Cortical lesion load associates with progression of disability in multiple sclerosis

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Cortical inflammatory lesions have been correlated with disability and cortical atrophy in multiple sclerosis. The extent to which cortical lesion load is associated with longer-term physical and cognitive disability in different multiple sclerosis phenotypes has not yet been investigated. Thus, a 5-year prospective longitudinal study was carried on in a large group of patients with multiple sclerosis. Three hundred and twelve consecutive patients suffering from multiple sclerosis (157 relapsing remitting, 35 paediatric, 45 benign, 44 primary progressive and 31 secondary progressive) were enrolled in a 5-year prospective clinical and neuroimaging study. Several magnetic resonance parameters (including cortical lesion number and volume, contrast-enhancing cortical lesions and grey matter atrophy) were analysed to find associations with clinical and cognitive outcomes. Patients with high cortical lesion load had higher Expanded Disability Status Scale increase (median = 1.5; range = 0–3) during the study than both patients with low cortical lesion load (median = 1.0; range = 1–3, P < 0.001) and without cortical lesions (median = 0.5; range = −1 to 2, P < 0.001). Compared with clinically stable patients, 101 (32.4%) patients showing clinical progression at 5 years had the highest rate of cortical lesion accumulation (P < 0.001). Stepwise regression analysis revealed significant and independent contributions from age (β = 0.55), cortical lesion volume (β = 0.58), T2 white matter lesion volume (β = 0.34) and grey matter fraction (β = 0.42) as predictors (final model with $r^2 = 0.657$, P < 0.001) of Expanded Disability Status Scale change. Disease duration (β = 0.52, P < 0.001), cortical lesion volume (β = 0.67, P < 0.001), grey matter fraction (β = 0.56, P < 0.001) and T2 white matter lesion volume (β = 0.31, P = 0.040) at baseline were found to be independent predictors of cognitive status at the end of the study. While confirming the relevance of cortical pathology in all multiple sclerosis phenotypes, but benign, our study suggests that grey matter and white matter changes in multiple sclerosis occur, at least, partly independently, and that grey matter, more than white matter, damage is associated with physical and cognitive disability progression. Thus, the combination of grey and white matter parameters gives a more comprehensive view of multiple sclerosis pathology and allows a better understanding of the progressive phase of the disease, which, however, seems more related to cortical damage than to subcortical white matter changes.

Keywords: cortical lesions; disability progression; multiple sclerosis; double inversion recovery; MRI

Abbreviations: EDSS = Expanded Disability Status Scale; T0 = start of study; T5 = end of study (5 years)
Introduction

Accumulation of focal inflammatory cortical lesions and progressive thinning characterize cortical pathology of all clinical subsets of multiple sclerosis (Kidd et al., 1999; Peterson et al., 2001; De Stefano et al., 2003; Geurts et al., 2005; Calabrese et al., 2007a, b, 2009b, c; Ramasamy et al., 2009; Absinta et al., 2011). Cortical lesions and grey matter atrophy can be detected by means of MRI (Calabrese et al., 2007b) and, in some cases, may precede the appearance of white matter lesions (Calabrese and Gallo, 2009; Popescu et al., 2011). In cross-sectional studies, we and others have observed an association between cortical lesion load and both physical and cognitive disability in patients with multiple sclerosis (Calabrese et al., 2009a, c; Roosendaal et al., 2010; Nelson et al., 2011). Moreover, in a previous 3-year longitudinal study on 76 patients with relapsing remitting and 31 with secondary progressive multiple sclerosis, we observed that the accumulation of cortical lesions was associated with the development of a higher degree of disability in both phenotypes (Calabrese et al., 2010).

The extent to which MRI parameters of cortical pathology may be used for clinical purposes needs to be confirmed in longer longitudinal prospective studies based on larger numbers of patients. We therefore designed a 5-year prospective study on >300 patients with multiple sclerosis, some of whom were paediatric, and affected with different disease phenotypes, including benign and primary progressive multiple sclerosis. The aim of the study was to assess whether correlation exists between the degree of cortical pathology and the rate of disability progression in multiple sclerosis.

