Voxel-Based Assessment of Differences in Damage and Distribution of White Matter Lesions Between Patients With Primary Progressive and Relapsing-Remitting Multiple Sclerosis

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Background: Several studies have reported lower focal demyelination and inflammatory activity in primary progressive multiple sclerosis (PPMS) than in relapsing-remitting MS (RRMS). However, very little is known about possible differences in damage and distribution that may occur within lesions visible on magnetic resonance imaging in the 2 forms of the disease.

Objective: To evaluate differences in spatial distribution and structural damage of focal demyelinating lesions in patients with PPMS and RRMS.

Design: We acquired conventional magnetic resonance and magnetization transfer images in 24 PPMS and 36 RRMS patients (matched for sex, age, and disease duration) and 23 healthy sex- and age-matched controls. In each participant, we measured T2- and T1-weighted lesion volumes and magnetization transfer ratios in lesional and nonlesional brain tissues. The spatial distribution of focal demyelination was assessed using T2- and T1-weighted lesion probability maps in each patient group. Voxel-based procedures were performed.

Setting: University hospital.

Results: Patients with PPMS had greater disability than those with RRMS, with 70% of PPMS patients and 11% of RRMS patients having relevant motor symptoms. The T1- and T2-weighted lesion volumes were higher in PPMS than in RRMS patients (P < .001). T1- and T2-weighted lesion probability maps showed that the maximum probability for lesions was higher in PPMS (peak probability, 45% and 29%, respectively) than in RRMS (peak probability, 33% and 19%, respectively) patients and was localized in the corona radiata. Voxelwise analysis of lesional magnetization transfer ratios gave overlapping results.

Conclusions: Differences in cerebral pathologic involvement exist between RRMS and PPMS and contribute to variations in clinical disability.

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Focal demyelination is one of the fundamental pathologic processes of MS. Demyelinating lesions of MS are pathologically heterogeneous\textsuperscript{22} and have a predilection for particular areas of the central nervous system.\textsuperscript{23} In particular, they cluster around the brain lateral ventricles and within the corpus callosum. Pathologic studies have reported differences in focal demyelination and lower inflammation between PPMS patients and those with other forms of MS.\textsuperscript{24,25} A number of MR studies have consistently reported a lower burden of cerebral WM lesions and signs of less inflammation in PPMS than in all other main clinical MS types.\textsuperscript{9,16,26} Despite this, very little is known about possible structural differences that may occur within lesions visible on MRI in different MS forms. In particular, no MR studies have focused on possible topographic differences in brain-lesion distribution between the different forms of MS.

Recent studies have used lesion distribution images to describe differences across populations in probabilistic terms.\textsuperscript{27-32} The MRI-based lesion probability map (LPM) is a powerful tool for studying in vivo MS lesion distribution, providing indirect clues regarding the mechanisms of lesion development.\textsuperscript{27-29} Furthermore, in previous studies, the measure provided by magnetization transfer imaging (the magnetization transfer ratio [MTR]) has provided accurate, quantitative estimates of brain tissue damage.\textsuperscript{33,34} This is particularly important in lesions visible on MRI. While areas of tissue damage (e.g., demyelination, remyelination, and axonal damage) are all equally hypointense on T2-weighted images, MTR changes are more strictly related to the underlying pathology.\textsuperscript{34,35}

On this basis, we planned to ascertain differences in brain pathologic involvement between 2 demographically matched groups of patients with PPMS and RRMS, assessing the spatial distribution and structural damage of focal demyelinating lesions. To do this, we used fully automated methods to produce LPMs\textsuperscript{32} and to assess MTRs within focal WM lesions\textsuperscript{35} in the 2 patient groups.

**METHODS**

**STUDY POPULATION**

We studied 24 patients with PPMS and 36 with RRMS. All the patients, consecutively selected and referred to MS clinics at the University of Siena and Hospital of Empoli, had clinically definite MS\textsuperscript{37} and also fulfilled established criteria for definite PPMS\textsuperscript{38} and RRMS.\textsuperscript{39} An effort was made to have comparable sex, age, and disease duration in the 2 patient groups (PPMS: 14 women and 10 men; median age, 49 [range, 28-68] years; mean [SD] disease duration, 8.3 [6] years. None of the MS patients had been taking steroids (and RRMS patients were relapse free) for at least 1 month before study entry. Twenty of 36 RRMS patients were being treated with beta interferons or glatiramer acetate and none of the PPMS patients were being treated with disease-modifying therapies at study entry. In each patient, neurologic evaluation, which included disability rating using the Expanded Disability Status Scale,\textsuperscript{40} was performed within 24 hours of the MR examination by an experienced observer blinded to the MRI results. The latter were compared with the MRI results of 23 healthy demographically matched controls (8 men and 15 women; median age, 45 [range, 30-62] years) who were recruited among laboratory and hospital workers and were included if they had normal neurologic examination results and no history of neurologic disorders. The study was approved by the ethics committee of the medical faculty of the University of Siena and informed consent was obtained from all participants.

