Lack of Correlation Between Cortical Demyelination and White Matter Pathologic Changes in Multiple Sclerosis

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Background: Histopathologic studies have shown that subpial cortical demyelination is extensive in chronic multiple sclerosis (MS).

Objective: To study whether subpial cortical demyelination in MS is associated with focal or diffuse white matter (WM) pathologic features on magnetic resonance imaging (MR imaging).

Design: Comparison of postmortem MR imaging findings with histopathologic findings.

Setting: Brain donations from a general community.

Patients: Three patients with MS with extensive cortical demyelination and 3 patients with minor cortical demyelination were selected from an MS autopsy data set. The postmortem MR imaging and histopathologic data of the patients were compared.

Main Outcome Measures: Two observers blinded to the results of each other assessed the presence, extent, and distribution of focal and diffuse pathologic changes in WM by MR imaging and by histopathology.

Results: Extensive subpial demyelination was not associated with a significant increase in the area of focal and diffuse WM pathologic changes as assessed by Luxol fast blue histochemistry or by MR imaging or with the presence or extent of juxtacortical abnormalities on MR imaging.

Conclusions: The lack of association of MS gray matter demyelination with diffuse or focal WM changes indicates that gray matter demyelination in MS occurs largely independent of WM pathologic changes. The extent or distribution of WM abnormalities cannot be used to identify extensive cortical demyelination in the clinical setting.

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Multiple sclerosis (MS) is a disease of the central nervous system histopathologically characterized by multifocal areas of myelin, oligodendrocyte, and axonal loss. Although MS is regarded as a white matter (WM) disease, gray matter (GM) demyelination has also been described. Immunohistochemical studies identified widespread subpial cortical demyelination in MS. Subpial lesions were shown to be the most common cortical lesion type; these are grossly underestimated by standard histochemical techniques. Autopsy studies indicate that a subgroup of patients with chronic MS has a pattern of general cortical subpial demyelination, with subpial demyelination in all neocortical areas. Other patients with MS have almost as extensive but not generalized subpial changes; we use the term extensive subpial demyelination (ESD) herein to refer to these patterns of MS pathology. Subpial cortical lesions are largely undetectable by standard magnetic resonance imaging (MRI) techniques. Therefore, they are thought to contribute to so-called clinicoradiological dissociation, the poor correlation between pathologic changes observed on MR imaging and clinical deficits. The clinical significance of ESD in MS may not be known until MR imaging methods more sensitive to GM demyelination are developed. A clinical correlate may be cognitive dysfunction, which affects approximately 50% of patients with MS.

Little is known about whether a possible association exists between MR imaging–visible WM abnormalities and ESD. The objective of this study was to investigate whether GM demyelination is related to the extent and distribution of focal and diffuse WM pathologic features as observed on MR imaging and histopathologic examination.
PATIENTS AND AUTOPSY PROCEDURE

Following the autopsy procedure at the VU University Medical Center, Amsterdam, the Netherlands, 10-mm-thick coronal sections were removed from each brain, immersion fixed for 6 to 12 weeks, and subjected to MR imaging. From 19 patients with MS, 5 to 12 widely scattered brain specimens from these sections, plus 1 full-coronal brain section or 2 half-coronal brain sections, were processed for paraffin embedding. The small specimens were selected on the basis of signal intensity changes in WM at postmortem T2-weighted MR imaging, while the full-coronal and half-coronal sections were selected independent of visible pathologic features. This procedure was performed in cooperation with the Netherlands Brain Bank. In total, 178 tissue sections were selected for analysis. On the basis of the results of immunohistochemical myelin staining, the 3 patients with the highest extent and the 3 patients with the lowest extent of cortical demyelination were selected for further study (Table 1).

MR IMAGING

Standard dual-echo T2-weighted images (repetition time, 2755 milliseconds; echo times, 90 milliseconds [first echo] and 45 milliseconds [second echo]; number of signals acquired, 2; in-plane resolution, 0.5×0.5 mm²; and 3-mm section thickness) of the 10-mm brain sections were acquired, using a 1.5-T scanner (Siemens Vision, Erlangen, Germany). Myelin basic protein–stained tissue sections were matched to the postmortem T2-weighted spin-echo images, using a procedure described in detail previously.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Immunolabeling with monoclonal antomyelin antibodies (antimyelin basic protein [Boehringer-Mannheim Biochemica, Mannheim, Germany] and antiproteolipid protein [Se-rotec, Oxford, England]) was performed using a standard immunohistochemical ABC procedure, as described previously. Luxol fast blue histochemistry was performed using standard procedures.

DATA ANALYSIS

Numbers of juxtacortical, periventricular, and deep WM lesions were scored independently on T2-weighted images and in histopathologic sections. In addition, the percentage areas of WM and GM with high-intensity signal changes were noted. The pathology reader (L.B.) scored the percentage area with complete demyelination using the antimyelin basic protein–stained or antiproteolipid protein–stained tissue sections, as well as the percentage area with diffusely lighter staining in WM using the Luxol fast blue–stained sections. Only the half-hemispheric or full-hemispheric sections were analyzed for the extent of WM and GM demyelination, as these were obtained independent of macroscopically or MR imaging–visible lesions. The GM lesion distribution was classified as mixed WM and GM (type 1), intracortical (type 2), or subpial (type 3). The GM and WM areas and the areas of GM and WM demyelination were measured using morphometry software (Scion Image; Scion Corporation, Frederick, Md) on digital images of the tissue sections.