Materials and methods

Study population

During the period 1 April 2005 to 1 May 2006, 327 consecutive patients with multiple sclerosis (McDonald et al., 2001) were enrolled in a 5-year prospective study consisting of programmed neurological examinations [Expanded Disability Status Scale (EDSS)] (Kurtzke, 1983) every 6 months or, in the case of relapse, a complete magnetic resonance assessment (including sequences for grey matter analysis) at study entry (T0) and after 5 years (T5) (range = 63 ± 2 months). Patients presenting with clinical relapse within the previous 6 months and therefore with an evolving neurological status were excluded from the study. During the study period, 12 patients moved to other cities/regions and were lost to follow-up. Thus, 35 patients (aged <16 years) with paediatric relapsing remitting multiple sclerosis, 157 adults with relapsing remitting multiple sclerosis, 48 patients with benign multiple sclerosis, 44 with primary progressive multiple sclerosis and 31 with secondary progressive multiple sclerosis were included in the analysis. Patients with ‘benign multiple sclerosis’ were defined as having at least 15 years of disease duration, an EDSS (Kurtzke, 1983) score <3.0 and normal cognition. At the end of the follow-up, however, 3 of 48 patients with benign multiple sclerosis had an EDSS score >3.0. We therefore decided to apply a ‘more restricted’ definition of benign multiple sclerosis and included in the final analysis only 45 patients with benign multiple sclerosis, i.e. those having an EDSS ≤3.0 after 20 years of disease duration. Demographic and clinical features of the final 312 patients analysed are summarized in Table 1.

During the study, 21 (67.7%) patients with secondary progressive multiple sclerosis and 25 (56.8%) patients with primary progressive multiple sclerosis received immunosuppressive drugs, whereas 153 (97.5%) patients with relapsing remitting multiple sclerosis, 24 (53.3%) with benign multiple sclerosis, 33 (94.3%) with paediatric multiple sclerosis and eight (25.8%) patients with secondary progressive multiple sclerosis were treated with immunomodulatory agents. Thus, 264 (84.6%) patients received a disease-modifying treatment during the study. Disability progression was defined as an increase of at least 1.0 point in EDSS compared with baseline, not related to a relapse, observed at any time during the 5-year period and sustained over at least 1 year of observation. Patients with increased EDSS due to relapses and patients showing cognitive impairment but stable EDSS were excluded from the analysis on clinical progression. The local Ethic Committee approved the study. Informed consent was obtained from the patients.

Neuropsychological assessment

Neuropsychological assessment was performed using Rao’s Brief Repeatable Battery (Rao et al., 1990; Amato et al., 2006) of Neuropsychological Test, version A, at T0 and T5. This evaluation was not performed on the paediatric population. The neuropsychologist was blind to both clinical and MRI data. Patients who scored 2 standard deviations (SDs) below mean normative values on at least one test of the Brief Repeatable Battery were considered cognitively impaired. A cognitive index was also computed for each patient, to counterbalance the limitation of such categorization (Camp et al., 1999). The index is a discrete variable obtained through the use of a grading system applied to the patient’s score on each cognitive test and dependent on the number of SDs below the mean normative value (Amato et al., 2006). The sum of grades across all tests gives an overall measure of cognitive dysfunction for each patient.

Image acquisition protocol

All images were acquired using a 1.5-T scanner (Achieva, Philips Medical Systems) with 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: (i) double inversion recovery: repetition time = 15 631 ms, echo time = 25 ms, inversion time = 3 400 ms, delay = 325 ms, echo train length = 17, 50 contiguous axial slices with a thickness = 3 mm, a matrix size = 130 × 256 and a field of view = 250 × 200 mm2; (ii) Fluid attenuated inversion recovery (FLAIR): repetition time = 10 000 ms, echo time = 120 ms, inversion time = 2500 ms, echo train length = 23, 50 contiguous axial slices with a thickness = 3.0 mm, a matrix size = 172 × 288 and a field of view = 250 × 200 mm2; and (iii) two volumetric fast-field echo sequences: 120 contiguous axial slices, repetition time = 25 ms, echo time = 4.6 ms, flip angle = 30°, slice thickness = 1.0 mm, matrix size = 256 × 256 and a field of view = 250 × 250 mm2 were acquired before and 5 min after gadolinium-EDTA (0.1 mmol/kg) intravenous administration. At follow-up, subjects were carefully repositioned according to published guidelines for serial MRI studies of multiple sclerosis (Miller et al., 1991).
was 1.2%, whereas inter-observer variability was 4.3%.

