
<td>.64</td>
</tr>
</tbody>
</table>

Abbreviations: CIS, clinically isolated syndrome; DT, diffusion tensor; FA, fractional anisotropy; LV, lesion volume; MD, mean diffusivity; MRI, magnetic resonance imaging; NA, data not applicable; NAGM, normal-appearing gray matter; NAWM, normal-appearing white matter; NBV, normalized brain parenchymal volume.

*Data are given as mean (SD).
†The difference between the 2 groups was significant.
Several postmortem studies of MS have shown that astrocytic hyperplasia, patchy edema, perivascular infiltration, myelin thinning, and axonal loss can occur in the WM of the brain outside macroscopic T2-visible lesions.6,7 The GM is also not spared by the MS pathological processes,28 although most cortical lesions may go undetected on conventional MRIs.29 Such brain damage occurring beyond the resolution of conventional imaging might influence the evolution of MS. In the past few years, considerable effort has been spent to investigate the patterns of normal-appearing brain tissue changes in MS.6,7 In this context, DT MRI proved to be extremely sensitive in detecting otherwise occult MS-related brain abnormalities, given its ability to quantify microstructural changes that modify the integrity of brain tissues.6

Increasing evidence indicates that the extent and severity of NAWM and NAGM damage, as detected by quantitative MRI techniques, significantly contribute to the development of irreversible neurological impairment in MS patients.6 Nevertheless, little is known about the characteristics of normal-appearing brain tissue damage in patients with CIS suggestive of MS at the earliest clinical stage. Preliminary cross-sectional magnetization transfer MRI studies of CIS have achieved conflicting results.9-12 Among the possible explanations for these discrepant results, methodological issues, such as the duration of the clinical follow-up, the sample size, and the patients' characteristics at study enrollment, should be considered. More recently, a study with single-voxel proton magnetic resonance spectroscopy13 has reported a significant increase of the concentration of myo-inositol (a marker of glial activity) in the NAWM of CIS patients. To our knowledge, only 1 study has investigated the brain diffusion characteristics of CIS,13 by measuring the apparent diffusion coefficient in several regions of interest from the NAWM of 19 patients. In this study, significantly higher NAWM apparent diffusion coefficient values were found in patients than in healthy controls only 1 year after CIS onset, when most patients had developed clinically definite MS.

Our main finding is that DT MRI discloses the presence of NAWM damage in CIS patients, which seems to be significantly, but only partially, related to the burden of brain MRI-visible abnormalities. All CIS patients enrolled in the present study had to have paraclinical signs of disease DIS, according to recent consensus criteria,20 and, as a consequence, none of them had a normal brain MRI result. Thus, they had a high risk of developing MS, as also confirmed by the high frequency (64%) of MRI disease DIT after 3 or 12 months of follow-up. Admittedly, more than one third of the patients in this study did not meet the criteria of McDonald et al20 for a diagnosis of MS after a follow-up of 1 year. Nevertheless, alternative diagnoses have been carefully excluded and all had clinical pictures suggestive of MS at presentation. Our patients were also selected for having disease DIS at presentation (ie, MRI and/or CSF findings consistent with a diagnosis of MS).20 As a consequence, we believe that all of them are at high risk to ultimately progress to clinically definite MS.3 This may explain why our results are consistent with those of previous magnetization transfer MRI studies9-12 of CIS cohorts with similar characteristics. When compared with the study by Caramia et al,13 our study differs on several aspects, including the following: (1) more patients; (2) a shorter time to first DT MRI after CIS onset; (3) the acquisition of a more sophisticated sequence, with reconstruction of the tensor; and (4) a histogram-based instead of a region of interest–based analysis, providing an overall evaluation of NAWM diffusivity characteristics. All of these aspects might explain why we found significant DT MRI abnormalities in the NAWM of CIS patients soon after clinical disease onset, in contrast to what was reported by the former study.13 Because inflammatory changes and gliosis can potentially restrict water molecular motion, we believe that myelin and axonal damage11 are the most likely contributors to the loss of barriers limiting water motion (increased MD) and to the tissue structural organization (decreased FA) we found in the NAWM of this patient cohort.

