Assessment of Normal-Appearing White and Gray Matter in Patients With Primary Progressive Multiple Sclerosis

A Diffusion-Tensor Magnetic Resonance Imaging Study

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Background: Diffusion-tensor magnetic resonance imaging is sensitive to the more destructive aspects of multiple sclerosis (MS) evolution occurring outside and within T2-visible lesions and, as a consequence, holds promise for providing a more complete picture of primary progressive (PP) MS–related tissue damage than conventional magnetic resonance imaging.

Objective: To improve our understanding of PPMS by assessing the extent of occult pathological features in the normal-appearing white and gray matter of the brain using diffusion-tensor magnetic resonance imaging.

Methods: Ninety-six patients with PPMS, 47 patients with secondary progressive (SP) MS, and 44 healthy control subjects were studied. T2-hyperintense and T1-hypointense lesion volumes were calculated, and the volume of the whole brain tissue was measured. Diffusion-tensor magnetic resonance imaging scans were postprocessed and analyzed to obtain the mean diffusivity and fractional anisotropy histograms from the brain and from the normal-appearing white and gray matter in isolation.

Results: The mean T2-hyperintense and T1-hypointense lesion volumes were lower in patients with PPMS than in patients with SPMS, while the mean absolute brain volumes were similar in the 2 groups. The average lesion diffusivity was significantly higher in patients with SPMS than in patients with PPMS (P < .001). Histogram-derived metrics of the brain tissue and normal-appearing white and gray matter were significantly different between patients with PPMS and healthy subjects (range, P = .004 to < .001). Average diffusivity values were significantly higher in patients with SPMS than in patients with PPMS for all the tissues studied (range, P = .001 to < .001). Fractional anisotropy histogram–derived quantities did not significantly differ between the 2 patient groups (range, P = .94 to .03).

Conclusion: This study confirms that, in patients with PPMS, normal-appearing white and gray matter are not spared by disease-related pathological processes, although they are affected to a lesser degree than in patients with SPMS.

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The mechanisms underlying multiple sclerosis (MS) evolution in patients with the primary progressive (PP) form of the disease are poorly understood.1 Despite the accumulation of irreversible neurological deficits, the burden and activity of lesions on T2-weighted and gadolinium-enhanced magnetic resonance imaging (MRI) scans of the brain are, on average, lower in patients with PPMS than in patients with relapsing-remitting or secondary progressive (SP) MS.2-7 That the pathological features of PPMS lesions consist of a predominant loss of myelin and axons with only mild inflammatory components8 can, at least partially, explain the relative paucity of conventional MRI-detectable activity3,4 and the discrepancy between clinical and MRI findings.

Several pieces of evidence6,11 have suggested that diffuse brain damage, which goes undetected when using conventional MRI, may be a relevant component of PPMS pathological features. All these studies are based on the use of quantitative MRI techniques, with increased specificity to the most destructive aspects of MS pathological features and increased sensitivity for the detection of subtle MS-related changes occurring in the normal-appearing white matter (NAWM). The severity of NAWM damage correlates with MS irreversible disability better than MRI-derived measures of macroscopic lesion burden do.11 More recently, the importance of gray matter pathological features in patients with MS has also been...
emphasized. Again, conventional T2-weighted MRI scans underestimate the burden of MS lesions located in the brain gray matter, as shown by several studies using fast fluid-attenuated inversion recovery, gadolinium-enhanced, magnetization transfer, or diffusion-tensor (DT) MRI. Measures derived from DT MRI include the mean diffusivity (D), which is affected by cellular size and integrity, and the fractional anisotropy (FA), which reflects the degree of alignment of cellular structures within fiber tracts and their structural integrity. By creating histograms of D and FA values from the corresponding brain maps, an estimation of MS-related damage can be achieved. Diffusion-tensor histogram-derived metrics differ between patients with MS and healthy subjects, and among patients with MS with varying disease phenotypes and clinical disability. In addition, using a technique based on FA thresholding to segment the white and gray matter on D maps of the brain, information on the presence and extent of MS abnormalities can be obtained from each of these 2 compartments separately.

