Detection of Cortical Inflammatory Lesions by Double Inversion Recovery Magnetic Resonance Imaging in Patients With Multiple Sclerosis

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Background: A significant inflammatory pathologic disorder in the cortex of patients with multiple sclerosis (MS) has been demonstrated by ex vivo studies.

Objective: To determine the frequency, time of appearance, and clinical relevance of intracortical lesions (ICLs) in MS in vivo.

Design: Double inversion recovery sequence study.

Setting: Multiple Sclerosis Centre of the Veneto Region.

Patients: We enrolled 380 patients (116 with clinically isolated syndrome [CIS], 163 with relapsing-remitting MS [RRMS], and 101 with secondary progressive MS [SPMS]) and 40 age- and sex-matched healthy volunteers between May 1, 2005, and December 31, 2006.

Main Outcome Measures: We assessed the frequency and number of ICLs and brain parenchyma fraction, white matter T2 lesion volume, and clinical disability.

Results: Although never observed in healthy volunteers, ICLs were detected in 58% of patients (36% of patients with CIS, 64% of patients with RRMS, and 73% of patients with SPMS). The number of ICLs was higher in patients with SPMS than in those with CIS or RRMS (P < .001), and patients with ICLs had a higher Expanded Disability Status Scale score (P = .004), a higher white matter T2 lesion volume (P = .008), a lower brain parenchyma fraction (P = .009), and a higher frequency of IgG oligoclonal bands (IgGOBs) (P < .001) than patients without ICLs. Patients positive for IgGOBs had more ICLs than patients negative for IgGOBs (P = .02). The number of ICLs correlated with the Expanded Disability Status Scale score (r = 0.48, P < .001), white matter T2 lesion volume (r = 0.38, P = .001), and brain parenchyma fraction (r = −0.47, P = .001). A significant association between ICLs and male sex was observed.

Conclusions: Although more frequent in patients with SPMS, ICLs were observed from the early disease stages. The ICLs were more frequently detected in patients with IgGOBs and were associated with a higher clinical disability score and male sex. The ICLs may help to define MS clinical heterogeneity and prognosis in clinical settings.

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the disease they occur, (3) whether they are related to WM pathologic conditions, and (4) whether they are relevant to clinical disability.

### METHODS

#### PATIENT POPULATION

We included in the study 380 patients (284 women and 96 men; female to male ratio, 2.9; age range, 18-60 years) who consecutively presented to the Multiple Sclerosis Centre of the Veneto Region in Padova from May 1, 2005, through December 31, 2006, and 40 age- and sex-matched healthy volunteers (Table 1). On the basis of established diagnostic and clinical criteria, patients were stratified into the following disease groups: clinically isolated syndrome (CIS; n = 116), relapsing-remitting MS (RRMS; n = 163), and secondary progressive MS (SPMS; n = 101). Among patients with CIS, 71 (61.5%) had evidence of dissemination in the space of the lesions (ie, MRI according to the criteria of Barkhof et al and Tintore et al or 2 or more MRI lesions plus demonstration of intrathecal IgG synthesis) and were considered to possibly have MS. Each patient was clinically assessed at the time of the MRI examination with the Kurtzke Expanded Disability Status Scale (EDSS).

All patients with MS underwent lumbar puncture for CSF examination at the time of the diagnosis. This examination included the determination of intrathecal synthesized IgG by the demonstration of IgG oligoclonal bands (IgGOBs) by means of isoelectric focusing and specific immunofixation. In patients with CIS, MRI was obtained within 2 months of symptom onset. Steroid therapy in the month before MRI acquisition was an exclusion criterion. Informed consent was obtained from all patients and healthy volunteers. The study was approved by the local ethics committee.