M.C. evaluated the double inversion recovery scans of 20 patients to reproducibly identify cortical lesions. A single observer (M.C.) evaluated the double inversion recovery images (M.C.) of their respective subgroup. Patients had a low cortical lesion load if they had a number of cortical lesions above the third quartile (Q3) of their respective subgroup. Patients who were blind to patient identity and to the date of image acquisition.

Grey matter fraction evaluation

Three-dimensional fast-field echo images at baseline and at 5 years were segmented into white matter, grey matter, and CSF using SPAMM as previously described (Chard et al., 2002; Miller et al., 2002). Using in-house software, lesion masks were obtained by contouring lesions on the 3D fast-field echo scans; these masks were then subtracted from white matter, grey matter, and CSF masks to obtain four mutually exclusive tissue masks with their volumes in millilitres. Total intracranial volume was calculated as the sum of white matter + grey matter + CSF + lesion masks with their volumes in millilitres. Total intracranial volume was calculated as grey matter volume/total intracranial volume at T0.

White matter lesion number and volume

The same software used to measure cortical lesion volumes (Pham et al., 2000) was applied to segment white matter lesions, previously identified on FLAIR images, thus obtaining a T2-weighted white matter lesion volume at baseline and follow-up. The number of contrast-enhancing lesions was also evaluated.

Statistical analysis

Differences among groups were assessed through analysis of variance, including age and treatment as covariates and post hoc Tukey’s Honestly Significant Difference procedure to account for multiple comparisons. Because cortical lesions and EDSS were not normally distributed, Mann-Whitney tests were used to compare populations with

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paediatric multiple sclerosis (n = 35)</th>
<th>RRMS (n = 157)</th>
<th>Benign multiple sclerosis (n = 45)</th>
<th>PPMS (n = 44)</th>
<th>SPMS (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.2 ± 2.0 (8:16)</td>
<td>37.4 ± 7.3 (18:55)</td>
<td>43.1 ± 8.9 (32:54)</td>
<td>41.1 ± 5.0 (23:58)</td>
<td>41.1 ± 4.1 (22:55)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>0.8 ± 0.5 (0.2:2)</td>
<td>4.1 ± 3.0 (1:12)</td>
<td>18.2 ± 6.1 (15:25)</td>
<td>7.5 ± 2.1 (2:13)</td>
<td>11.6 ± 2.3 (5:14)</td>
</tr>
<tr>
<td>EDSS</td>
<td>1.5 (0.2:5)</td>
<td>2.0 (1.0:5.5)</td>
<td>1.5 (1.0:3.0)</td>
<td>4.0 (3.0:6.5)</td>
<td>4.5 (2.5:6.0)</td>
</tr>
<tr>
<td>Cognitive score</td>
<td>NA</td>
<td>6.4 ± 2.0 (0.18)</td>
<td>3.2 ± 1.0 (0.6)</td>
<td>11.5 ± 3.0 (4:20)</td>
<td>12.7 ± 4.0 (5:20)</td>
</tr>
<tr>
<td>Cortical lesion number</td>
<td>1.2 ± 2.0 (0.8)</td>
<td>2.5 ± 3.0 (0.18)</td>
<td>1.0 ± 1.6 (0.9)</td>
<td>6.2 ± 3.8 (0.24)</td>
<td>11.3 ± 4.2 (1:20)</td>
</tr>
<tr>
<td>Cortical lesions Gadolineum +</td>
<td>10</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patients with cortical lesions</td>
<td>12 (34.3%)</td>
<td>111 (70.7%)</td>
<td>24 (53.3%)</td>
<td>35 (79.5%)</td>
<td>28 (90.3%)</td>
</tr>
<tr>
<td>Cortical lesion volume (cm³)</td>
<td>0.3 ± 0.2 (0.1:1.0)</td>
<td>1.0 ± 0.3 (0.2:6)</td>
<td>0.2 ± 0.2 (0.1:1.1)</td>
<td>1.1 ± 0.7 (0.3:4.3)</td>
<td>2.0 ± 0.8 (0.3:3.8)</td>
</tr>
<tr>
<td>Grey matter fraction (%)</td>
<td>NA</td>
<td>50.0 ± 2.0 (46.4:56.2)</td>
<td>47.2 ± 2.8 (40.8:52.2)</td>
<td>45.9 ± 3.0 (40.1:50.3)</td>
<td>44.8 ± 3.1 (40.5:51.0)</td>
</tr>
<tr>
<td>WMLV (cm³)</td>
<td>1.2 ± 2.1 (0.3:13.2)</td>
<td>6.6 ± 7.8 (0.4:40.2)</td>
<td>7.4 ± 4.0 (2.2:20.6)</td>
<td>4.8 ± 2.2 (2.1:10.4)</td>
<td>9.8 ± 3.5 (4.1:15.4)</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD (range). For the EDSS and EDSS change, median and (range) are provided.