**MR EXAMINATIONS**

All participants were examined using an identical MR protocol. Acquisitions of brain MRIs were obtained in a single session using a Philips Gyroscan (Philips Medical Systems, Best, the Netherlands) operating at 1.5 T. A sagittal survey image was used to identify the anterior commissure and posterior commissure. A dual-echo, turbo spin-echo sequence (repetition time/echo time 1/echo time 2=2075/30/90 milliseconds, 256×256 matrix, 1 signal average, 250-mm field of view, 50 contiguous 3-mm slices) yielding proton density- and T2-weighted images was acquired in the transverse plane parallel to the line connecting the anterior commissure and posterior commissure. Subsequently, a magnetization transfer sequence was performed acquiring 2 transverse T1-weighted, gradient-echo images, one without and one with magnetization transfer saturation pulses (repetition time/echo time = 35/10 milliseconds, 256×256 matrix, 1 signal average, 230-mm field of view). This sequence yielded image volumes of 50 slices 3-mm thick oriented to exactly match the proton density- and T2-weighted images. The magnetization transfer pulse was a 1.2-millisecond, on-resonance, 121-binomial pulse (radio-frequency field strength, 20 µT) placed just before each slice-selective excitation.\textsuperscript{41} Postgadolinium T1-weighted images were not acquired. Monthly quality assurance sessions and no major hardware upgrades were carried out on the scanner during the time of the study.

**MR DATA ANALYSIS**

**Lesion Volumes**

Classification of T2- and T1-weighted lesion volume was performed in each patient by a single observer, unaware of participants' identities, employing a segmentation technique based on user-supervised local thresholding. For the T2-weighted lesion volume classification, lesion borders were determined primarily on proton density-weighted images, but information from T2- and T1-weighted images were also considered because the software used (Jim 3.0, Xinapse System, Leicester, England) offered the ability to toggle between the proton density– and the T2- and T1-weighted images, providing the operator with convenient access to the information in both data sets while defining lesions. Hypointense WM T1-weighted lesions were defined as lesions with signal intensity between that of grey matter and cerebrospinal fluid on T1-weighted scans.\textsuperscript{42} In both T2- and T1-weighted images, the value of total brain lesion volume was calculated by multiplying lesion area by slice thickness.

**Lesion Probability Maps**

We created an LPM for each patient group as previously described,\textsuperscript{27,32} using imaging analysis tools implemented in the Functional Magnetic Resonance Imaging of the Brain's Software Library (University of Oxford, Oxford, England).\textsuperscript{43} Briefly, the original proton density–weighted images were registered to the Montreal Neurological Institute (MNI152) standard space image, using affine registration with Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool.\textsuperscript{43} The resulting matrices were then applied to the lesion mask.
images to put these into standard space. All registrations were checked visually to exclude alignment failures. Lesion mask images in standard space were binarized (all segmented voxels within the traced regions of interest were considered). Voxel-wise statistics were then calculated to give LPMs at each standard space voxel. Within these maps, the probability of finding a lesion in any given voxel is defined by the relative voxel intensity. For the purpose of this study, we characterized the spatial deployment of T2- and T1-weighted WM lesions in the 2 MS groups at 3 levels. First, we used LPMs by combining the lesion segments across participants. These are reported descriptively to quantify the spatial profile of T2- and T1-weighted lesion occurrence in each patient group. Second, we measured the probability that lesional voxels of a given participant were lesions in the corresponding participant group. This was obtained by performing the equation $LPM_i = \mu \times LPM_{\text{cor}}$, in which $\mu$ is the binarized, segmented lesion mask of the participant, $LPM_{\text{cor}}$ is the LPM of the corresponding patient group, $\mu$ is the resulting mean, and $i$ is the LPM index. Therefore, $LPM_i$ obtained was in 2 steps: (1) by multiplying the binarized lesion (T2- and T1-weighted) masks of each participant with the LPM of his/her patient group and (2) by averaging the value of each voxel different from zero in each participant. As a result of this procedure, $LPM_i$ is higher when the participant’s lesion mask is localized in brain regions with a high probability of being a lesion for that group. Third, we tested for regionally specific differences in the expression of lesions among different groups using a voxel-based morphometry approach.

