Associations Between Cervical Cord Gray Matter Damage and Disability in Patients With Multiple Sclerosis

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Objectives: To assess in vivo the volume and the magnetization transfer magnetic resonance imaging (MRI)–detectable damage of the cervical cord gray matter in patients with relapsing-remitting multiple sclerosis (RRMS) and to evaluate whether such damage correlates with disability.

Design: Cervical cord conventional and magnetization transfer MRI scans were acquired from 18 patients with RRMS (and no T2-visible cervical cord lesions) and 13 age- and sex-matched healthy controls. After ad hoc image postprocessing, characterized by high intraobserver reproducibility, the average magnetization transfer ratio and volume of the cervical cord gray matter from patients and controls were calculated and compared using a 2-tailed t test with Bonferroni correction. The correlation between MRI metrics and Expanded Disability Status Scale score was assessed using the Spearman rank correlation coefficient.

Results: Compared with healthy controls, patients with RRMS had a lower cervical cord gray matter average magnetization transfer ratio (P = .009). No cervical cord gray matter atrophy was detected. In patients with RRMS, the gray matter average magnetization transfer ratio was correlated with the degree of disability (r = −0.48, P = .048).

Conclusions: Cervical cord gray matter is not spared by MS pathology, and such damage is an additional factor contributing to the disability of these patients.

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METHODS

During the past few years, several postmortem and in vivo magnetic resonance imaging (MRI) studies have provided convincing evidence that multiple sclerosis (MS) pathology does not spare brain gray matter. In this context, magnetization transfer MRI aids in grading the extent of overall gray matter damage in the brain of patients with MS, thus contributing to a better understanding of the nature and severity of such damage.

Although it is established that the spinal cord is a clinically eloquent site that is frequently involved in MS (postmortem studies have indeed found cord lesions in up to 90% of patients with MS), that cord gray matter can harbor MS lesions, and that high-quality magnetization transfer MRI of the cervical cord is feasible, the extent of gray matter injury in the spinal cord of patients with MS has never been quantified.

The present study was performed to assess in vivo the volume and the magnetization transfer MRI–detectable damage of the cervical cord gray matter in patients with relapsing-remitting (RR) MS and to evaluate whether such damage correlates with clinical disability.

We evaluated MRI findings from the cervical cord of 18 patients with RRMS (6 men and 12 women; mean age, 39 years; range, 24-55 years; mean disease duration, 8 years; range, 1-14 years; median Expanded Disability Status Scale score, 3.5; range, 1-5.0) and 13 age- and sex-matched healthy controls (4 men and 9 women; mean age, 38 years; range, 24-50 years). Using a machine operating at 1.5 T and a standard matrix neck coil, we acquired the following pulse sequences:

- Dual-echo turbo spin echo (repetition time [TR], 2000 milliseconds; echo time [TE], 30/145 milliseconds; flip angle [FA], 150°; echo train length, 23; field of view [FOV], 300 × 300 mm; matrix size, 256 × 320; 7 sagittal contiguous slices with a thickness of 4 mm)
- Two-dimensional gradient echo (TR, 600 milliseconds; TE, 25 milliseconds; FA, 20°; FOV, 180 × 180 mm; matrix size, 128 × 128; 20 axial contiguous slices with a thickness of 4 mm) with and without a magnetization transfer saturation pulse (offset frequency, 1500 Hz;
Gaussian envelope duration, 7.68 milliseconds; FA, 50°; frequency bandwidth, 250 Hz)
• Sagittal T1-weighted 3-dimensional magnetization-prepared rapid acquisition gradient echo (MP-RAGE) (TR, 11.6 milliseconds; TE, 4.2 milliseconds; FA, 15°; FOV, 115 × 230 mm; matrix size, 256 × 256; slice thickness, 0.9 mm)