#### IMAGE ACQUISITION

All MRIs were acquired with a 1.5-T Philips Achieva scanner (Philips Medical Systems, Best, the Netherlands). No major hardware upgrades were performed on the scanner during the study, and weekly quality assurance sessions took place to guarantee measurement stability. The following sets of images were obtained: DIR: 2-dimensional multisection sequences with 50 contiguous axial sections, without any interpolation techniques (repetition time, 15 631 milliseconds; inversion time, 3400 milliseconds; delay, 325 milliseconds; section thickness, 3 mm; matrix, 256 × 256; gap, 0; acquisition time, 5.43 minutes); 3-dimensional fast field echo, 3-dimensional sequence with 120 contiguous axial sections with the off-center positioned on zero (repetition time, 25 milliseconds; echo time, 4.6 milliseconds; flip angle, 30°; section thickness, 1.2 mm; matrix, 256 × 256); fluid-attenuated inversion recovery, 2-dimensional sequence with 50 contiguous axial sections (repetition time, 10 000 milliseconds; echo time, 120 milliseconds; inversion time, 2500 milliseconds; section thickness, 3.0 mm; matrix, 256 × 256; gap, 0), and finally conventional turbo-spin echo proton density/T2 sequences.

#### IDENTIFICATION OF ICLs

An experienced neuroradiologist (A.M.) and a neurologist (M.C.) interpreted the images by consensus, with a count and classification interobserver variability of 0.1%. The neuroradiologist and the neurologist were blinded to the clinical features and the results of the paraclinical tests. On DIR images, the lesions were recognized for their high-signal intensity. Lesions had different locations: infratentorial, periventricular WM, deep WM, juxtacortical WM (not entering the cortical GM), mixed WM-GM, and intracortical. The ICLs were distinguishable as lesions confined to the cortical ribbon without involving the underlying subcortical WM (Figure 1B-F). Of course, considering the actual resolution of our images, the possibility that some ICLs extend into the juxtacortical WM cannot be excluded. Possible artifacts were evaluated on DIR images of healthy control subjects.

#### T2 LESION VOLUME AND BRAIN PARENCHYMA FRACTION MEASUREMENTS

Using a semiautomated thresholding technique included in a software program developed at the National Institutes of Health called Medical Images Processing, Analysis and Visualization (http://mipav.cit.nih.gov), lesions were segmented on the fluid-attenuated inversion recovery images, providing a hyperintense WM T2 lesion volume (T2LV). The presence of possible fluid-attenuated inversion recovery–related artifacts was controlled for on DP and T2 images. Brain parenchyma fraction (BPF) was computed as previously described on the 3-dimensional fast field echo image with the Medical Images Processing, Analysis and Visualization program.

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**Table 1. Demographic Characteristics of Study Population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy Volunteers (n = 40)</th>
<th>Patients With CIS (n = 116)</th>
<th>Patients With RRMS (n = 163)</th>
<th>Patients With SPMS (n = 101)</th>
<th>All Patients (n = 380)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female to male ratio</td>
<td>30:10 (3.0)</td>
<td>85:31 (2.7)</td>
<td>125:38 (3.2)</td>
<td>73:27 (2.7)</td>
<td>284:96 (2.9)</td>
</tr>
<tr>
<td>Age, mean ± SD (range), y</td>
<td>36.2 ± 7.2 (18-55)</td>
<td>31.7 ± 6.9 (18-51)</td>
<td>37.4 ± 7.3 (18-55)</td>
<td>44.1 ± 8.8 (28-60)</td>
<td>37.4 ± 7.9 (18-60)</td>
</tr>
<tr>
<td>IgGOBs in CSF, No. (%)</td>
<td>ND</td>
<td>73 (62.9)</td>
<td>127 (77.9)</td>
<td>82 (81.1)</td>
<td>277 (72.6)</td>
</tr>
<tr>
<td>Disease duration, mean ± SD (range), y</td>
<td>ND</td>
<td>0.8 ± 0.7 (0.1-1)</td>
<td>5.1 ± 5.0 (1-13)</td>
<td>12.1 ± 6.1 (5-25)</td>
<td>5.6 ± 5.1 (0.1-25)</td>
</tr>
<tr>
<td>No. receiving therapy</td>
<td>None</td>
<td>0</td>
<td>111</td>
<td>20</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Immunoamodulatory</td>
<td>0</td>
<td>117</td>
<td>20</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>Immunosuppressive</td>
<td>0</td>
<td>13</td>
<td>70</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>EDSS score, mean ± SD (range)</td>
<td>ND</td>
<td>1.2 ± 0.9 (0-3.5)</td>
<td>2.0 ± 1.2 (1.0-5.5)</td>
<td>5.5 ± 1.0 (4.0-7.0)</td>
</tr>
<tr>
<td></td>
<td>T2 lesion volume, mean ± SD, cm³</td>
<td>ND</td>
<td>2.1 ± 3.2 (0.2-8.8)</td>
<td>6.6 ± 7.8 (0.4-40.2)</td>
<td>12.2 ± 15.0 (4.0-52.7)</td>
</tr>
<tr>
<td></td>
<td>No. of ICLs, mean ± SD (range)</td>
<td>0</td>
<td>1.4 ± 3.3 (0-13)</td>
<td>2.2 ± 3.5 (0-20)</td>
<td>4.7 ± 4.5 (0-16)</td>
</tr>
<tr>
<td></td>
<td>Patients with ICLs, No. (%)</td>
<td>0</td>
<td>43 (36.8)</td>
<td>105 (64.6)</td>
<td>71 (70.1)</td>
</tr>
</tbody>
</table>