PPMS = primary progressive multiple sclerosis; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; WMLV = T2 white matter lesion volume.

Image analysis

Cortical lesion number and volume

All images were assessed by consensus by two experienced observers who were blind to patient identity and to the date of image acquisition. At T0 and T5, the number of cortical lesions and the number of new cortical lesions were assessed on double inversion recovery images (Fig. 1) following the recent recommendations for cortical lesion scoring in patients with multiple sclerosis (Geurts et al., 2011). As previously described (Calabrese et al., 2010), to test the intra-observer reproducibility of cortical lesion identification, a single observer (M.C.) evaluated the double inversion recovery scans of 20 patients with multiple sclerosis three times. Intra-observer variability was calculated using the coefficient of variation, defined as the SD of a random variable divided by its mean value. To test inter-observer reproducibility, two observers evaluated the double inversion recovery scans of 20 patients with multiple sclerosis once. Mean intra-observer variability was 1.2%, whereas inter-observer variability was 4.3%.

Cortical lesion volume was calculated using a semi-automatic thresholding technique based on fuzzy C-mean algorithm (Pham et al., 2000) included in software developed at the National Institutes of Health, Medical Images Processing, Analysis and Visualization (http://mipav.cit.nih.gov). The number of cortical lesions and their volumes were calculated for each multiple sclerosis clinical subgroup. Then, a restrictive definition of ‘high cortical lesion load’ was applied: within each group, patients were defined as having a high cortical lesion load if they had a number of cortical lesions above the third quartile (Q3) of their respective subgroup. Patients who were blind to patient identity and to the date of image acquisition.

White matter lesion number and volume

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Statistical analysis

Differences among groups were assessed through analysis of variance, including age and treatment as covariates and post hoc Tukey’s Honestly Significant Difference procedure to account for multiple comparisons. Because cortical lesions and EDSS were not normally distributed, Mann-Whitney tests were used to compare populations with
Cortical lesions and multiple sclerosis progression

Respect to their cortical lesion volume, cortical lesion number, EDSS and EDSS change. Pearson chi square was applied to test the difference between patients with and without cortical lesions, and between patients with and without clinical worsening.

Univariate correlations between continuous variables were assessed using the Pearson correlation coefficient; those including discrete variables were assessed using the Spearman rank correlation coefficient.

A general linear model (logistic model) was applied to evaluate the association between EDSS progression (binary: worsened/stable) and cognitive status at T5 (binary: impaired/preserved) as outcome variables, and all demographic (age, age at onset, gender), clinical (EDSS at T0) and MRI variables at T0. Once the significant variables were identified, a separate general linear model was performed to evaluate their prognostic value. Leave-one-out cross-validation prediction error for general linear models was applied to estimate the goodness of the model: the general linear model was fit to data, omitting one patient a time; then the predicted response for the omitted patient was compared with the observed ones and the error rate was estimated.

