In vivo assessment of cervical cord damage in MS patients: a longitudinal diffusion tensor MRI study

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Cervical cord damage is likely to contribute to the accumulation of disability in multiple sclerosis (MS) and can be quantified in vivo using MRI. We used conventional and diffusion tensor (DT) MRI to: (a) define the temporal evolution of intrinsic tissue injury and atrophy in the cervical cord from MS patients, (b) investigate how these two aspects of cord damage are interrelated and (c) assess the correlation of cord MRI metrics with concomitant brain damage and disability. Conventional and DT MRI of the brain and cervical cord were obtained from 42 MS patients and 9 healthy controls at baseline and after a mean follow-up of 2.4 years. At each time-point, we measured: cervical cord lesion number, cross-sectional area, mean diffusivity (MD) and fractional anisotropy (FA). Brain T2 lesion volume, grey matter MD, normal appearing white matter (NAWM) MD and FA, as well as longitudinal normalized percentage brain volume changes were also measured.

In MS patients, cervical cord cross-sectional area (P < 0.001) and FA (P = 0.01) decreased, and cervical cord MD increased (P < 0.001) during follow-up. Cord FA decrease, but not cord cross-sectional area and MD, was significantly higher (P = 0.05) in primary progressive MS patients than in those with either relapsing–remitting or secondary progressive MS. At baseline and follow-up, moderate correlations were found between intrinsic cord diffusivity abnormalities and cord cross-sectional area (r values ranging from 0.34 to 0.58), but not between their changes over time. No cross-sectional and longitudinal correlations were found between these MRI metrics and the number of cord T2-visible lesions. Brain NAWM MD (P = 0.03) and brain volume (P < 0.001) also changed in patients. There was no significant correlation between cord and brain MRI metrics at both time-points, as well as between their changes occurred over the follow-up. Baseline cord cross-sectional area (r = −0.40, P = 0.01) and FA (r = −0.40, P = 0.03) correlated with increase in disability at follow-up.

This study shows that both progressive tissue loss and injury to the remaining tissue occur in the cervical cord of MS patients, and that these two components of cord damage are not strictly interrelated, thus suggesting that a multiparametric MRI approach is needed to achieve more accurate estimates of such a damage. MS cord pathology also seems to be independent of concomitant brain changes, to develop at different rates according to disease phenotype, and to be associated to medium-term disability accrual.

Keywords: multiple sclerosis; cervical cord; magnetic resonance imaging; diffusion tensor; disability

Abbreviations: DE= dual-echo; DT = diffusion tensor; DW = diffusion-weighted; EDSS = Expanded Disability Status Scale; EP = echo planar; ETL = echo train length; FA = fractional anisotropy; Fast-STIR = fast-short-tau inversion recovery; FOV = field of view; FU = follow up; GM = grey matter; LV = lesion volume; MD = mean diffusivity; MP-RAGE = magnetization-prepared rapid acquisition gradient echo; MR = magnetic resonance; MRI = magnetic resonance imaging; MS = multiple sclerosis; NA = normal appearing; NAWM = normal appearing white matter; PBVC = percentage brain volume change; PPMS = primary progressive multiple sclerosis; RRMS = relapsing–remitting multiple sclerosis; SD = standard deviation; SE = spin echo; SENSE = sensitivity encoded; SIENA = Structural Imaging Evaluation of Normalised Atrophy; SPMS = secondary progressive multiple sclerosis; TE = echo time; TR = repetition time; WM = white matter

Introduction
The spinal cord is a common site of pathology in multiple sclerosis (MS) (Ikuta and Zimmermann, 1976) and conventional MRI can detect cord lesions in up to 90% of patients with established disease (Lycklama à Nijeholt et al., 2003). However, conventional MRI is unable to grade the extent of tissue injury within macroscopic lesions, as well as to detect and quantify the ‘occult’ damage known to occur in the normal-appearing tissues of patients with MS (Filippi and Grossman, 2002).