Abbreviations: CIS, clinically isolated syndrome; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; ICLs, intracortical lesions; IgGOBs, IgG oligoclonal bands; ND, not determined; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
STATISTICAL ANALYSIS

Differences among patients with CIS, RRMS, and SPMS were assessed with analysis of variance followed by post hoc pairwise comparison using the Tukey honestly significant difference procedure to account for multiple tests. Since the comparison between patients with and without ICLs could be affected by the different numbers of patients with CIS, RRMS, and SPMS who compose the 2 groups, when patients with and without ICLs were compared, we performed a Monte Carlo simulation for balancing the sample sizes of the patient groups. Thus, we randomly eliminated patients from both groups (with and without ICLs) to obtain 2 groups of 127 patients, with each group composed of 42 patients with CIS, 58 with RRMS, and 27 with SPMS. Then the comparison of the clinical variable means between these 2 groups was performed with a nonparametric Mann-Whitney test. We repeated this procedure 10 times, and the average \(P\) values across the entire set of Monte Carlo-simulated groups were reported (Table 2).

Association between the presence of ICLs and IgGOBs and sex has been tested using a table of \(\chi^2\) test for contingency and through a determination of the odds ratio. To analyze the correlation among discrete and continuous variables, the Pearson and Spearman indices were used, respectively. \(P < .05\) was considered statistically significant. All statistical analyses were performed using SPSS statistical software version 12 (SAS Institute Inc, Cary, North Carolina) and R, an open source statistical package available at http://www.r-project.org. Data are presented as mean±SD.

RESULTS

LACK OF ICLs IN HEALTHY VOLUNTEERS

Intracortical lesions were never observed in healthy volunteers. However, some artifacts that resembled ICLs were found on DIR sequences. These artifacts were ribbon-like hyperintense images, were often located in extracortical regions, were sometimes bilateral or symmetric, and had shapes that changed in contiguous sections but maintained the orientation of their major axis along with the MRI phase direction (Figure 1A). Other hyperintense signals on DIR come from sinuses and bigger vessels, but these are easily identified.
Intracortical lesions could be identified in 58.1% of all patients included in the study. Although they were more commonly detected in patients with RRMS or SPMS (64.4% and 73.2% of the cases, respectively), they were not infrequently observed at clinical onset (36.2% of patients with CIS). In the whole MS group, the mean number of ICLs was 2.6±3.4 and was significantly higher in patients with SPMS (4.7±4.5) than in those with CIS and RRMS (1.4±3.1 and 2.2±3.2, respectively; P<.001 for both comparisons) (Figure 2). When only patients with ICLs were taken into account, ICLs (mean±SD number, 5.1±3.2) were again more frequent in patients with SPMS than in the other groups (mean±SD number, 3.9±2.9 in patients with CIS, 4.2±3.5 in patients with RRMS, and 7.2±3.1 in patients with SPMS; P<.001 for all comparisons).