The prediction capability of the estimated model (goodness of fit) is given by the error rate of the classifying patient (Mark and Goldberg, 2001). All statistical analyses were performed using SPSS v.18 and R, a statistical package available at http://www.r-project.org.

Results

Clinical and cognitive findings

During the study, one or more relapses were observed in 122 patients with relapsing remitting multiple sclerosis, 15 patients with paediatric multiple sclerosis, 13 patients with benign multiple sclerosis and one patient with secondary progressive multiple sclerosis. Relapse therapy consisted of 1g methylprednisolone/day for 5 days. Among these, 28 patients with relapsing remitting multiple sclerosis, one patient with paediatric multiple sclerosis, 11 patients with benign multiple sclerosis and one patient with secondary progressive multiple sclerosis showed an increased EDSS score. Increased EDSS not related to relapse was observed in 101 of 312 (32.4%) patients: 36 (22.9%) with relapsing remitting multiple sclerosis, 28 (90.3%) with secondary progressive multiple sclerosis and 37 (84.1%) with primary progressive multiple sclerosis.

At T0, 91 patients (53 with relapsing remitting multiple sclerosis, 20 with primary progressive multiple sclerosis and 18 with secondary progressive multiple sclerosis) showed cognitive impairment. During the study, 27 additional patients (18 with relapsing remitting multiple sclerosis, five with primary progressive multiple sclerosis and four with secondary progressive multiple sclerosis) developed cognitive impairment (24/27 also had an increase in EDSS). Thus, 118 of 312 patients (37 with relapsing remitting multiple sclerosis (45.2%), 25 with primary progressive multiple sclerosis (56.8%) and 22 with secondary progressive multiple sclerosis (71.0%)) were cognitively impaired at T5.

Magnetic resonance imaging findings

Cortical lesion load at T0 and at T5

At T0, 1103 cortical lesions were detected in 210 of 312 (67.3%) patients (Table 1). Forty-two gadolinium-enhancing lesions (32 in patients with relapsing remitting multiple sclerosis and 10 in patients with paediatric multiple sclerosis) were identified. At T5, 913

![Figure 1 Axial double inversion recovery of (A) paediatric, (B and C) relapsing remitting, (D) benign, (E) secondary progressive and (F) primary progressive multiple sclerosis. Several cortical lesions (thin arrows), some of which extend into the subcortical white matter (thick arrows), are depicted. Some artefacts are also evident (arrowhead).](https://academic.oup.com/brain/article-abstract/135/10/2952/299411)
new cortical lesions (36 enhancing) were identified in 237 of 312 (75.9%) patients (Table 1). Thus, both the number and volume of cortical lesions significantly increased during the follow-up ($P < 0.001$ for each comparison).

No difference in the rate of cortical lesion accumulation was observed among relapsing remitting multiple sclerosis, primary progressive multiple sclerosis and secondary progressive multiple sclerosis groups, but paediatric and benign groups accumulated fewer cortical lesions compared with other groups ($P < 0.001$ for both comparisons), and the percentage of patients that accumulated new cortical lesions in these groups was significantly lower than that of patients with relapsing remitting multiple sclerosis ($P < 0.001$ for both comparisons, Table 1). However, when the analysis was restricted only to patients who developed new cortical lesions during the follow-up, the rate of cortical lesion accumulation was almost the same in patients with paediatric multiple sclerosis (mean ± SD = 4.0 ± 1.9), relapsing remitting multiple sclerosis (4.0 ± 3.3), primary progressive multiple sclerosis (4.2 ± 3.5) and secondary progressive multiple sclerosis (3.8 ± 2.7), whereas patients with benign multiple sclerosis had a significantly lower cortical lesion accumulation (2.5 ± 2.4, $P = 0.007$).