In an attempt to overcome these limitations and to define new MR markers more closely linked to the most disabling pathological features of MS (i.e. irreversible demyelination and neuroaxonal injury), diffusion tensor (DT) MRI has received considerable attention (Rovaris et al., 2005a). The development of a novel DT MRI sequence (Cercignani et al., 2003) has recently made possible to achieve an accurate estimate of the extent of cervical cord damage (Agosta et al., 2005; Valsasina et al., 2005, 2007; Benedetti et al., 2006), which have been shown by cross-sectional studies to correlate with the level of disability associated to demyelinating (Valsasina et al., 2005; Benedetti et al., 2006) and degenerative (Valsasina et al., 2007) conditions. Whereas a few longitudinal DT MRI studies of the brain have been performed in patients with MS (Caramia et al., 2002; Cassol et al., 2004; Schmierer et al., 2004; Oreja-Guevara et al., 2005; Rovaris et al., 2005b), the temporal evolution of cervical cord diffusivity changes has not been investigated yet in these patients.

Against this background, we performed a longitudinal, conventional and DT MRI study of the cervical cord in MS patients to: (a) define the temporal evolution of intrinsic tissue injury and atrophy in the cervical cord from MS patients, (b) investigate how these two aspects of cord damage are interrelated and (c) assess the correlation of cord MRI metrics with concomitant brain damage and disability.

Patients and methods
Patients
We studied 42 patients with MS (Polman et al., 2005) [26 females and 16 males; mean age (SD) = 44.8 (13.2) years; median disease duration (range) = 12 (3–40) years]. Thirteen patients had relapsing–remitting (RRMS), 14 secondary progressive (SPMS) and 15 primary progressive (PPMS) MS (Lublin and Reingold, 1996). In SPMS patients, the mean time since progression was 6.5 (range = 2–17) years. At study entry, 17 patients (nine RRMS, seven SPMS, one PPMS) were receiving an immunomodulatory drug, whereas nine patients (two RRMS, four SPMS, three PPMS) were in immunosuppressive treatment. Nine healthy controls [7 females and 2 males; mean age (SD) = 38.9 (13.2) years] with no history of neurological disorders and a normal neurological exam served as controls. At the time MRI scans were performed, patients were assessed neurologically by a single physician, unaware of the MRI results, and disability was measured using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).

At follow-up, patients were considered clinically worsened if they had an EDSS score increase $\geq 1.0$, when baseline EDSS was $<6.0$ or an EDSS score increase $\geq 0.5$, when baseline EDSS was $\geq 6.0$. The mean follow-up length was 2.4 years (range = 1.5–3.0 years). During the study period, the occurrence of relapses was assessed by the same physician. Local Ethical Committee approval and written informed consent from all individuals were obtained before study initiation.

Image acquisition
MRI scans of the brain and cervical cord from all subjects were obtained at study entry and follow-up, using the same 1.5 Tesla machine, which did not undergo any upgrade during the study period and was under a regular program of maintenance. At follow-up, patients were repositioned following published guidelines (Miller et al., 1991). During each session, the following sequences were acquired:

1. Cervical cord: (a) fast-short-tau inversion recovery (fast-STIR) [repetition time (TR) = 2288 ms, echo time (TE) = 60 ms, inversion time = 110 ms, echo train length (ETL) = 11, field of view (FOV) = 280 × 280 mm², matrix size = 264 × 512, number of signal averages = 4, 8 sagittal slices with thickness = 3 mm and interslice gap = 0.3 mm]; (b) sagittal T1-weighted 3D magnetization-prepared rapid acquisition gradient echo (MP-RAGE) (TR = 9.7 ms, TE = 4 ms, flip angle = 128°, FOV = 280 × 280 mm², slab thickness = 160 mm, number of partitions = 128) and (c) pulsed-gradient, diffusion-weighted (DW) sensitivity encoded (SENSE) single-shot echo planar (EP) (reduction factor = 2, TR = 7000 ms, TE = 100 ms, FOV = 240 × 90 mm², matrix = 128 × 48, echo train = 40 ms, 5 sagittal slices with thickness = 4 mm). This sequence collects 16 images per section, including two images with no diffusion weighting ($b \approx 0$ s/mm²) and 14 images with the same $b$ factor of 900 s/mm², but with gradients applied in different directions. The non-diffusion-weighted images are needed to compute the DT, and the gradient orientations were chosen according to the algorithm proposed by Jones et al. (1999), which was designed to optimize DT MRI acquisition. The measurement was repeated four times to improve the signal-to-noise ratio. Three saturation bands were used, positioned in the anterior part of the neck and transversely at the edges of the FOV in the superior–inferior direction. A detailed description of this sequence is given elsewhere (Cercignani et al., 2003).