**Patients with ICLs**

When compared with patients without ICLs (after balancing the sample sizes of the patient groups), patients with ICLs were characterized by higher EDSS scores (P=.004), higher WM T2LVs (P=.008), and lower BPFs (P=.009) (Table 2). The intrathecal synthesis of IgG0Bs (Table 2) resulted significantly more often in patients with ICLs (88.2%, 195/221) than in patients without ICLs (51.6%, 82/159), and patients with IgG0Bs showed a higher frequency of ICLs (70.4%, 195/277) than patients without IgG0Bs (29.2%, 26/103). Thus, the risk of having ICLs was significantly higher in the presence of IgG0Bs (χ²=62.909; P<.001; odds ratio, 6.7; 95% confidence interval, 4.2-11.7). This finding was confirmed in each patient subgroup (Table 2). Moreover, IgG0B-positive patients had a significantly higher number of ICLs than did IgG0B-negative patients (mean±SD, 2.5±3.6 vs 1.0±2.7; P=.02).

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**FREQUENCY AND NUMBERS OF ICLs**

Notably, 79.2% of men had ICLs (men with ICLs/men without ICLs, 76/20), whereas only 51.0% of women had ICLs (with/without, 145/139). In the group of patients without ICLs, the female to male ratio was 5:1 (ie, men accounted for only 13% of these patients), whereas in the group of patients with ICLs the female to male ratio was 2:1. Thus, the risk of having ICLs was significantly higher in men than in women (χ²=23.298; P<.001; odds ratio, 3.6; 95% confidence interval, 2.1-6.2). Moreover, the number of ICLs was statistically significantly (P=.001) higher in men (mean±SD, 3.4±3.2) than in women (mean±SD, 2.2±3.5).
A moderate positive correlation was found between the number of ICLs and the WM T2LV in the whole MS group ($r=0.38$, $P=.001$) and in each patient subgroup (patients with CIS: $r=0.37$, $P=.001$; patients with RRMS: $r=0.41$, $P<.001$; and patients with SPMS: $r=0.37$, $P=.005$). Similar findings were also observed between the number of ICLs and BPF (whole patient group: $r=−0.47$, $P=.001$; patients with CIS: $r=−0.40$, $P=.005$; patients with RRMS: $r=−0.52$, $P=.001$; and patients with SPMS: $r=−0.49$, $P=.002$).

A significant correlation between the number of ICLs and the EDSS score was also observed in the whole MS group ($r=0.48$, $P<.001$), patients with RRMS ($r=0.49$, $P=.001$), patients with SPMS ($r=0.51$, $P=.001$), and more weakly in patients with CIS ($r=0.28$, $P=.008$). Finally, in the whole MS group, the number of ICLs weakly correlated with disease duration ($r=0.28$, $P=.053$), whereas no correlation was observed with patient age ($r=0.12$, $P=.15$).

MORPHOLOGIC CHARACTERISTICS OF ICLs

We attempted a differentiation of ICLs on the basis of their morphologic features. Four different types of ICLs could be recognized: (1) round or ovoid (Figure 1B) with clear-cut margins, similar to WM lesions, sometimes extending across the full width of the cortex (accounting for approximately half of all detectable ICLs); (2) worm shaped (Figure 1C and D), following the profile of 1 or more gyri (approximately 25%); (3) wedge shaped, accounting for approximately 20% of the ICLs (Figure 1E); and (4) clusters of microgranular lesions (Figure 1F), infrequent and almost exclusively observed in patients with SPMS.

Although a limited number of the inflammatory lesions that we believe to occur in the cortex of the brain affected by MS can be disclosed by DIR, this innovative MRI sequence constitutes a significant step forward in the demonstration of focal cortical pathologic conditions, including inflammatory ICLs in patients with MS. We applied DIR in a large number of clinically stratified patients with MS with the primary aims of assessing whether and at what disease stages ICLs could be observed and their clinical relevance. We observed that ICLs (1) can be detected in more than half of the MS population, (2) are more numerous and frequent at later disease stages but can be found from disease onset, (3) are signific-
cantly more evident in patients with more pronounced brain tissue damage (ie, increase in WM T2LV and decrease in BPF), and (4) are relevant to clinical disability. Moreover, the risk of having ICLs was found to be higher in men and in the presence of IgG0Bs in the CSF.