### White matter lesion load at T0 and at T5

At T5, new $T_2$ white matter lesions were observed in 188 of 312 patients (60.2%, Table 1). A total number of 1212 $T_2$ white matter lesions (52 enhancing) were scored (245 in paediatric multiple sclerosis, 659 in relapsing remitting multiple sclerosis, 26 in benign multiple sclerosis, 57 in primary progressive multiple sclerosis and 46 in secondary progressive multiple sclerosis), and the white matter lesion volume significantly increased from T0 to T5 in all multiple sclerosis groups ($P < 0.001$ for all comparisons, Table 1).

### Magnetic resonance imaging profile of patients with clinical and cognitive worsening

Compared with clinically stable patients, the 101 (37.8%) who showed EDSS progression (unrelated to relapses) at T5 were characterized by: (i) higher number ($P < 0.001$) and volume ($P < 0.001$) of cortical lesions at T0; (ii) higher rates of cortical lesion accumulation ($P < 0.001$); and (iii) higher increase in cortical lesion volume ($P = 0.002$) (Table 2). No significant increase in volume and number of white matter lesions was observed in patients clinically worsened compared with those who remained clinically stable during follow-up (Table 2). MRI characteristics of patients with or without cognitive dysfunction at T5 are broken down by disease subtype in Table 3.

### Clinical and cognitive profile of patients with high cortical lesion load at T0

Seventy-seven patients (nine with paediatric multiple sclerosis, 39 with relapsing remitting multiple sclerosis, 11 with benign multiple sclerosis, 10 with primary progressive multiple sclerosis and eight with secondary progressive multiple sclerosis) had a high cortical lesion load at T0. These patients had worse clinical evolution (median EDSS change = 1.5, range = 0–3) compared with patients with low cortical lesion load (median EDSS change = 1.0, range = 1–3, $P < 0.001$) and with patients without cortical lesions (median EDSS change = 0.5, range = −1 to 2, $P < 0.001$). Moreover, their cognitive score was higher compared with patients with low cortical lesion load or without cortical lesions both at T0 (median = 12.0; range = 3.0–20.0) and at T5 (median = 15.5; range = 3.0–20.0; $P < 0.001$ for each comparison).

Table 4 summarizes clinical, cognitive and MRI features of these three groups of patients.

### Cortical lesion load and grey matter atrophy

After age correction, the grey matter fraction at T0 was significantly lower in secondary progressive multiple sclerosis and primary progressive multiple sclerosis compared with relapsing remitting multiple sclerosis and benign multiple sclerosis ($P < 0.001$ for both comparisons); no difference was observed between relapsing remitting multiple sclerosis and benign multiple sclerosis ($P = 0.121$) (Table 1). At T5, grey matter fraction change was smaller in benign multiple sclerosis ($P = 0.002$) and greater in primary progressive multiple sclerosis ($P < 0.001$) and secondary progressive multiple sclerosis ($P = 0.012$) compared with relapsing remitting multiple sclerosis. Patients with high cortical lesion load showed a significant reduction of grey matter fraction at T0 compared with patients with low cortical lesion load ($P = 0.004$) and without cortical lesions ($P < 0.001$, Table 4). Those patients who accumulated new cortical lesions during the follow-up showed a greater grey matter fraction change (2.0 ± 1.6; range = 0.6–4.9) than patients with no evidence of new cortical lesions (1.4 ± 1.0; range = 0.6–3.2; $P = 0.004$).

### Correlation analysis

The EDSS at T0 correlated with age ($r = 0.46$, $P < 0.001$), disease duration ($r = 0.49$, $P < 0.001$), number of cortical lesions ($r = 0.48$, $P < 0.001$) and with patients with or without cognitive dysfunction at T5 ($r = 0.49$, $P < 0.001$).
Cortical lesions and multiple sclerosis progression

