Cortical Lesions and Atrophy Associated With Cognitive Impairment in Relapsing-Remitting Multiple Sclerosis

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**Background:** Neuropsychological deficits in patients with multiple sclerosis (MS) have been shown to be associated with the major pathological substrates of the disease, ie, inflammatory demyelination and neurodegeneration. Double inversion recovery sequences allow cortical lesions (CLs) to be detected in the brain of patients with MS. Modern postprocessing techniques allow cortical atrophy to be assessed reliably.

**Objective:** To investigate the contribution of cortical gray matter lesions and tissue loss to cognitive impairment in patients with relapsing-remitting MS.

**Design:** Cross-sectional survey.

**Setting:** Referral, hospital-based MS clinic.

**Patients:** Seventy patients with relapsing-remitting MS.

**Main Outcome Measures:** Neuropsychological performance was tested using the Rao Brief Repeatable Battery of Neuropsychological Tests, version A. Patients who scored 2 SDs below the mean normative values on at least 1 test of the Rao Brief Repeatable Battery of Neuropsychological Tests, version A, were considered to be cognitively impaired. A composite cognitive score (the cognitive impairment index) was computed. T2 hyperintense white matter lesion volume, contrast-enhancing lesion number, CL number and volume, normalized brain volume, and normalized neocortical gray matter volume were also assessed.

**Results:** Twenty-four patients with relapsing-remitting MS (34.3%) were classified as cognitively impaired. T2 hyperintense white matter lesion volume and contrast-enhancing lesion number were not different between cognitively impaired and cognitively unimpaired patients. Cognitively impaired patients had a higher CL number (P=.01) and volume (P<.001) and decreased normalized brain volume (P=.02) and normalized neocortical gray matter volume (P=.002) when compared with cognitively unimpaired patients. Multivariate analysis revealed that age (β=0.228; P=.02), CL volume (β=0.432; P<.001), and normalized neocortical gray matter volume (β=0.349; P<.001) were independent predictors of the cognitive impairment index (r^2=0.55; F=23.903; P<.001).

**Conclusion:** The burden of CLs and tissue loss are among the major structural changes associated with cognitive impairment in relapsing-remitting MS.

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Cognitive impairment affects 40% to 65% of patients with multiple sclerosis (MS) and contributes significantly to their disability status. Although it is more frequent and severe in the progressive forms of MS, such an impairment can be present since the early clinical stages of the disease and typically includes deficits of long-term memory, attention and concentration, executive functioning, efficiency of information processing, and processing speed.

Despite the fact that magnetic resonance imaging (MRI) has been largely applied to investigate MS, the factors associated with cognitive dysfunction in this disease have not been fully elucidated yet. Indeed, the strength of the relationship between white matter (WM) lesion burden and cognitive impairment is modest at best, and measures of brain atrophy have been found to correlate only moderately with MS-related cognitive impairment. Cortical involvement related to MS is heterogeneous since it may arise from local demyelinating lesions, meningeal inflammation, neuronal injury, and Wallerian or transsynaptic degeneration. In the past few years, a large effort has been devoted to the development of MRI techniques with the ability to characterize in vivo the different substrates of gray matter (GM) damage and to improve our understanding of its clinical consequences.
cent MRI studies, which assessed the extent of brain tissue loss on a regional basis, have suggested that cortical volume loss is more closely associated with cognition than whole-brain atrophy. More recently, the application of double inversion recovery (DIR) sequences has convincingly demonstrated that cortical lesions (CLs) are a frequent finding in patients with MS, even at the earliest clinical stages. The CLs were found to correlate with physical disability, reduced brain parenchymal fraction, and T2 hyperintense lesion volume (LV).

Against this background, we wished to test whether in vivo assessment of the 2 major pathological aspects of the MS-related cortical damage (ie, the burden of focal lesions and the amount of tissue loss) would improve our understanding of cognitive impairment in relapsing-remitting (RR) MS. To this end, in a large sample of patients with RRMS, we quantified the extent of CLs detectable on DIR images and cortical atrophy to improve our understanding of their relative contributions to disease-related cognitive impairment.