Lesion numbers and percentage areas with pathologic signal scored on the T2-weighted spin-echo images were compared with each other and with the lesion numbers and the percentage of diffuse or focal pathologic areas obtained from the matched histopathologic tissue sections. Clinical data were evaluated in all available medical records by one of us (C.P.); age at onset, MS subtype, and time to Expanded Disability Status Scale score of 6 were assessed. It was noted whether cognitive deficit had been described in the medical records; however, cognitive function had not been systematically assessed in any of the patients studied.

STATISTICAL ANALYSIS

The means of the focal and diffuse WM demyelinated areas were compared between cases with low and high cortical demyelination using nonparametric statistics (Mann-Whitney test [SPSS version 9.0; SPSS Inc, Chicago, Ill]). Statistical analyses were performed, with 2-tailed P <.05 considered statistically significant.

HISTOPATHOLOGY

The pattern of demyelination was established by myelin immunohistochemistry on 9 to 11 widely scattered small (approximately 1–2 mm²) brain tissue blocks, plus 1 full-coronal or 2 half-coronal tissue sections, from each patient. In total, 11 large coronal and 60 small tissue blocks were studied. All 3 patients with extensive cortical demyelination had a similar pattern of large subpial MS lesions; in 1 of these patients, subpial demyelination was

Table 1. Autopsy and Clinical Data From Patients With Multiple Sclerosis (MS)

<table>
<thead>
<tr>
<th>Patient No./Sex/Age at Death, y</th>
<th>Clinical Diagnosis</th>
<th>Disease Duration From First Symptom to Death, y</th>
<th>Time From First Symptom to EDSS Score of 6, y</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/69</td>
<td>SPMS</td>
<td>27</td>
<td>10</td>
<td>Viral infection</td>
</tr>
<tr>
<td>2/F/84</td>
<td>SPMS</td>
<td>49</td>
<td>42</td>
<td>Euthanasia</td>
</tr>
<tr>
<td>3/F/72</td>
<td>SPMS</td>
<td>14</td>
<td>13</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>4/F/74</td>
<td>PPMS?</td>
<td>18</td>
<td>15</td>
<td>Cardiac arrest</td>
</tr>
<tr>
<td>5/M/59</td>
<td>SPMS</td>
<td>15</td>
<td>4</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>6/M/73</td>
<td>SPMS</td>
<td>22</td>
<td>17</td>
<td>Ileus</td>
</tr>
<tr>
<td>7/F/80</td>
<td>SPMS</td>
<td>28</td>
<td>1</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>8/M/66</td>
<td>SPMS</td>
<td>36</td>
<td>9</td>
<td>Euthanasia</td>
</tr>
<tr>
<td>9/F/65</td>
<td>SPMS</td>
<td>30</td>
<td>5</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>10/F/71</td>
<td>SPMS</td>
<td>18</td>
<td>12</td>
<td>Ileus</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS, Expanded Disability Status Scale; ESD, extensive subpial demyelination; PPMS, primary progressive MS; SPMS, secondary progressive MS.
ubiquitous (Figure 1). Subpial cortical demyelination could only be detected by myelin immunohistochemistry, not by Luxol fast blue histochemistry. The numbers of GM and WM lesions were scored in the full-coronal and half-coronal sections. These tissue sections were from the frontal region (3 specimens from the ESD group and 4 specimens from the non-ESD group) and the parieto-occipital regions (2 regions for both groups). The total number of GM lesions was 116 among the 3 patients in the ESD group and 10 among the 3 patients in the non-ESD group. The most frequent cortical lesion type was subpial lesions for both groups, with 96 lesions in the ESD group and 8 lesions in the non-ESD group. The total number of WM lesions was 23 in the ESD group and 8 in the non-ESD group. There was 1 subcortical WM lesion in the ESD group; there were 2 in the non-ESD group.

CLINICAL DATA

Key clinical data are summarized in Table 1. One patient with extensive cortical demyelination (patient 2) had a particularly long disease duration (49 years) and a long time to an Expanded Disability Status Scale score of 6 (42 years); the 2 other patients in the ESD group had disease durations and times to an Expanded Disability Status Scale score of 6 comparable to those of the patients in the non-ESD group. Cognitive problems during at least 1 time point were noted in the medical records for all the patients in the ESD group and for 1 of the patients in the non-ESD group.

MR IMAGING

The immersion fixed-tissue sections had a low variability of blood and cerebrospinal fluid content in cerebral sulci, giving consistent results on MR imaging. Examination of postmortem MR images revealed no specific pattern of WM abnormalities associated with general or extensive cortical demyelination (Figure 2). The extensive subpial lesions were not visible on MR imaging. The patients with extensive cortical lesions had no increased juxta-cortical demyelination on MR imaging (0.1% of the total WM area in both groups [Table 2]). There was no difference in the extent of diffuse WM changes (7.1% in
the ESD group vs 7.7% in the non-ESD group). The extent of focal WM lesions was higher in the ESD group than in the non-ESD group (5.3% vs 2.7%); this difference was not statistically significant (P = .28).