Table 3 MRI characteristics of patients with or without cognitive dysfunction at T5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RRMS cognitive impaired (n = 86)</th>
<th>RRMS cognitive preserved (n = 22)</th>
<th>PPMS cognitive impaired (n = 19)</th>
<th>PPMS cognitive preserved (n = 9)</th>
<th>SPMS cognitive impaired (n = 25)</th>
<th>SPMS cognitive preserved (n = 71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WMLV–T5</td>
<td>2.0 ± 0.8 (0.3–4.2)</td>
<td>2.1 ± 0.9 (0.3–4.1)</td>
<td>5.2 ± 2.1 (2.4–10.2)</td>
<td>5.6 ± 2.6 (2.2–10.4)</td>
<td>5.8 ± 2.6 (3.6–9.6)*</td>
<td>5.6 ± 2.6 (2.0–3.8)</td>
</tr>
<tr>
<td>Cortical lesion volume–T5</td>
<td>1.6 ± 0.4 (0.3–2.0)</td>
<td>1.3 ± 0.5 (0.3–2.4)</td>
<td>2.1 ± 0.5 (0.2–2.4)</td>
<td>1.1 ± 0.3 (0–4.4)</td>
<td>1.1 ± 0.3 (0.3–2.4)</td>
<td>1.2 ± 0.4 (0.6–3.2)</td>
</tr>
<tr>
<td>New cortical lesions</td>
<td>1.8 ± 0.8 (0.3–2.4)</td>
<td>3.6 ± 3.7 (1–16)</td>
<td>4.2 ± 1.0 (0.2–10.2)</td>
<td>1.2 ± 0.3 (0.2–2.0)</td>
<td>2.7 ± 0.3 (0.2–2.0)</td>
<td>2.6 ± 0.5 (0.3–2.6)</td>
</tr>
<tr>
<td>Grey matter fraction–T5</td>
<td>1.8 ± 0.8 (0.3–2.0)</td>
<td>3.6 ± 3.7 (1–16)</td>
<td>4.2 ± 1.0 (0.2–10.2)</td>
<td>1.2 ± 0.3 (0.2–2.0)</td>
<td>2.7 ± 0.3 (0.2–2.0)</td>
<td>2.6 ± 0.5 (0.3–2.6)</td>
</tr>
<tr>
<td>Grey matter fraction change %</td>
<td>1.8 ± 0.8 (0.3–2.0)</td>
<td>3.6 ± 3.7 (1–16)</td>
<td>4.2 ± 1.0 (0.2–10.2)</td>
<td>1.2 ± 0.3 (0.2–2.0)</td>
<td>2.7 ± 0.3 (0.2–2.0)</td>
<td>2.6 ± 0.5 (0.3–2.6)</td>
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</table>

PPMS = primary progressive multiple sclerosis; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; WM LV = T2 white matter lesion volume.

When the cognitive status at T5 was set as an outcome variable (binary: impaired/preserved), disease duration ($\beta = 0.52$, $P < 0.001$), cortical lesion volume ($\beta = 0.67$, $P < 0.001$), grey matter fraction ($\beta = 0.56$, $P < 0.001$) and white matter lesion volume ($\beta = 0.31$, $P = 0.040$) were pointed out as its independent predictors (Table 6). In this case, the estimated model including
these variables correctly identified 65 patients with relapsing remitting multiple sclerosis, 21 with primary progressive multiple sclerosis and 18 with secondary progressive multiple sclerosis among the 118 (88.1%) patients with cognitive impairment, and correctly identified 78 patients with relapsing remitting multiple sclerosis, 14 with primary progressive multiple sclerosis and seven with secondary progressive multiple sclerosis among the 114 (86.4%) cognitive preserved patients (error rate = 12.5%).
Discussion

In the present 5-year longitudinal study, >300 patients with multiple sclerosis with different clinical phenotypes were evaluated using an advanced MRI acquisition protocol, including non-conventional sequences to study grey matter pathology. In such study, cortical lesion volume and grey matter fraction were found to be associated to each other and to physical and cognitive disability progression. Patients having high cortical lesion load at baseline showed the worse clinical evolution and a significant progression of cortical atrophy after 5 years. This was observed in all clinical subsets. Moreover, cognitive dysfunction was more frequently found in patients with high cortical lesion load. These results extend and strengthen previous cross-sectional and shorter longitudinal studies (Calabrese et al., 2009a, 2012) and confirm that high cortical lesion load characterizes patients with multiple sclerosis having the most severe grey matter atrophy and the worst physical and cognitive prognosis.