METHODS

PATIENTS

Seventy consecutive patients (45 women and 25 men; mean age, 34.8 years; age range, 18-55 years; mean educational level, 11.8 years; educational level range, 5-18 years; mean disease duration, 8.4 years; disease duration range, 1-18 years) with a diagnosis of definite MS and an RR course were enrolled into the study. All patients underwent brain MRI and were assessed clinically and neuropsychologically. To be included, patients had to be relapse- and steroid-free for at least 3 months and had to have no history of depression and drug or alcohol abuse. No patients were affected by visual deficits or upper limb sensorimotor impairment that could interfere with performance on neuropsychological tests. Sixty-seven patients were treated with an immunomodulatory drug (44 with interferon beta-1a, 9 with interferon beta-1b, and 14 with glatiramer acetate). Twenty-two healthy volunteers served as control participants (15 women and 7 men; mean age, 35.1 years; age range, 18-49 years; mean educational level, 12.3 years; educational level range, 5-18 years; mean disease duration range, 1-18 years). The study was approved by the local ethics committee, and written informed consent was obtained from all subjects enrolled in the study.

NEUROPSYCHOLOGICAL ASSESSMENT

Neuropsychological assessment using the Rao Brief Repeatable Battery of Neuropsychological Tests, version A, was performed, only in patients and within 48 hours from MRI examination by a neuropsychologist (P. Grossi) blinded to both clinical and MRI results. This battery includes tests of verbal immediate and delayed recall memory (Selective Reminding Test [SRT] and SRT-Delayed Recall), spatial immediate and delayed recall memory (10/36 Spatial Recall Test and 10/36 Spatial Recall Test-Delayed), sustained attention, concentration, and speed of information processing (Paced Auditory Serial Addition Test at 3 seconds and Symbol Digit Modalities Test), and verbal fluency on semantic stimuli (Word List Generation). Patients who scored 2 SDs below the mean normative values on at least 1 test of the Rao Brief Repeatable Battery of Neuropsychological Tests, version A, were considered cognitively impaired.

For each patient, a cognitive impairment index score was also computed as previously described. Briefly, the cognitive impairment index score is a continuous variable obtained by a grading system applied to each patient’s score on every cognitive test, dependent on the number of SDs below the mean normative value. The patient was given a grade of 0 if he or she scored at or above the mean score of the control participants. Grade 1 was assigned if the patient scored below the control participants’ mean score but at or above 1 SD below that mean. Grade 2 was assigned if the patient achieved a score more than 1 SD but 2 or fewer SDs below the control participants’ mean. Finally, grade 3 was assigned if the patient scored more than 2 SDs below the control participants’ mean. These grades were then summed across all variables to give an overall measure of cognitive dysfunction for each patient.

IMAGE ACQUISITION

All images were acquired using a 1.5-T machine (Achieva; Philips Medical Systems, Best, the Netherlands) with a 33-mT/m power gradient and a 16-channel head coil. No major hardware upgrades of the scanner occurred during the study period, and bimonthly quality assurance sessions took place to guarantee measurement stability.

The following images were acquired from each subject (both control participants and patients): (1) DIR: repetition time, 15,631 milliseconds; echo time, 25 milliseconds; inversion time, 3,400 milliseconds; delay, 325 milliseconds; echo train length, 17; 50 contiguous axial slices with a thickness of 3.0 mm; matrix size, 130 × 256; and field of view, 250 × 200 mm²; (2) fast fluid-attenuated inversion recovery: repetition time, 10,000 milliseconds; echo time, 120 milliseconds; inversion time, 2,500 milliseconds; echo train length, 23; 50 contiguous axial slices with a thickness of 3.0 mm; matrix size, 172 × 288; and field of view, 250 × 200 mm²; and (3) 3-dimensional fast field echo sequence: 120 contiguous axial slices with the off-center positioned on 0; repetition time, 23 milliseconds; echo time, 4.6 milliseconds; flip angle, 30°; slice thickness, 1.2 mm; matrix size, 256 × 256; and field of view, 230 × 230 mm². Finally, a postcontrast T1-weighted spin echo sequence (repetition time, 618 milliseconds; echo time, 10 milliseconds; flip angle, 90°; 20 contiguous axial slices with a thickness of 5.5 mm; matrix size, 224 × 256; and field of view, 230 × 230 mm²) was acquired only in patients and 5 minutes after gadolinium-EDTA (0.1 mmol/kg) intravenous administration.