We did not find significant DT MRI changes in the NAGM of patients when compared with healthy controls. This agrees with the results of a previous magnetization transfer MRI study of CIS,12 and with those of quantitative MRI studies of patients with established MS, in whom NAGM changes have often increased with disease duration and severity.19,32,33 These findings suggest...
that the accumulation of intrinsic GM pathological features in MS patients might be the consequence of retrograde degeneration of neurons, following the injury to axons passing through diseased WM regions.\textsuperscript{34} An additional explanation might be the progressive accumulation of GM lesions, most of which may go undetected when using conventional MRI. Demyelinated regions of the cerebral cortex from MS patients have also been found to harbor transected dendrites and axons and apoptotic neurons,\textsuperscript{25} and this also suggests that T2-undetectable cortical lesions might contribute to the observed increase of NAGM MD in patients with the more disabling forms of MS.\textsuperscript{10,32} The notion that significant GM damage may accompany the evolution of MS since its early stage\textsuperscript{33} is also supported by the recent observation of a decrease of GM volume in CIS patients developing MS after 3 years of follow-up.\textsuperscript{36}

The average normalized brain volume of our patients did not significantly differ from that of healthy controls, thus reasonably excluding that the observed diffusivity changes can be ascribed to partial volume effects from enlarged CSF spaces. Other cross-sectional studies did not report evidence of brain\textsuperscript{34} and corpus callosum atrophy\textsuperscript{27} in those with CIS. Two recent longitudinal studies have demonstrated that only CIS patients converting to MS during the study period developed significant ventricular enlargement and GM atrophy.\textsuperscript{36,38} The present study, and the recent demonstration of significant axonal pathological features at the earliest clinical stage of MS,\textsuperscript{14} suggests that intrinsic tissue damage may precede MRI-detectable atrophy.

Another intriguing finding of this study is that DT MRI-derived metrics do not predict the occurrence of either MRI-detectable inflammatory activity within 1 year after CIS onset or a relapse leading to the diagnosis of clinically definite MS. Although a longer follow-up might lead to different results, because the proportion of CIS patients developing MS continues to increase after 10 years\textsuperscript{3} and the prognostic value of MRI characteristics for the clinical evolution of MS seems to become stronger after longer periods of observation,\textsuperscript{39} our data agree with those of another study comparing magnetization transfer MRI data from recent-onset CIS patients with those from CIS patients not developing MS after 14 years of follow-up.\textsuperscript{12} Considering also that the NAWM and NAGM diffusivity characteristics did not differ between patients with and those without enhanced lesions on the same image, our findings suggest that diffuse DT MRI-detectable structural NAWM damage and discrete conventional MRI-visible inflammatory changes can be, at least partially, dissociated since the early phase of MS. Admittedly, however, the lack of association between DT MRI findings at CIS onset and the subsequent occurrence of disease DIT might also depend on the insufficient power of our study. All of this underpins the need for further studies with longer follow-up periods and larger sample sizes to properly address this issue.

Accepted for Publication: October 22, 2004.

Correspondence: Massimo Filippi, MD, Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy (filippi.massimo@hsr.it).

Author Contributions: Study concept and design: Rovaris, Ghezzi, Falini, Comi, and Filippi. Acquisition of data: Gallo, Riva, Benedetti, and Martinelli. Analysis and interpretation of data: Gallo, Rovaris, Ghezzi, Benedetti, and Filippi. Drafting of the manuscript: Gallo, Rovaris, and Filippi. Critical revision of the manuscript for important intellectual content: Gallo, Rovaris, Riva, Ghezzi, Benedetti, Martinelli, Falini, Comi, and Filippi. Statistical analysis: Filippi. Study supervision: Filippi.

REFERENCES