The stepwise regression analysis disclosed that both white matter and grey matter pathology are independent predictors of EDSS worsening. However, although disability is just slightly associated with white matter lesion volume in the relapsing remitting population and with grey matter fraction in the chronic progressive one, it is more associated with cortical lesions in all multiple sclerosis phenotypes. Thus, despite the evident clinical rebound of white matter pathology (i.e. lesion number and volume), our data strengthen the concept that disability progression in multiple sclerosis is more related to grey matter than to white matter pathology, in all disease phenotypes. This would be inline with previous studies conducted with different MRI methodologies and in relatively small patient populations (Agosta et al., 2006; Rovaris et al., 2006; Roosendaal et al., 2011; Tur et al., 2011; Calabrese et al., 2012). When cognitive status was set as clinical outcome, disease duration, cortical lesion volume, grey matter fraction and white matter lesion volume were retained as its independent predictors. These data confirm that patients with high
cortical lesion load also have a worse cognitive evolution and extend previous short-term observations in favour of a mixed cortical/subcortical origin of cognitive dysfunction in multiple sclerosis. Therefore, a comprehensive MRI assessment of both grey and white matter damage may better explain the heterogeneous clinical evolution of multiple sclerosis.

Surprisingly, our study discloses that cortical pathology evolves in a similar way in adult patients with relapsing remitting or chronic progressive multiple sclerosis. The difference in cortical lesion load at baseline can be explained in terms of different disease duration, whereas the rate of cortical lesion accumulation was similar among the different phenotypes. Although therapies (i.e. immunosuppressive versus immunomodulatory) might have partially influenced the results, cortical pathology appears to be largely independent of the clinical phenotypes (e.g. relapsing remitting versus chronic progressive), thus representing a further element of heterogeneity within each disease phenotype that might be useful for a better clinical stratification of patients.

In this regard, patients with benign and paediatric multiple sclerosis need separate comments. The benign group was characterized by the smallest number of cortical lesions at baseline and the lowest rate of cortical lesion accumulation during the study; such results probably reflect the peculiar immunological background of these patients. The question whether a more efficient regulatory arm of the immune network may explain the less aggressive disease evolution in these patients (Dalla Libera et al., 2011) merits further investigation. The low number of cortical lesions observed in paediatric multiple sclerosis at baseline, inline with a recent report (Absinta et al., 2011), can be explained by the short disease duration in these patients. The difference in lesion accumulation between paediatric and adult relapsing remitting multiple sclerosis observed in this study may be partially explained by the limited sample size of the paediatric group. However, differences in brain plasticity and reparative mechanisms between paediatric and adult patients should be taken into consideration.

The major limitation of double inversion recovery in detecting cortical lesions, especially subpial ones, is its low sensitivity compared with histology (Seewann et al., 2012). However, recent comparative histological/MRI studies have demonstrated that the ‘tip of the iceberg’ detected by MRI and its ‘bulk’ differ only in size, and that the number of detectable cortical lesions correlates with their overall number and with the overall percentage of cortical demyelination (Seewann et al., 2011). Thus, we feel that our double inversion recovery-based findings can be considered an acceptable assessment of grey matter pathology. Nevertheless, we are aware that further improvement in cortical lesion detection could be achieved, perhaps by combining different MRI sequences such as phase-sensitive inversion recovery and 3D MP-RAGE (Nelson et al., 2007, 2008). A second possible limitation of double inversion recovery lies in its high inter-observer and inter-centre variability when heterogeneous images are used (Geurts et al., 2011). Our study, however, was carried out at a single centre, is based on a highly homogeneous set of images and cortical lesion identification is the result of an agreement between two experienced observers (M.C. and A.F.). Finally, although a robust statistical analysis has been performed, the limited sample size of each phenotype, except relapsing remitting multiple sclerosis, might constitute a weakness when each subgroup is analysed separately.

In summary, our study indicates that the degree of cortical damage at baseline is associated with the rate of physical and cognitive disability progression. Thus, analysis of cortical pathology may help in early identification of patients with a worse prognosis and, given the relevant clinical and therapeutic implications, its prognostic value needs to be evaluated in detail.

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