IMAGE ANALYSIS

All images were assessed by consensus by 2 experienced observers (M.C. and M.A.) who were blinded to the patients’ identity. On DIR images, particular attention was devoted to identify artifacts; CLs were defined as those lesions confined to the cortical ribbon and not involving the underlying subcortical WM. The number of CLs was assessed on DIR images, and CL volumes were calculated using a semiautomatic thresholding technique based on the Fuzzy C-means algorithm included in the Medical Image Processing, Analysis, and Visualization software program (http://mipav.cit.nih.gov) developed by the National Institutes of Health. The same procedure was applied to fluid-attenuated inversion recovery images to identify and segment WM lesions, thus obtaining a T2 hyperintense WM LV. On postcontrast T1-weighted images, the number of contrast-enhancing lesions was measured. On 3-dimensional fast field echo images, the normalized brain volume (NBV) and the normalized neocortical GM volume (NCV) were measured using the cross-sectional version of the Structural Image Evaluation of Normalized Atrophy (SIENAX; FMRIB Centre, Oxford, England) software program.
Table 1. Demographic, Clinical, and Magnetic Resonance Imaging Characteristics of Cognitively Impaired and Cognitively Unimpaired Patients With Relapsing-Remitting Multiple Sclerosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cognitively Impaired (n=24)</th>
<th>Cognitively Unimpaired (n=46)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), y</td>
<td>36.1 (20-55)</td>
<td>34.2 (18-52)</td>
<td>.19</td>
</tr>
<tr>
<td>Women/men, No.</td>
<td>16/8</td>
<td>29/17</td>
<td>.77</td>
</tr>
<tr>
<td>Disease duration, mean (range), y</td>
<td>8.7 (1-16)</td>
<td>8.2 (1-18)</td>
<td>.35</td>
</tr>
<tr>
<td>EDSS score, mean (range)</td>
<td>2.9 (1.0-5.0)</td>
<td>1.9 (1.0-4.5)</td>
<td>.006</td>
</tr>
<tr>
<td>Cognitive impairment index score, mean (range)</td>
<td>12.0 (3.0-20.0)</td>
<td>5.1 (0.0-12.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>T2 hyperintense WM LV, mean (range), cm³</td>
<td>8.4 (1.3-14.5)</td>
<td>7.5 (1.0-12.1)</td>
<td>.08</td>
</tr>
<tr>
<td>CELs, mean (range), No.</td>
<td>0.2 (0-2)</td>
<td>0.2 (0-2)</td>
<td>.88</td>
</tr>
<tr>
<td>CLs, mean (range), No.</td>
<td>7.2 (0-20)</td>
<td>4.8 (0-17)</td>
<td>.01</td>
</tr>
<tr>
<td>CL volume, mean (range), cm³</td>
<td>7.3 (0-13.2)</td>
<td>4.1 (0-12.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NBV, mean (range), mL</td>
<td>1518 (1357-1748)</td>
<td>1623 (1373-1789)</td>
<td>.02</td>
</tr>
<tr>
<td>NCV, mean (range), mL</td>
<td>615 (580-672)</td>
<td>686 (651-734)</td>
<td>.002</td>
</tr>
</tbody>
</table>

Abbreviations: CEL, contrast-enhancing lesion; CL, cortical lesion; EDSS, Expanded Disability Status Scale; LV, lesion volume; NBV, normalized brain volume; NCV, normalized neocortical gray matter volume; WM, white matter.

A t test was used to compare demographic, clinical, and MRI findings between cognitively impaired and cognitively unimpaired patients with RRMS. To investigate the correlation between MRI and cognitive variables, univariate correlations between continuous variables were assessed using the Pearson correlation coefficient and those between discrete variables were assessed with the Spearman rank correlation coefficient. A stepwise linear regression analysis was performed to assess the relative contributions from age (and sex), clinical (disease duration), and MRI (T2 hyperintense WM LV, contrast-enhancing lesion number, CL volume, and NCV) variables in predicting the cognitive impairment index score. The CL number and NBV were not included in this model owing to their high correlation with CL volume and NCV, respectively. Forward and backward stepwise analyses were conducted using the Wald statistic as a criterion, with P=.05 for entry and P=.10 for removal. All statistical analyses were performed using SPSS version 13 statistical software (SPSS Inc, Chicago, Illinois) and R, a statistical software package available at http://www.r-project.org.

RESULTS

CLINICAL AND COGNITIVE FINDINGS

The median score of the cognitive impairment index was 7.0 (range, 0.0-20.0). Twenty-four patients (34.3%) were classified as cognitively impaired (ie, they scored 2 SDs below the mean normative values in ≥1 cognitive test) and 46 patients (65.7%) were classified as cognitively unimpaired. The rate of failure for each neuropsychological test is as follows: SRT—Delayed Recall and SRT—Long-term Storage, 9 patients (12.9%); SRT—Consistent Long-term Retrieval, Paced Auditory Serial Addition Test at 3 seconds, and Word List Generation, 7 patients (10.0%); 10/36 Spatial Recall Test—Delayed and Symbol Digit Modalities Test, 6 patients (8.6%); and 10/36 Spatial Recall Test, 1 patient (1.4%). Demographic and clinical characteristics of the 2 groups are reported in Table 1. No between-group difference was found in terms of age, sex, or disease duration. Cognitively impaired patients with RRMS had a significantly higher Expanded Disability Status Scale score compared with cognitively unimpaired patients (P=.006).