2. Brain: (a) dual-echo (DE) turbo spin echo (SE) (TR = 3300 ms, TE = 16/98 ms, ETL = 5); (b) T1-weighted conventional SE (TR = 768 ms, TE = 14 ms) and (c) pulsed-gradient DW EP (inter-echo spacing = 0.8 ms, TE = 123 ms, with diffusion-weighting gradients applied in eight different directions). As in the cervical cord, only two $b$ factors were used: $b_1 \approx 0$ and $b_2 = 1044$ s/mm² (Bito et al., 1995). For the brain DE and T1-weighted sequences, 24 contiguous axial slices were acquired with 3 mm slice thickness, 256 × 256 matrix and 250 × 250 mm² FOV. The slices were positioned to run parallel to a line that joins the most infero-anterior and infero-posterior parts of the corpus callosum. For the brain DW scans, 10 axial slices with 5 mm slice thickness, 128 × 128 matrix and 250 × 250 mm² FOV were acquired, with the same orientation as the DE scans, and with the
Medians EDSS at baseline (range) 4.0 (1.0–7.5) 2.0 (1.0–4.0) 5.5 (3.0–7.5) 5.5 (3.0–7.0)
Median EDSS at FU (range) 5.0 (1.0–8.5) 1.5 (1.0–4.0) 6.0 (3.0–8.5) 6.0 (3.0–8.0)

Abbreviations: RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; SD = standard deviation; EDSS = Expanded Disability Status Scale; FU = follow-up.

Table 1 Main demographic and clinical characteristics of patients at baseline and follow-up

<table>
<thead>
<tr>
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<th>All patients</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
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<tr>
<td>Number of patients</td>
<td>42</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Males/females</td>
<td>16/26</td>
<td>5/8</td>
<td>3/11</td>
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<tr>
<td>Mean age (SD) (years)</td>
<td>44.8 (13.2)</td>
<td>33.4 (5.5)</td>
<td>44.1 (10.6)</td>
<td>55.4 (12.0)</td>
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<td>Median disease duration (range) (years)</td>
<td>12 (3–40)</td>
<td>9 (3–16)</td>
<td>16 (11–40)</td>
<td>11 (3–35)</td>
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<td>2.0 (1.0–4.0)</td>
<td>5.5 (3.0–7.5)</td>
<td>5.5 (3.0–7.0)</td>
</tr>
<tr>
<td>Median EDSS at FU (range)</td>
<td>5.0 (1.0–8.5)</td>
<td>1.5 (1.0–4.0)</td>
<td>6.0 (3.0–8.5)</td>
<td>6.0 (3.0–8.0)</td>
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Longitudinal cord DT MRI changes in MS

Brain (2007), 130, 2211–2219 2213

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<td>2.0 (1.0–4.0)</td>
<td>5.5 (3.0–7.5)</td>
<td>5.5 (3.0–7.0)</td>
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<tr>
<td>Median EDSS at FU (range)</td>
<td>5.0 (1.0–8.5)</td>
<td>1.5 (1.0–4.0)</td>
<td>6.0 (3.0–8.5)</td>
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</tr>
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Abbreviations: RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; SD = standard deviation; EDSS = Expanded Disability Status Scale; FU = follow-up.

Results

No abnormalities were seen on the conventional brain and cervical cord MR images obtained from healthy controls. During the study period, all MRI metrics from healthy controls remained stable (data not shown).

In the whole cohort of patients, median EDSS scores were 4.0 (range = 1.0–7.5) at baseline and 5.0 (range = 1.0–8.5) at follow-up. During the follow-up, 7 RRMS patients experienced 14 relapses. All relapses were treated with intravenous methylprednisolone (1 g/d for 3–5 days without tapering). In these patients, follow-up MRI scans were always obtained at least 3 months after the attack. During the study period, in one SPMS and in three RRMS patients the treatment was changed from an immunomodulatory to an immunosuppressive drug. In one PPMS patient the immunosuppressive treatment was stopped because of intolerance. At follow-up 14 patients (six SPMS and eight PPMS) were considered clinically worsened. The main demographic and clinical characteristics of the three groups of patients at study entry and follow-up are presented in Table 1.