The pathogenesis of ICLs in MS is not clear. Typically, ICLs show less inflammation than WM lesions and are commonly classified, on the basis of their topographical localization, into juxtacortical, intracortical, or subpial lesions. The most common cortical lesion type is found to spread subpially, but the remaining 2 types are not at all rare. Our in vivo data, which agree with previous ex vivo studies, confirm that ICLs can be seen at different disease stages, with a significant increase from early to later stages, probably due to an inflammatory process developing in the cortex during an autoimmune demyelinating attack. The detection of similar numbers of ICLs in our C1S and RRMS groups may be explained by both the short disease duration of the RRMS group and the presence of a significant number of patients with dissemination in the space of the lesions among the C1S group. It might also be possible that some ICLs undergo rapid resolution and active remyelination, as observed in an experimental murine model of autoimmune demyelination. Since only prospective studies may confirm this possibility, a 2-year longitudinal study aimed at analyzing the persistence and the morphologic evolution of ICLs is in progress. Preliminary data at 6 and 12 months indicate that most ICLs persist over time and maintain their initial morphologic features.

After correction for unbalanced sample size, the possible differences between patients with MS who did or did not have ICLs were assessed. We found that patients with ICLs had higher WM T2LVs, more pronounced cerebral atrophy (decrease in the BPF), and more severe clinical disability. These findings seem to favor the occurrence of a more diffuse and severe pathologic process in patients with documented ICLs. This occurrence suggests that focal neocortical degeneration gives a major contribution to the clinical expression of the disease, which becomes more important in later disease stages when the central nervous system physiologic compensatory resources are exhausted.

An additional, intriguing finding of our work was the higher frequency of intrathecally synthesized IgG0Bs in the group of patients with ICLs compared with those without ICLs. This finding was confirmed in each patient group, and in general, patients with IgG0Bs had more than twice as many ICLs as patients without IgG0Bs. Since the WM T2LV did not differ in patients with or without IgG0Bs (data not shown), the presence of IgG0Bs does not merely reflect a more severe inflammatory profile involving both WM and GM. To the extent that B-cell infiltrates in MS and experimental autoimmune encephalomyelitis have been characteristically observed in leptomeningeal spaces close to the cortex and ICLs seem to differ from WM lesions in terms of T-cell infiltration, it might be possible that B cells and humoral immune mechanisms play a central role in the immunopathologic process that takes place in the cortex. However, regardless of the underlying mechanisms, the link between the intrathecal synthesis of IgG0Bs and the formation of ICLs is intriguing and needs to be further investigated.

Finally, the higher risk of ICLs observed in men compared with women deserves attention. The risk of having ICLs was considerable (odds ratio, 3.6) in men and was particularly significant in patients with IgG0Bs in the CSF (odds ratio, 7.0). These findings suggest that a more severe pathologic process characterizes the disease in men with IgG0Bs in the CSF and agree with previous epidemiologic data that suggest that male sex is a negative prognostic factor for MS. Despite the fact that the use of DIR sequencing has largely improved our ability to detect ICLs and that the limitations of MRI spatial resolution are great, we believe that most subpial lesions escape DIR detection and that a clear differentiation between mixed juxtacortical or intracortical lesions and pure ICLs is still difficult. Nevertheless, at least 4 different types of ICLs could be recognized in the brains of our patients on the basis of their morphologic features (Figure 1B-F). Whether the different shape of the lesions is related to differences in cellular content cannot be elucidated herein. However, the particular morphologic features of these lesions should be taken into account for a correct discrimination of ICLs on DIR sequences.

In conclusion, we show that ICLs, as detected in vivo by DIR, are significantly more frequent and numerous at later MS stages but can be demonstrated in early disease phases, even in patients with a first attack suggestive of MS. Considering their increase in frequency and the number of patients with more pronounced brain tissue damage, clinical disability, and a CSF inflammatory profile, the identification of ICLs in vivo should be taken into account for a more precise definition of MS clinical heterogeneity and evolution.

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REFERENCES


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