Magnetic resonance images were transferred to a workstation for postprocessing, which was performed by 2 experienced observers unaware of subjects’ identity. Because T2-visible lesions can be misclassified and wrongly assigned to the gray matter, only patients without macroscopic cervical cord abnormalities on the conventional MRI scans were selected for this study. First, images with (“magnetization transfer” images) and without (“reference” images) the off-saturation pulse were coregistered to compensate for any mismatch due to patient movement during scanning. To this end, we used a 3-dimensional rigid transformation10 with image reslicing (one forward and the other backward halfway). Then, the cord tissue and the surrounding cerebrospinal fluid were accurately extracted from the transformed images using a semiautomatic contouring technique.11 The subsequent step consisted of the calculation of a 2-dimensional transformation (allowing translations in plane x and y and a rotation along the z axis), which was applied to each slice to compensate for additional subtile cord motion. Finally, the cervical cord magnetization transfer ratio (MTR) maps were produced using the coregistered magnetization transfer and reference images, as previously described.5 Cervical cord gray matter outlines were manually drawn on the transformed reference images where the gray matter shape had enough contrast on the white matter tissue and the contamination from the white matter was further minimized by considering in the analysis only pixels that were away from the edge of the cord gray matter. The gray matter outlines were then transferred onto the MTR maps and used to extract the gray matter MTR maps and to calculate the average MTR of the overall cervical cord gray matter. To test the intraobserver reproducibility of this approach, a single observer calculated cord gray matter MTR twice from all healthy controls (the 2 measurement sessions were separated by an interval of 1 month), and the intraobserver variability was assessed using the coefficient of variation, defined as the standard deviation of a random variable divided by its mean value.

Using the approach of Losseff et al.,12 cervical cord gray matter volume was measured from 6 slices upward using the center of the C2-C3 disc as the caudal landmark. The original MP-RAGE images were coregistered to the transformed reference images using the graphical interface of the VTK CISG tool13; the transformation was initialized, and after the coregistration ended, the caudal landmark was identified on the transformed MP-RAGE images and then transferred onto the reference images. The volume of regions of interest previously drawn to select cervical cord gray matter on MTR images was calculated for the corresponding reference images. A patients was classified as having cervical cord gray matter abnormalities if the gray matter average MTR was considered abnormal.6 The present study shows that gray matter injury of the cervical cord also involves the gray matter in terms of focal lesion, “diffuse” tissue abnormalities, and irreversible tissue loss.1,2

The difference of cervical cord volume between patients and controls was not significant, and no individual patient was found to have cervical cord atrophy. In patients with RRMS, gray matter average MTR was correlated with the degree of disability ($t=-0.48, P=.048$) (Figure).

No abnormalities were seen on the cervical cord conventional MRI scans obtained from healthy controls and RRMS patients. The mean value of the intraobserver coefficient of variation for cord gray matter MTR measurement was 0.9% (range, 0.7%-1.2%). The Table reports the average MTR and volume of the cervical cord gray matter from healthy controls and RRMS patients. Compared with healthy controls, patients with RRMS had a lower cervical cord gray matter average MTR ($P=.009$). The Table 3 shows that the average MTR of cervical cord gray matter from healthy controls and RRMS patients. Compared with healthy controls, patients with RRMS had a lower cervical cord gray matter average MTR ($P=.009$). The difference of cervical cord volume between patients and controls was not significant, and no individual patient was found to have cervical cord atrophy. In patients with RRMS, gray matter average MTR was correlated with the degree of disability ($t=-0.48, P=.048$) (Figure).

**COMMENT**

In MS, a growing body of evidence indicates that tissue damage in the brain is not restricted to white matter but also involves the gray matter in terms of focal lesion, “diffuse” tissue abnormalities, and irreversible tissue loss.1,2 The present study shows that gray matter injury of the spinal cord is another feature of the disease, which might contribute to the presence of disability.

Imaging the spinal cord in vivo is challenging for several reasons, which include the small size of the target tissue and the presence of motion artifacts, such as cardiac pulsation and respiration. In detail, the cervical cord cross-sectional area is only about 1 cm in size, and submillimeter spatial resolution is necessary to distinguish

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**Table. Average Magnetization Transfer Ratio and Volumetry of the Cervical Cord Gray Matter From Healthy Controls and Patients With MS**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Healthy Controls</th>
<th>Patients With MS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average MTR (SD), %</td>
<td>24.8 (1.5)</td>
<td>23.5 (1.2)</td>
<td>.009</td>
</tr>
<tr>
<td>Volume (SD), mL</td>
<td>510.8 (106.9)</td>
<td>486.7 (94.9)</td>
<td>.40</td>
</tr>
</tbody>
</table>

Abbreviations: MS, multiple sclerosis; MTR, magnetization transfer ratio.

a Two-tailed t test for unpaired data with Bonferroni correction.