At baseline, 84 cervical cord lesions were found in 33 patients (79%, median number of lesions/patient = 2, range = 0–5). At follow-up, eight patients (three RRMS, three SPMS and two PPMS) had at least one new cord lesion (six patients had one new cord lesions and two patients had two new cord lesions). Table 2 reports cervical cord cross-sectional area and DT MRI histogram-derived quantities for MS patients at baseline and follow-up.

Statistical analysis

The baseline and follow-up values of conventional and DT MRI-derived metrics were compared using a repeated measures analysis of variance (ANOVA) model, adjusted for age and, in case of patients, clinical phenotype. Cross-sectional area of the cervical cord was also used as a covariate in the comparison between baseline and follow-up DT MRI quantities of the cervical cord. The percentage changes over time of MRI quantities were adjusted for age and cord cross-sectional area whenever needed. A ‘time × group’ interaction analysis was performed to assess the heterogeneity of MRI changes over time among clinical phenotypes; the results were displayed using forest plots. Univariate correlations were assessed using the Spearman Rank Correlation Coefficient. An univariate logistic regression model, adjusted for follow-up duration, was used to investigate the role of clinical and MRI quantities as independent predictors of the probability to have an EDSS worsening at follow-up.

Image analysis

All MRI post-processing was performed by two experienced observers by consensus, unaware to whom the scans belonged. Cervical cord MS lesions were first identified on the hardcopies of fast-STIR scans. Then, fast-STIR scans were reviewed in a chronological order and the new lesions seen on follow-up images counted. After off-line reconstruction of cervical cord DT MRI data, cervical cord mean diffusivity (MD) and fractional anisotropy (FA) maps were derived and MD and FA histograms produced, as previously described (Valsasina et al., 2005). For each histogram, only the average MD and FA were a priori chosen to enter the analysis, to minimize the number of comparisons and hence reduce the risk of type I error. Cervical cord cross-sectional area was measured using a semi-automated technique developed by Losseff et al. (1996).

Following the identification of brain T2-hyperintense lesions on DE scans, T2 lesion volume (LV) was measured using a computer-assisted technique (Rovaris et al., 1997). Brain MD and FA maps were derived for every pixel, as for the cord. Using SPM2 and maximum image in-homogeneity correction (Ashburner and Friston, 1997), brain grey matter (GM), white matter (WM) and CSF were automatically segmented from the DE images. Each pixel was classified as GM, WM or CSF, dependent on which mask had the greatest probability at that location. This generated mutually exclusive masks for each tissue, which were superimposed onto the MD and FA maps (on which hyperintense lesions were masked out previously), and the corresponding MD histograms of the NAWM and GM and FA histogram of NAWM produced (Rovaris et al., 2005b). FA histograms were derived only for the NAWM, since no preferential direction of water molecular motion is expected to occur in GM, due to the absence of a microstructural anisotropic organization of this compartment. As for the cord, only the average MD and FA entered the analysis. Longitudinal percentage brain volume change (PBVC) was calculated using brain T1-weighted images and the Structural Imaging Evaluation of Normalised Atrophy (SIENA) software (Smith et al., 2002).

Eleven patients stopped because of intolerance. At follow-up 14 patients (seven SPMS and four PPMS) had at least one new cord lesion (six patients had one new cord lesion and two patients had two new cord lesions). Table 2 reports cervical cord cross-sectional area and DT MRI histogram-derived quantities for MS patients at baseline and follow-up.
Table 2  Cervical cord conventional and DT MRI findings at baseline and follow-up in MS patients

<table>
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<tr>
<th></th>
<th>All patients</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area—baseline (mm³)</td>
<td>Mean (SD)</td>
<td>76.5 (11.6)</td>
<td>83.1 (11.9)</td>
<td>71.6 (11.6)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>49.8–101.7</td>
<td>579–101.7</td>
<td>498–95.7</td>
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<tr>
<td>Cross-sectional area—FU (mm³)</td>
<td>Mean (SD)</td>
<td>72.9 (10.8)</td>
<td>77.7 (9.7)</td>
<td>69.4 (12.5)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>46.7–96.2</td>
<td>54.8–96.2</td>
<td>46.7–90.0</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average MD—baseline (×10⁻³ mm²/s)</td>
<td>Mean (SD)</td>
<td>1.26 (0.13)</td>
<td>1.22 (0.11)</td>
<td>1.30 (0.17)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
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<td>1.04–1.38</td>
<td>0.95–1.57</td>
</tr>
<tr>
<td>Average MD—FU (×10⁻³ mm²/s)</td>
<td>Mean (SD)</td>
<td>1.37 (0.12)</td>
<td>1.34 (0.16)</td>
<td>1.38 (0.11)</td>
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<td></td>
<td>Range</td>
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<td>1.08–1.73</td>
<td>1.19–1.50</td>
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<tr>
<td>P</td>
<td></td>
<td>&lt;0.001</td>
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</table>

*Adjusted for age (see text for further details).