In the present study, DT MRI was used to assess the presence and severity of normal-appearing gray matter (NAGM) and NAWM damage in a large sample of patients with PPMS and to compare their damage with that of age-matched healthy subjects and of a group of patients with SPMS with similar clinical characteristics.

PATIENTS AND METHODS

PATIENTS

Patients with PPMS were consecutively selected from the populations attending the MS clinics in the participating centers. To be included, patients had to be corticosteroid free for at least 3 months before study enrollment. The disease course was classified according to the criteria of Lublin et al, who were consecutively referred to the MS clinics in the participating centers. To be included, patients with SPMS had to be relapse- and corticosteroid-free for at least 3 months before study enrollment. Their mean age was 47.4 (range, 31-61) years, their median disease duration was 16.0 (range, 3-29) years, and the median EDSS score, assessed by the same rater and with the same modalities as for patients with PPMS, was 6.0 (range, 3.5-8.0). At enrollment, 23 patients with SPMS were untreated, 14 were treated with interferon beta-1b, 8 were treated with pulses of intravenous mitoxantrone, and 2 were treated with glatiramer acetate.

All of the subjects signed a written informed consent form before study enrollment, and the study was approved by the local ethical committees.

IMAGE ACQUISITION

Using a 1.5-T system, the following pulse sequences were acquired: (a) dual-echo turbo spin-echo (repetition time, 3300 ms; echo time, 16 ms; first echo) and 98 ms (second echo); and echo train length, 5; (b) T1-weighted conventional spin-echo (repetition time, 768 ms; and echo time, 14 ms); and (c) pulsed-gradient spin-echo echo-planar (interecho spacing, 0.8; and echo time, 123 ms), with diffusion gradients applied in 8 noncollinear directions, chosen to cover 3-dimensional space uniformly. The duration and maximum amplitude of the diffusion gradients were 25 ms and 21 mT/m (mT indicates millitesla), respectively, giving a maximum b factor in each direction of 1044 s/mm². To optimize the measurement of diffusion, only 2 b factors were used (b₀ = 0 and b₁ = 1044 s/mm²). Fat saturation was performed using a 4-radius frequency pulse bimodal pulse train to avoid chemical shift artifacts. Twenty-four contiguous axial slices, with a 5-mm thickness, a 192 × 256 matrix size, and a 188 × 250-mm field of view were obtained for dual-echo and T1-weighted scans. For pulsed-gradient spin-echo echo-planar scans, 10 axial slices with a 5-mm thickness, a 128 × 128 matrix size, and a 250 × 250-mm field of view were acquired, with the same orientation as the other scans and the second-last caudal slice positioned to match exactly the central slices of these sets.

IMAGE ANALYSIS

Two experienced observers (M.R. and M.B.), without knowing to whom the scans belonged, identified by consensus the hypointense lesions on T1-weighted scans. T2-weighted images were always used to increase confidence in lesion identification. On proton density- and T1-weighted images, total lesion volumes (TLVs) were measured by a single observer, using a local thresholding technique for lesion segmentation. On T1-weighted images, the absolute volumes of the whole brain were measured using a segmentation technique based on signal intensity thresholding and characterized by a high intraobserver reproducibility.

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From DT MRI scans, \( \hat{D} \) and FA maps were created and coregistered to the dual-echo images following a method described elsewhere.\(^1\)\(^8\)\(^,\)\(^33\)\(^-\)\(^37\) Lesion outlines on proton density–weighted images were automatically transferred onto the coregistered \( \hat{D} \) and FA images, and the area and \( \hat{D} \) and FA of each lesion were measured. Then, for each patient, the average lesion \( \hat{D} \) and FA, weighted by lesion area,\(^1\)\(^8\) were calculated.

Normalized histograms of \( \hat{D} \) and FA maps were created as previously described.\(^1\)\(^8\) For all the histograms, the average \( \hat{D} \) and FA values were calculated, as were the heights and locations of their peaks. Diffusivity histograms were derived from the brain tissue (including T2-visible MS lesions and normal–appearing tissue), NAWM, and NAGM. To obtain \( \hat{D} \) histograms of NAWM and NAGM, MS lesion outlines from T2-weighted scans were automatically transferred onto the coregistered \( \hat{D} \) maps and then nulled out. The segmentation of NAWM and NAGM from the resulting \( \hat{D} \) maps was obtained using an automated technique based on FA thresholding, which has been previously validated in healthy controls and in patients with MS.\(^1\)\(^8\) Because FA maps were used for the segmentation of NAWM and NAGM, FA histograms were derived only from the brain tissue.