In this study, the extreme ends of the spectrum of cortical demyelination in chronic MS were selected to identify a possible association with the extent of WM demyelination. The results showed that the presence, distribution, and extent of WM changes were independent of the extent of GM pathologic features. This indicates that there are no major differences in the extent of WM pathologic features on MR imaging that may be used to discriminate patients with ESD in clinical practice.

Other histopathologic studies6,8,10 found no correlation between the extent of GM demyelination and focal WM demyelination in MS. A recent study6,8 found a correlation between the extent of cortical demyelination and WM inflammation and microglial activation in MS; however, the correlation coefficient was low. The results of our study support the hypothesis that GM demyelination in MS is a process occurring largely independent of WM demyelination. It was reported that a subgroup of patients with MS may have such extensive subpial cortical demyelination that it constitutes a pattern of general cortical subpial demyelination.6,8 This is confirmed in the present study, as subpial demyelination was detected in patient 1 (Table 1 and Figure 1) in all neocortical areas by extensive histopathologic sampling at autopsy. Our data indicate that this may represent one end of a spectrum rather than a distinct subgroup, as other patients having MS with almost as extensive but not general subpial cortical demyelination were identified. Patients with extensive or general cortical demyelination have typical MS WM lesions, indicating that this pathologic pattern represents MS and not a separate disease entity.6

The sensitivity of standard MR imaging methods for subpial lesions is low, approximately 5%.9,11 The present data do not allow any firm conclusions regarding the clinical effects of extensive cortical demyelination, as the group examined is small and the postmortem information collected from nursing homes or primary care physicians was insufficiently detailed. However, in all patients with ESD, cognitive dysfunction had been noted. The question of a possible association between MS cortical demyelination and cognitive dysfunction could be further resolved by prospectively and systematically recording cognitive function in a larger group of brain bank donors or by developing more sensitive MR imaging methods for cortical lesion detection in vivo. The presence and extent of so-called juxtacortical lesions on MR imaging may not be helpful, because it was found in this study that this does not reflect the presence or the extent of superficial cortical demyelination. In previous studies,16,18 the main MR imaging correlate of cognitive deficit was global atrophy measures, with only a moderate contribution of WM lesion load. Moreover, MR imaging studies19,20 indicate that cortical atrophy has an effect on cognitive deficit independent of the extent of WM pathologic features. It is unknown whether this effect is due to focal GM lesions or to the extent of diffuse pathologic changes of GM. We are investigating the postmortem application of potentially more sensitive MR imaging methods for the detection of GM lesions, such as high-field MR imaging and the double inversion recovery technique. The absence of a spatial association between intracortical lesions and subcortical WM lesions or an association between the extent of WM demyelination and the extent of GM demyelination indicates that GM lesions are not likely to be caused by neuronal pathologic features secondary to axonal loss within WM lesions.

### CONCLUSIONS

We show that extensive cortical demyelination in MS is not associated with an increased area of WM focal or diffuse signal abnormalities on MR imaging, nor is it associated with a specific pattern of signal abnormalities on MR imaging. This indicates (1) that the extent of cortical demyelination is largely independent of WM myelin loss and (2) that the extent or pattern of WM changes as assessed by MR imaging cannot aid in the identification of patients with extensive cortical myelin loss in the clinical setting.

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Author Contributions: Study concept and design: Bo and Geurts. Acquisition of data: Bo, Geurts, van der Valk, Polman, and Barkhof. Analysis and interpretation of data: Bo and Geurts. Drafting of the manuscript: Bo. Critical revision of the manuscript for important intellectual content: Bo, Geurts, van der Valk, Polman, and Barkhof. Administrative, technical, and material support: Bo and Geurts. Study supervision: Bo, van der Valk, Polman, and Barkhof.

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Table 2. Histopathologic and Magnetic Resonance (MR) Imaging Data From Patients With (ESD Group) and Without (Non-ESD Group) Extensive Subpial Demyelination*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Focal Lesions, %</th>
<th>Diffuse WM Lesions, %</th>
<th>Lesions, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR imaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESD group</td>
<td>5.3</td>
<td>7.1</td>
<td>0.1†</td>
</tr>
<tr>
<td>Non-ESD group</td>
<td>2.7</td>
<td>7.7</td>
<td>0.1†</td>
</tr>
<tr>
<td>Histopathologic examination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESD group</td>
<td>3.5</td>
<td>10.1</td>
<td>32.6‡</td>
</tr>
<tr>
<td>Non-ESD group</td>
<td>1.5</td>
<td>11.0</td>
<td>1.1‡</td>
</tr>
</tbody>
</table>

Abbreviation: WM, white matter.

*No differences between the ESD and non-ESD groups were statistically significant (P > .05 for all).
†Juxtacortical lesions as percentage of total WM area.
‡Gray matter lesions as percentage of total gray matter area.
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REFERENCES