**CLUSTER ANALYSIS**

To test for differences of both LPM and lesonal MTR measures in the PPMS and RRMS groups (also taking into account differences in Expanded Disability Status Scale score, sex, and treatment), we used the randomize program within the Functional Magnetic Resonance Imaging of the Brain’s Software Library to carry out permutation-based testing.35 Clusters were formed according to a defined threshold and corrected for age and multiple comparisons (across space) within the permutation framework by building up the null distribution of the maximum cluster size (for each permutation). Multi-scale smoothing and appropriate correction for multiple comparisons were applied. From the raw t image, at each spatial smoothing scale, clusters were defined and each cluster size was converted into a P value through the use of permutation testing. The optimal P value over different scales was then kept. $P < .05$ was considered significant.

**GENERAL STATISTICAL ANALYSIS**

The nonparametric Mann-Whitney test was used for comparisons of the 2 groups of MS patients. Values of MR measures for RMSS and PPMS patients were compared with those of a healthy control group. All analyses were age and sex corrected. Differences between the patient and healthy control groups were assessed using analysis of variance followed by pairwise post hoc comparison using the Tukey test to account for multiple comparisons. Clinical and MR metrics of the 2 patient groups were correlated using the Pearson correlation. We assessed significant differences between homologous correlates in the 2 patient groups after a Fisher transformation. Data were considered significant at the 0.05 level. The SYSTAT software, version 9 (SPSS Inc, Chicago, Illinois), was used to perform statistical calculations.

**RESULTS**

**DEMOGRAPHIC AND CLINICAL DATA OF PPMS AND RRMS PATIENTS**

Demographic, clinical, and MR data of the 2 patient groups are summarized in Table 1 and Table 2. The patient groups were similar for age, sex, and disease duration ($P > .5$), but PPMS patients had significantly higher ($P < .001$) Expanded Disability Status Scale scores (median, 4.5 [range, 2.5-8.0]) than RRMS patients (median, 2 [range, 1-5]). In particular, about 70% of PPMS (17 of 24) and 11% of RRMS (4 of 36) patients had relevant motor symptoms. In contrast, none of the PPMS patients and about 66% of RRMS patients (24 of 36) had either no or minimal clinical disability (Expanded Disability Status Scale score ≤ 2).

### Table 1. Demographic, Clinical, and MRI Data of PPMS and RRMS Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PPMS Patients</th>
<th>RRMS Patients</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>46.6 (11)</td>
<td>46.5 (7)</td>
<td>.5</td>
</tr>
<tr>
<td>Sex, M/F, No.</td>
<td>14/10</td>
<td>24/12</td>
<td></td>
</tr>
<tr>
<td>EDSS score</td>
<td>5.0 (1.7)</td>
<td>2.1 (1.1)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>8.1 (6.8)</td>
<td>8.5 (6.2)</td>
<td>.8</td>
</tr>
<tr>
<td>T2-weighted LV, cm$^3$</td>
<td>6.6 (8)</td>
<td>10.6 (13)</td>
<td>.1</td>
</tr>
<tr>
<td>T1-weighted LV, cm$^3$</td>
<td>2.8 (2.6)</td>
<td>2.1 (3.0)</td>
<td>.4</td>
</tr>
<tr>
<td>T1-weighted to T2-weighted LV ratio</td>
<td>0.42 (0.16)</td>
<td>0.20 (0.13)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS, Expanded Disability Status Scale; LV, lesion volume; MRI, magnetic resonance imaging; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis.
LESION LOAD AND DISTRIBUTION

Measures of T2-weighted lesion volume tended to be higher in RRMS patients than in PPMS patients, but this did not reach significance (P = .1) (Table 1). The T1-weighted hypointense lesion volume was similar in the 2 patient groups (P = .4) (Table 1). However, the T1-weighted to T2-weighted lesion volume ratio was significantly higher in PPMS than in RRMS patients (P < .001) (Table 1).

The analysis of the probabilistic distribution of T2- and T1-weighted lesion volume showed that, in both patient groups, the areas with the highest probability of having lesions corresponded anatomically to superior and posterior regions of the corona radiata (Figure 1 and Figure 2). However, the maximum local probability for lesions was higher in PPMS patients (45% peak probability for T2-weighted LPs; 29% peak probability for T1-weighted LPs) (Figures 1A and 2A) than in RRMS patients (33% peak probability for T2-weighted LPs; 19% peak probability for T1-weighted LPs) (Figures 1B and 2B).