**STATISTICAL ANALYSIS**

Group comparisons were assessed using the Mann-Whitney test for nonparametric data or the \( t \) test for parametric data. After Bonferroni correction, significant \( P \) values were \( P<.004 \) for comparisons between patients with PPMS and healthy controls and \( P<.003 \) for comparisons between patients with PPMS and those with SPMS.

Univariate correlations were assessed using the Spearman rank correlation coefficient. Composite MRI scores\(^1\)\(^8\)\(^,\)\(^26\)\(^,\)\(^28\)\(^,\)\(^29\) were generated using a linear combination of MRI variables, which were chosen a priori based on biological considerations. The first score included MRI measures of macroscopic lesion burden and intrinsic lesion damage (ie, T2-hyperintense LV, T1-hypointense LV, and average lesion \( \hat{D} \)). The second score was composed by average brain, NAWM, and NAGM \( \hat{D} \) (ie, by measures reflecting diffuse tissue damage). A third score included quantities reflecting MS tissue damage within and outside T2-visible lesions (ie, average lesion, NAWM, and NAGM \( \hat{D} \)). The weight of each MRI variable resulted from the coefficients estimated by a linear regression model, with EDSS score as the dependent variable. The magnitude and the significance of the correlation between composite MRI scores and EDSS score were evaluated by a nonparametric Spearman rank correlation analysis, because EDSS score does not satisfy the assumptions of continuity and normality for a valid inference in linear regression models. For all the correlations, \( P<.05 \) was considered significant. Statistical analysis was performed using a statistical package (Statistical Product and Service Solutions, version 9.0; SPSS Inc, Chicago, Ill).

RESULTS

Nine or more T2-hyperintense lesions\(^30\) were seen on the scans from 88 (92%) of the 96 patients with PPMS and on the scans from all patients with SPMS; fewer lesions (range, 2-8) were visible in the remaining patients with PPMS. In the patients with PPMS, median T2-hyperintense and T1-hypointense LVs were 11.3 (25th-75th percentile range [PR], 4.5-30.4) mL and 2.5 (25th-75th PR, 0.4-7.1) mL, respectively. In the patients with SPMS, the corresponding quantities were 26.4 mL (25th-75th PR, 16.0-38.6 mL; \( P<.001 \) vs patients with PPMS, Mann-Whitney test) and 4.5 mL (25th-75th PR, 2.6-9.1 mL; \( P = .004 \) vs patients with PPMS, Mann-Whitney test), respectively. The median values of average lesion \( \hat{D} \) and FA were 1.07 (25th-75th PR, 0.98-1.16) mm²/s per 10⁻³ and 0.26 (25th-75th PR, 0.23-0.28) in patients with PPMS and 1.26 (25th-75th PR, 1.14-1.37) mm²/s per 10⁻³ and 0.23 (25th-75th PR, 0.20-0.27) in patients with SPMS, respectively. The average lesion \( \hat{D} \) was significantly higher in patients with SPMS than in patients with PPMS (\( P<.001 \), Mann-Whitney test).

The mean (SD) values of brain volume were 1094.8 (113.5) mL in patients with PPMS, 1155.4 (89.3) mL in healthy subjects, and 1096.1 (117.8) mL in patients with SPMS. In patients with PPMS, the mean brain volume was significantly lower than in healthy subjects (\( P = .001 \), \( t \) test), while no significant differences were found between patients with PPMS and those with SPMS (\( P = .95 \)).

**Table 1** and **Table 2** report the mean values of histogram-derived quantities from brain tissue, NAWM, and NAGM (Figure) in patients with PPMS, healthy controls, and patients with SPMS. All the differences remained statistically significant after adjusting for brain volume. **Table 3** reports the correlations between DT MRI–derived metrics and T2-hyperintense or T1-hypointense LV; the strongest correlation was that between average brain \( \hat{D} \) and T2-hyperintense LV (\( r = 0.74 \), \( P < .001 \)).