MRI FINDINGS

Healthy Control Participants

No CLs were observed on DIR images in any of the healthy control participants. Although DIR signal changes resembling CLs were found on control participants’ images, they were easily identifiable as artifacts because they were the following: (1) ribbonlike hyperintense areas, often located in extracortical regions (sometimes bilateral or symmetric), (2) vascular and flow artifacts around sines and large vessels (Figure 1A), and (3) areas possibly due to cerebrospinal fluid effusion in the periventricular WM (Figure 1B).

Patients With RRMS

On patients’ DIR images, artifacts similar to those observed in healthy control participants were detected, but again they were easily identified as such (Figure 1). In Table 1, MRI characteristics of cognitively impaired and cognitively unimpaired patients are shown. T2 hyperintense WM LV (P=.08) and contrast-enhancing lesion number (P=.88) were not different between the 2 groups. We detected CLs in 18 of 24 cognitively impaired patients (75.0%) and 44 of 46 cognitively unimpaired patients (95.7%) (P=.01). Cognitively impaired patients also had higher CL volume (P<.001). In cognitively impaired patients as compared with cognitively unimpaired patients, NBV (P=.02) and NCV (P=.002) were significantly decreased. Similar results were obtained when patients who failed at 2 or more neuropsychological tests were considered cognitively impaired (data not shown).

CORRELATION ANALYSES

The correlations between MRI measures and cognitive findings are reported in Table 2 and Figure 2. Both the forward and backward stepwise regression revealed significant contributions from age (P=.02), CL volume (P<.001), and NCV (P<.001) as independent predictors of the cognitive impairment index (r²=0.55;
Table 4 shows the correlations of CL number and volume and NCV with T2 hyperintense WM LV and NBV. The CL number and volume significantly correlated with T2 hyperintense WM LV, NBV, and NCV. The NCV significantly correlated with T2 hyperintense WM LV and NBV.

**COMMENT**

This study unambiguously shows that cortical damage is associated with MS-related cognitive impairment. Indeed, patients with RRMS and cognitive deficits showed more cortical lesions and more severe cortical atrophy than patients who were cognitively preserved. Our results confirm and extend those of previous pathological11 and imaging1,12 studies, which demonstrated cortical volume reduction in cognitively impaired patients with RRMS but did not investigate the role of focal demyelinating lesions in the cortex or elucidate how the burden of CLs and the extent of cortical atrophy are interrelated.

The most intriguing finding of this study was that the CL number and volume are higher in cognitively impaired patients compared with those without cognitive deficits. Similar results were also obtained when using a...
conservative approach in defining what constitutes MS-related cognitive impairment (ie, failure at ≥2 neuropsychological tests). Furthermore, the CL number and volume were found to correlate with both the cognitive impairment index score and deficits in attention and concentration, speed of information processing, and memory. Although the cognitive impairment index has not yet been validated to rate MS-related cognitive impairment, the use of such a global score strengthened the notion that, independent of the criterion used for binary classification of patients into those who are cognitively impaired and those who are cognitively unimpaired, neuropsychological dysfunction occurs in MS in association with increased cortical damage. Interestingly, CL volume showed the strongest correlation with speed processing (as measured with the Paced Auditory Serial Addition Test at 3 seconds and the Symbol Digit Modalities Test). This finding is likely to be consistent with the prominent involvement of the frontal cortex in patients with MS.31-36 Future studies assessing the topographical distribution of focal GM damage are needed to confirm this finding. In patients with MS, mechanisms leading to focal cortical damage are likely to be at least partially different from those associated with lesion formation in the WM.10 As a matter of fact, histopathological studies indicated that CLs, as opposed to lesions in the WM, are characterized by a modest increase in blood-brain barrier permeability and harbor only little inflammation changes and gliosis.10 Meningeal inflammation as a possible cause of subpial cortical demyelination is yet another pathological feature of cortical MS lesions that is currently being explored.37 Our findings, by showing that focal lesions in the cortex of patients with RRMS are clinically meaningful, call for additional research to clarify how demyelination of the cortex develops in these patients and hence to identify possible future therapeutic venues.