The LPM index, which is an index of the probability that lesional voxels of a given participant are lesions in the corresponding patient group, was significantly higher (P < .001) in PPMS (mean [SD], 16% [4%] for T2-weighted lesions and 9% [2%] for T1-weighted lesions) than in RRMS (8% [2%] for T2-weighted lesions and 4.5% [1%] for T1-weighted lesions) patients.

Finally, we performed a 2-sample t test comparing the proportion of WM lesions at each voxel between the 2 patient groups. In the T2-weighted lesion masks, we identified specific WM areas to be more frequently involved in PPMS than RRMS (significant clusters, P < .01 [corrected]) (Figure 3). These areas corresponded anatomically to right posterior regions of the corona radiata and tapetum (Figure 3A).

LESIONAL MTR, NAWM MTR, AND CORTICAL MAGNETIZATION TRANSFER

As expected, the MTR values of T2- and T1-weighted lesional regions were significantly lower (P < .001) in both PPMS and RRMS patients than in the WM of healthy controls (Table 2). In both lesional MTR measures, there were no differences between the 2 patient groups (P > .5). Compared with those in controls, NAWM MTR values were significantly lower in RRMS patients (P = .04) and showed a trend toward significant decrease in the PPMS group (P = 1), whereas there was no difference between the 2 patient groups (P = .8). Similarly, cortical MTR values were significantly lower in both patients with RRMS and PPMS than in controls (P < .05), with no differences between the 2 patient groups (P = .9).

We also performed, in lesional MTR data, a 2-sample t test comparing the lesional MTR values at each voxel between the 2 patient groups. In the T2-weighted lesional MTR masks, areas that were lower in PPMS than RRMS (significant clusters, P < .01 [corrected]) (Figure 3) were identical to those that were found to be more frequently involved in the T2-weighted LPM (ie, right posterior regions of the corona radiata and tapetum) (Figure 3B).

CORRELATIONS BETWEEN CLINICAL AND MR MEASURES

In general, there was no close correlation among MTR values, T2- and T2-weighted lesion volume, and clinical measures in either patient group. However, a close correlation was found in RRMS between T2-weighted lesion volume and both NAWM MTR (r = −0.56, P < .001) and cortical MTR (r = −0.69, P < .001). Both correlations differed significantly (P = .05 and 0.01, respectively) from those observed in the PPMS group (NAWM MTR, r = −0.20; cortical MTR, r = −0.21; P > .2). Similarly, significantly close correlation was found in RRMS patients between T1-weighted lesion volume and both NAWM MTR (r = −0.50, P < .005) and cortical MTR (r = −0.63, P < .001). Also, in this case, both correlations were different (P = .06 and 0.01, respectively) from those observed in the PPMS group (NAWM MTR, r = −0.12; cortical MTR, r = −0.13; P > .2).

COMMENT

Owing to the impact of disease-modifying treatments on disease progression, it has become very important to understand whether and to what extent PPMS is part of the disease spectrum. Presently, patients with PPMS remain orphans with regards to disease-modifying treatments and any clue that could help to clarify issues related to this disease form can therefore be very important.

In our study, with the aim to stress potential differences in the brains of PPMS and RRMS patients, we assessed differences in spatial distribution and structural damage in the focal demyelinating lesions of 2 demographically matched groups of patients with these clinically defined forms of the disease. To do this, we estimated, at voxel level, the differences in LPMs and lesional MTRs in these 2 groups of patients with definite PPMS and RRMS. We found that (1) though there were no clear differences in the burden of hyperintense T2-weighted lesions and hypointense T1-weighted lesions, the probabilistic distribution of these lesions was different in the brains of the 2 patient groups, being more related to anatomical areas of motor involvement in PPMS; and (2) both PPMS and RRMS patients had lower lesional (and non-
lesional) MTR values than controls, but these values were not different between the 2 demographically matched patient groups.