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**Figure. Correlation between cervical cord gray matter average magnetization transfer ratio (MTR) and the Expanded Disability Status Scale (EDSS) score in patients with relapsing-remitting multiple sclerosis.**
with relative accuracy gray matter from white matter. Furthermore, the sensitivity of MTR to detect pathology depends on the available signal-to-noise ratio, and it is necessary to minimize the motion between scans with and without off-resonance saturation pulse. In an attempt to overcome these limitations, we optimized our MTR map creation procedure, which has been described previously, by adding 2 coregistrations of magnetization transfer and reference images to improve both gross and fine motion correction. The segmentation of cord gray matter also has a unique advantage in that it is affected only a very tiny amount by cerebrospinal fluid averaging because only the inner border of the gray matter is contiguous to the cerebrospinal fluid contained in the central canal. The pixels at the larger outer edge of the cord are contiguous to those of the white matter, which is characterized by higher MTR values than gray matter (thus, if it had any effect, partial volume averaging is likely to have reduced the difference between patients and controls and not the opposite). For the same reasons, we decided a priori to select patients without focal cord lesions (which are known to have MTR values lower than those of normal-appearing tissues) and not to study cord white matter MTR and volumetry. As a consequence, although we cannot exclude contamination of gray matter MTR data from partial volume averaging from the surrounding white matter, we do believe this did not affect our results a great deal; at any rate, it might have worked against the detection of gray matter MTR changes. Furthermore, we acknowledge that these results were obtained from patients with no T2-visible cervical cord lesions and, therefore, might not be representative of the general population of patients with RRMS. Nevertheless, it is conceivable that intrinsic cord lesions might be associated with even more damage to the gray matter due to wallerian and anterograde degeneration.

The main result of this study was showing that patients with RRMS have a lower average MTR of overall cervical cord gray matter than healthy controls. Because reduced MTR values indicate an increased proportion of “free” protons over that of protons bound to the cord tissue matrix molecules (such as those of the myelin sheaths and neuroaxonal membranes), this finding suggests the presence of cord gray matter disruption due to MS pathology. Postmortem studies have indeed shown that MTR is strongly associated to the percentage of residual axons and the degree of demyelination in the brain and spinal cord and that extensive demyelination can occur in the cord gray matter of patients. Clearly, because we do not have direct histopathological confirmation, we can only speculate on the pathological nature of the damage underlying the observed cord MTR changes. Nevertheless, as it is the case for the brain, it is conceivable that the presence of tiny discrete gray matter lesions (which may go undetected when imaging the cord with conventional T2-weighted sequences) and the degeneration of gray matter neurons (caused by axonal transection in the white matter) are both likely to play a role. Interestingly, cord gray matter volume did not differ between patients and controls. This finding, on one hand, confirms once again that the observed gray matter MTR differences between patients and controls is not attributable to partial volume effects, and on the other hand, it fits with the results from a recent postmortem study that showed that spinal cord atrophy in MS is virtually caused exclusively by white matter volume loss.

The second intriguing result of this study is the moderate correlation found between the severity of cervical cord gray matter injury and the degree of disability. This finding suggests that such an injury may yet be an additional relevant factor in the causation of the irreversible clinical manifestations of the disease, possibly through a disregulation of the complex facilitatory/inhibitory interneuronal network of the sensorimotor network of the cord. Because we studied only patients with RRMS and the patients had a relatively narrow ranges of disabilities, it is likely that the magnitude of such a correlation may be even greater in cohorts of patients with both the relapsing and the progressive forms of the disease. Although this finding needs to be replicated, taken together with recent magnetization transfer MRI data of the brain, it strengthens the notion that gray matter damage of the entire central nervous system is one of the key factors associated with “fixed” disability in MS.

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REFERENCES


New Initiatives: Clinical Trials and Videos

We have embarked on 2 new initiatives: Clinical Trials and video presentations. We welcome manuscripts that describe double-blind, randomized, placebo-controlled clinical trials as our primary area of interest. Open-label studies will also receive our special attention. We plan on expediting the review process and time to publication and to include them online ahead of print as these studies are time sensitive and of direct benefit to our patients. We hope you will take advantage of this new initiative. Please refer to the Instructions for Authors when submitting a Clinical Trials paper, including the requirement to register the trial with an accepted clinical trials site.

We plan to utilize videos as part of published papers that highlight and provide convincing information about the observational and visual features of a patient’s neurologic findings. Please refer to Instructions for Authors for instructions on submitting video presentations.