Our study also showed that patients with RRMS who were cognitively impaired at the Rao Brief Repeatable Battery of Neuropsychological Tests, version A, also had a lower NCV than those who were cognitively unimpaired and that the degree of cortical atrophy significantly correlates with the composite cognitive score and performance on a number of neuropsychological tests. This is consistent with previous neuroimaging studies showing that cortical atrophy is one of the main correlates of cognitive decline in MS.13-20 A selective decrease of cortical volume was found in patients with RRMS and mild cognitive deficits; this was associated with poorer performance on tests of verbal and spatial memory, attention and concentration, and verbal fluency.13 Two other studies17,20 found a correlation between tissue loss in the temporal and prefrontal cortices and performance on several cognitive tasks. Finally, a recent longitudinal study with a 2.5-year follow-up18 showed that cognitive deterioration proceeds in parallel with the decrease of cortical volume in patients with RRMS.

Although we found that WM lesion burden correlates with individual neuropsychological test scores and the cognitive impairment index score, T2 hyperintense WM LV and contrast-enhancing lesion number were not significantly different between the 2 groups of patients with RRMS, but only a trend for increased mean T2 hyperintense WM LV in cognitively impaired patients vs cognitively unimpaired patients was found. Previous studies assessing the relationship between the extent of WM damage and cognitive impairment in patients with MS gave conflicting results as a significant correlation was found by some studies but not by others.13 Although the small sample size of cognitively impaired subjects may be among the reasons
for such a finding, our results are compatible with those of previous studies and seem to indicate that the overall burden of focal WM lesions does not fully account for the severity of cognitive impairment in MS. Such a conclusion is also supported by the results of the multivariate analysis, which revealed that only CL volume and, even if at a lesser extent, NCV independently predicted the overall cognitive performance of our patients.

We also investigated the correlations between CLs and cortical atrophy as well as between cortical damage and other MRI metrics. We found that the extent of cortical focal damage accounts for almost half of the variance of cortical tissue loss in our patient sample, which was not the case for WM lesion burden. Recent longitudinal studies have suggested that in the early stage of disease GM atrophy is likely to be secondary to WM injury, but as the disease progresses GM tissue loss seems to evolve independently of WM damage. However, previous pathological and MRI studies left the question of whether atrophy can occur as a result of focal demyelination in the GM unanswered. In a recent study assessing the association between cortical atrophy and type 1 (leuko-cortical) lesions, the overall reduction of cortical thickness was not related to cortical demyelination, although neuronal, glial, and synaptic losses were prominent in leukocortical lesions. However, Wegner et al did not look at the contribution of intracortical and subpial lesions to cortical atrophy. Clearly, because we do not have direct histopathological confirmation, we can only speculate on the relationship between cortical demyelination and tissue loss. Nevertheless, it is tempting to suggest that CLs are at least one of the factors with the potential to contribute to the development of cortical atrophy in patients with RRMS, which in turn may lead to more severe cognitive decline.

Admittedly, in our sample, cognitive performance was not entirely explained by CL burden and degree of cortical atrophy. However, this does not come as a surprise. First, while pathological studies have reported that the number of CLs can amount to almost 60% of the total lesion count, it is known that DIR sequences allow only a limited harvest of such lesions. In addition, the sensitivity of DIR sequences in CL detection has been suggested to be higher for certain lesion types (ie, types 1, 2, and 4) than for others (ie, type 3). Nevertheless, DIR sequences are at present those which provide the most precise quantification of CL burden in patients with MS. Second, owing to time constraints, we did not acquire additional sequences such as those allowing us to measure the diffusivity properties of the cortex. As a consequence we were unable to investigate other aspects of MS-related cortical damage (ie, diffuse pathology outside focal CLs) that are likely to influence a patient’s cognitive performance. Finally, we did not assess damage to deep GM structures, which has been indicated as a possible factor contributing to MS-related cognitive impairment. Further investigations are needed to determine the mechanism by which site-specific differences in GM pathology contribute to cognitive dysfunction in MS. Despite these limitations, our findings suggest that cortical pathology correlates better than WM damage with cognitive deficits in patients with RRMS and that both the presently available MRI markers of cortical demyelination and degeneration might be helpful in identifying patients at risk for cognitive deterioration.
Author Contributions: Dr Filippi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Calabrese, Gallo, and Filippi. Acquisition of data: Calabrese, F. Rinaldi, Mattisi, Grossi, Favaretto, Atzori, Bernardi, Barachino, L. Rinaldi, and Perini. Analysis and interpretation of data: Calabrese, Agosta, F. Rinaldi, Atzori, and Filippi. Drafting of the manuscript: Calabrese, Agosta, F. Rinaldi, Mattisi, Grossi, Favaretto, Atzori, Bernardi, Barachino, L. Rinaldi, Perini, Gallo, and Filippi. Critical revision of the manuscript for important intellectual content: Calabrese, Agosta, Gallo, and Filippi. Statistical analysis: Calabrese and Filippi. Study supervision: Filippi. Financial Disclosure: None reported.