No significant correlations were found between EDSS score and any MRI- or DT MRI–derived variable (range, \( P = .99 \) to .26). No significant differences in any of the MRI-

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**Table 1.** \( \hat{D} \) and FA Histogram–Derived Metrics of the Brain Tissue From All Study Subjects\(^*\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients With PPMS</th>
<th>Healthy Control Subjects</th>
<th>( P ) Value‡‡</th>
<th>Patients With SPMS</th>
<th>( P ) Value‡§</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \hat{D} )</td>
<td>0.99 (0.96-1.03)</td>
<td>0.91 (0.87-0.94)</td>
<td>&lt;.001</td>
<td>1.04 (0.99-1.09)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Average</td>
<td>83.7 (73.9-94.2)</td>
<td>105.4 (100.3-112.9)</td>
<td>&lt;.001</td>
<td>76.2 (61.0-84.3)</td>
<td>.002</td>
</tr>
<tr>
<td>Peak height</td>
<td>0.78 (0.75-0.81)</td>
<td>0.75 (0.72-0.79)</td>
<td>&lt;.001</td>
<td>0.78 (0.75-0.84)</td>
<td>.25</td>
</tr>
<tr>
<td>FA</td>
<td>0.20 (0.19-0.21)</td>
<td>0.24 (0.23-0.25)</td>
<td>&lt;.001</td>
<td>0.19 (0.18-0.20)</td>
<td>.03</td>
</tr>
<tr>
<td>Average</td>
<td>47.9 (44.4-51.3)</td>
<td>39.2 (37.3-41.2)</td>
<td>&lt;.001</td>
<td>48.4 (45.3-51.2)</td>
<td>.94</td>
</tr>
<tr>
<td>Peak height</td>
<td>0.09 (0.09-0.10)</td>
<td>0.11 (0.11-0.13)</td>
<td>&lt;.001</td>
<td>0.09 (0.08-0.10)</td>
<td>.92</td>
</tr>
</tbody>
</table>

*Data are given as median (25th-75th percentile range) unless otherwise indicated. \( \hat{D} \) indicates mean diffusivity; FA, fractional anisotropy; PPMS, primary progressive multiple sclerosis; and SPMS, secondary progressive multiple sclerosis.

†For group comparisons between patients with PPMS and healthy control subjects.

‡The Mann-Whitney test was used.

§For group comparisons between patients with PPMS and patients with SPMS.
The clinical characteristics of our sample of patients with PPMS are similar to those of other populations enrolled in previous large-scale studies, but the predominance of patients with definite PPMS is likely to be the explanation for the relatively higher brain T2-hyperintense LV we found compared with those previously reported. A diagnosis of definite PPMS actually requires the presence of at least 9 brain T2-hyperintense lesions or 4 to 8 brain lesions when spinal cord lesions are detected. Consistent with the results of another study, we found that brain T1-hypointense LVs did not differ significantly between patients with PPMS and those with SPMS, thus indicating that the overall burden of macroscopic lesions affected by severe tissue damage may overlap in the 2 progressive forms of MS. However, the average D of T2-visible lesions was significantly higher in patients with SPMS than in those with PPMS. These findings suggest that, when only a binary classification of MS lesions as either T1 hypointense or T1 isointense is performed, relevant information about the pathological heterogeneity of “black holes” is inevitably lost. Our results also prompt speculations on how intrinsic lesion MS pathology may have a different functional impact in patients with the progressive forms of the disease. Given the greater amount of tissue that is involved by T2-visible lesions in patients with SPMS, in these patients the severity of intrinsic lesion damage might play an important role in the accumulation of irreversible disabil-
ity. This agrees with longitudinal data showing a continuous increase of tissue damage within newly formed lesions in patients with SPMS. On the contrary, the few T2-visible lesions and the observation that the severity of intrinsic tissue damage within individual lesions is lower in patients with PPMS than in patients with SPMS suggest that other factors in addition to the presence of lesions affected by marked tissue disruption should act in determining the dynamics of PPMS evolution.