Transformation of images into standard anatomical coordinate space has allowed us to generate, for each patient group, LPMs based on T2- and T1-weighted lesion maps of individual patients. These standardized images showed that, in T2- and T1-weighted LPMs, specific brain regions (ie, superior and posterior regions of the corona radiata) were more frequently classified as lesions in PPMS than in RRMS, a finding that would not have been noted in images from individual patients. This was substantially confirmed on cluster analysis of T2-weighted lesion masks (Figure 3A). Given the anatomical characteristics of the corona radiata, a region where axonal projections converge, making significant contributions to the corticospinal tract, this result is particularly interesting. In effect, the greater damage in PPMS patients in eloquent areas of motor functions could explain the large differences in Expanded Disability Status Scale scores of our demographically matched patient groups, with up to 70% of PPMS patients and only 11% of RRMS patients presenting signs or symptoms of motor involvement. Previously reported findings of prominent damage of long-tract axons and ongoing axonal reduction in PPMS give additional support to this interpretation.

We also performed a voxelwise analysis of MTR data by using a previously described method. In both PPMS and RRMS patients, lesional and nonlesional MTR measures were lower than those of controls, confirming that structural damage occurs in lesions and normal-appearing brains in the 2 different forms. However, NAWM MTR and cortical MTR values showed, in general, only small decreases, and this did not reach significance for NAWM MTRs of PPMS patients. This could be because of the specific characteristics of our patient groups or, because MTR data of normal-appearing tissue on MRI are heavily dependent on partial volume and distance from lesions, it might be related to the very conservative quantification analysis performed here (the method uses high thresholds to avoid, at best, partial volume estimation at cerebrospinal fluid/gray matter and gray matter/WM interfaces and includes only voxels not adjacent to lesions). In addition, we did not find clear differences in lesional and nonlesional MTR measures between the 2 patient groups in our study, with exception of a small cluster of lesional MTR voxels that was significantly lower in PPMS than in RRMS patients (Figure 3). This is in substantial agreement with previous MTR studies comparing RRMS with PPMS, suggesting that similar structural damage occurs in lesions and normal-appearing brains in the 2 different forms of the disease. Thus, although differences in lesion characteristics (as shown by the significantly higher T1-weighted to T2-weighted lesion volume ratio of PPMS) and distribution (as shown by the more frequent involve-
ment of areas related to motor functions in PPMS) suggest that variations in cerebral pathology do exist between the 2 patient groups, the pathologic damage underlying the WM lesions does not seem different.

Interestingly, in this study, values of both T2- and T1-weighted lesion volume showed a moderately close correlation with NAWM MTRs and cortical MTRs in RRMS patients, whereas this correlation was not found in the PPMS group. This adds to findings of our previous study that reported a general decrease in the cortical volume that was occurring regardless of the WM lesion load in PPMS and, in contrast, was related to that in RRMS. Taken together, these results suggest that WM lesions are much more relevant to the damage occurring in normal-appearing brains in RRMS than in PPMS patients. The more widespread distribution of WM lesions in RRMS patients (Figure 1) and its relative influence on MTR values in normal-appearing tissue may provide a good explanation for this.

A potential limitation of the study lies in the voxel-based analysis of both MTR data and LPMs. Several studies have explored limitations and strengths of voxel-based analysis to quantitative data. In our study, the potential bias coming from errors in registration has been minimized by the use of an optimized technique and by visually checking all registrations to ensure that there were no failures of alignment and consequent misclassification of tissues. This is particularly easy for WM lesions. For the normal-appearing brain, the use of very conservative thresholds during tissue segmentation has strongly minimized the possibility of tissue misclassification. Another caveat that needs to be considered in the interpretation of our results is that the contrast created between the 2 groups was based on an unequal and relatively low numbers of patients. However, the presence of similar brain lesion load in the 2 patient groups makes it unlikely that the limited sample of participants could have significantly influenced our results. In any case, one of the advantages of permutation methods (used here for the cluster analysis) lies in their applicability even when the assumptions of a parametric approach are weak.

In summary, we found similar lesional and nonlesional MTR decreases in patients with RRMS and PPMS, suggesting that similar structural damage occurs in lesions and normal-appearing brains in the 2 different forms of the disease. In contrast, there were differences in the probabilistic distribution of WM lesions between RRMS and PPMS patients, with a prevalent involvement of anatomical areas of motor function in PPMS. This matched with the more pronounced motor deficits and higher Expanded Disability Status Scale score of this patient group. The finding of differences in lesion distribution, associated with that of differences in lesion characteristic (given
by the significantly higher T1-weighted to T2-weighted lesion volume ratio in PPMS), leads to the conclusion that differences between RRMS and PPMS do not rely exclusively on spinal cord pathology. Differences in cerebral pathology between the 2 patient groups exist and contribute, at least in part, to differences in clinical disability.

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