Brain D and FA histogram analysis confirmed the presence of diffuse abnormalities in patients with PPMS, for whom the histographic quantities were all significantly lower than those of healthy subjects. Admittedly, the DT MRI acquisition scheme we used allowed us to obtain limited brain coverage. Nevertheless, we covered a large portion of the central brain, where most of the MS abnormalities are typically located. The severity of brain tissue damage was, however, greater in patients with SPMS than in those with PPMS. Although patients with PPMS showed a significant decrease of brain parenchymal volume vs healthy controls, the results of group comparisons for D and FA histogram-derived quantities did not change after correcting for this factor, thus indicating that the observed changes are not attributable to the inclusion of pixels with significant partial volume effects from the CSF. The correlation we found between T2-hyperintense LVs and average brain D or FA suggests that, at least partially, wallerian degeneration of axons that are transverse to macroscopic lesions might contribute to MRI-undetectable brain abnormalities in patients with PPMS. Nevertheless, given the paucity of T2-visible lesions in patients with PPMS, the occurrence of multiple discrete lesions beyond the resolution of conventional scanning should also be considered. This important issue should be addressed by future postmortem studies because, to our knowledge, no pathological data are available supporting the presence of more diffuse occult damage in patients with PPMS than in those with other MS phenotypes.

Although much of the examined brain tissue is constituted of NAWM and, as a consequence, diffuse NAWM damage is likely to be the major contributor to the observed histogram changes, recent studies¹⁹,²⁰ have suggested that gray matter damage is not a negligible aspect of PPMS pathological features. For this reason, after removal of T2-visible lesions, we obtained D histograms from the NAWM and NAGM separately.

In a previous study,¹⁸ it was demonstrated that a segmentation process based on FA thresholding allows accurate separation between white and gray matter, based on their different microstructural properties. That this segmentation technique works properly in healthy subjects and in patients with MS is indicated by the previous finding that the mean ratios between pixels attributed to NAGM and those attributed to NAWM were similar in these 2 samples, and by the lack of significant differences between region of interest-based and histogramic analysis of DT MRI characteristics from the NAGM and NAWM of patients with MS.²⁸ We found that NAWM and NAGM D histogram-derived quantities were different between patients with PPMS and age-matched healthy subjects. Again, tissue damage in both of the compartments seemed to be more pronounced in patients with SPMS than in patients with PPMS.

Our finding of NAWM D abnormalities is consistent with those of other magnetization transfer MRI and magnetic resonance spectroscopy studies,⁶-¹¹ showing that tissue damage occurs outside T2-visible lesions in patients with PPMS. Although these results indicate a net loss and disorganization of structural barriers to water molecular motion in the NAWM, we can only speculate on the possible pathological substrates, and postmortem correlative studies are needed to clarify this issue. Nevertheless, valuable information can be derived from DT MRI studies of other neurological conditions. Increased D values have been described in the brain tissue and NAGM from patients with Alzheimer disease,²² which can be considered a condition characterized by diffuse degenerative brain changes. Increased D has also been found in patients with cerebrovascular abnormalities, such as leukoaraiosis,³¹ systemic lupus erythematosus,⁴¹ and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.⁴⁵ Although the magnitude of DT MRI histogram abnormalities observed in our patients is less pronounced than in patients with Alzheimer disease, forty most of the subtle pathological changes known to occur in the NAWM from patients with MS, including diffuse astrocytic hyperplasia, patchy edema, perivascular infiltration, and abnormally thin myelin and axonal loss,⁴⁶ have the potential to determine increased D values.

Several observations²²-²⁴ have recently emphasized the potential role of gray matter tissue damage in patients with MS. Studies with positron emission tomography and quantitative¹⁶,²¹ MRI techniques have consistently shown functional and structural abnormalities in the NAGM of patients with MS. In a postmortem study,⁴⁶ gray matter MS lesions had a reduced amount

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**Table 3. Univariate Correlations Between Quantities Derived From DT MRI and T2-Hyperintense or T1-Hypointense LVs in 96 Patients With PPMS**

<table>
<thead>
<tr>
<th>LV</th>
<th>Average Lesion</th>
<th>Average Brain</th>
<th>Average D</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 hyperintense</td>
<td>D: 0.56 (.001)</td>
<td>FA: -0.11 (.30)</td>
<td>D: 0.74 (.001)</td>
</tr>
<tr>
<td>T1 hypointense</td>
<td>D: 0.54 (.001)</td>
<td>FA: -0.18 (.01)</td>
<td>D: 0.72 (.001)</td>
</tr>
</tbody>
</table>

Data are given as Spearman rank correlation coefficients (P-value). DT indicates diffusion-tensor; MRI, magnetic resonance imaging; LV, lesion volume; PPMS, primary progressive multiple sclerosis; D, mean diffusivity; FA, fractional anisotropy; NAWM, normal-appearing white matter; and NAGM, normal-appearing gray matter.
of inflammation when compared with those located in the white matter of the same patients, thus supporting the hypothesis that MS pathological features might follow different patterns in these 2 tissue compartments. Our results confirm that, in patients with PPMS, the brain NAGM is not spared by the pathological process. There are at least 2 factors that may contribute to the increased $D$ values found in the NAGM of patients with PPMS. First, there is the presence of discrete MS lesions, which may go undetected when using T2-weighted imaging.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\) That demyelinated regions of the cerebral cortex from patients with MS harbored transected dendrites, transected axons, and apoptotic neurons\(^6\)\(^7\) also suggests that T2-undetectable cortical lesions might provoke a significant increase of gray matter $D$. Second, an alternative, but not mutually exclusive, explanation of the observed $D$ changes might be the presence of wallerian degeneration of gray matter neurons, secondary to the damage of fibers that are transverse to MS white matter lesions.\(^8\) However, the modest correlation we observed between macroscopic lesion load and average NAGM $D$ suggests that such a mechanism is likely to account only for a limited part of DT MRI findings from the NAGM. Because the results of NAGM histogram analysis did not change after correcting for brain volume, we conclude that, even though in patients with PPMS and SPMS, due to the presence of brain atrophy, pixels with significant partial volume effect from the CSF might have been introduced in the gray matter pixel pool, such a factor is likely not to affect NAGM $D$ a great deal.

Disappointingly, we did not find any significant correlation between DT MRI–derived measures and EDSS score. This might be because of the limitations of EDSS score, including that it is heavily weighted toward locomotor disability, which is likely to be largely dependent on the amount of spinal cord damage. Accordingly, conventional and magnetization transfer MRI studies\(^6\)\(^7\)\(^8\)\(^9\)\(^10\) have suggested that a prevalent involvement of the cervical cord might, at least partially, explain the discrepancy between brain MRI and clinical findings in patients with PPMS. In this study, the absence of imaging data from the cervical cord might, therefore, account for the poor clinical and MRI correlations. Interestingly, when the subgroup of patients with PPMS with clinical presentations other than a spinal cord syndrome was considered in isolation, we found a stronger correlation between the EDSS score and a composite MRI score based on measures of tissue damage within and outside T2-visible lesions. The paucity of the correlation between NAWM/NAGM pathological features and disability might also be secondary to the interpatient variability of adaptive cortical reorganization, with the potential to limit the clinical impact of structural PPMS damage, as shown by recent functional MRI studies.\(^10\)\(^11\)

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Author contributions: Study concept and design (Drs Rovaris, Ghezzi, Caputo, Montanari, Bertolotto, and Filippi); acquisition of data (Drs Bozzali, Iannucci, Ghezzi, Caputo, Montanari, Bertolotto, Bergamaschi, Capra, Mancardi, and Martinelli); analysis and interpretation of data (Drs Rovaris, Bozzali, Iannucci, Bergamaschi, Capra, Mancardi, Comi, and Filippi); drafting of the manuscript (Dr Rovaris); critical revision of the manuscript for important intellectual content (Drs Bozzali, Iannucci, Ghezzi, Caputo, Montanari, Bertolotto, Bergamaschi, Mancardi, Martinelli, Comi, and Filippi); statistical expertise (Drs Bozzali, Bergamaschi, and Filippi); obtained funding (Drs Ghezzi, Capra, Mancardi, Comi, and Filippi); administrative, technical, and material support (Drs Rovaris, Iannucci, Caputo, Montanari, Bertolotto, and Martinelli); study supervision (Dr Filippi).

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