Abbreviations: RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; SD = standard deviation; FU = follow-up; MD = mean diffusivity; FA = fractional anisotropy.

In Fig. 1, the forest plot of the same quantities is presented for the overall sample of patients and for each of the three disease phenotypes. The x-axis reports magnetic resonance imaging metrics percentage changes. The bars represent the 95% confidence intervals of measurements. Note that the forest plot for FA does not necessarily show the same trend as the histogram-derived quantities for MS patients at baseline and follow-up. In Fig. 2, the forest plot of the same quantities is presented for the overall sample of patients and for each of the three disease phenotypes. An increase of average NAWM MD (P = 0.03), which was independent of clinical phenotype (P for ‘time × group’ interaction = 0.35), was found. Brain NAWM FA significantly decreased only in SPMS patients (P for ‘time × group’ interaction = 0.02).

In Fig. 1, the forest plot of the same quantities is presented for the overall sample of patients and for each of the three disease phenotypes. Cord area decreased at follow-up (P < 0.001) at a similar rate in all the three patient groups (P for ‘time × group’ interaction = 0.44). An increase of cord average MD (P < 0.001) and a decrease of average cervical cord FA (P = 0.01) were also observed in the patient cohort; the MD increase was again independent of clinical phenotype (P for ‘time × group’ interaction = 0.80), whereas the FA decrease was associated to patient age (P for ‘time × age’ interaction = 0.02, being higher for younger patients) and clinical phenotype (P for ‘time × group’ interaction = 0.05), being higher in PPMS patients than in the other two groups. As shown in Fig. 1, the mean FA change (estimated at the average age for each patient group) was −4.2% (standard error = 2.9%) for PPMS, +1.1% (standard error = 4.2) for SPMS and −0.2% (standard error = 2.8%) for RRMS patients. This did not change after adjusting for disease duration (data not shown). Also, none of the conventional and DT MRI changes differed significantly between patients with and without relapses during follow-up or between patients receiving or not receiving disease-modifying treatment (data not shown).

Brain T2 LV did not change significantly over the study period [mean T2 LV (SD) was 17.7 (16.6) ml at baseline and 18.3 (16.2) ml at follow-up]. The mean PBVC was −1.40 (SD = 1.15). Table 3 reports brain DT MRI histogram-derived quantities for MS patients at baseline and follow-up. In Fig. 2, the forest plot of the same quantities is presented for the overall sample of patients and for each of the three disease phenotypes. An increase of average NAWM MD (P = 0.03), which was independent of clinical phenotype (P for ‘time × group’ interaction = 0.35), was found. Brain NAWM FA significantly decreased only in SPMS patients (P for ‘time × group’ interaction = 0.02).

At baseline and follow-up, average MD and FA of the cervical cord did not correlate with the number of cord lesions seen at the two time-points and their changes over time did not differ significantly between patients with and without new cord lesions at follow up (r values ranged from −0.15 to 0.23). Baseline average MD and FA of the cervical cord correlated with cervical cord area at baseline (MD: r = −0.34, P = 0.03; FA: r = 0.58, P < 0.001).
Table 3  Brain DT MRI changes at baseline and follow-up in MS patients

<table>
<thead>
<tr>
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<th>All patients</th>
<th>RRMS</th>
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<tbody>
<tr>
<td>NAWM average MD—baseline (×10⁻³ mm²/s) Mean (SD)</td>
<td>0.86 (0.06)</td>
<td>0.85 (0.05)</td>
<td>0.87 (0.09)</td>
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<tr>
<td>Range</td>
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<td>NAWM average MD—FU (×10⁻³ mm²/s) Mean (SD)</td>
<td>0.88 (0.11)</td>
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<td>0.92 (0.16)</td>
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<td>0.80–1.36</td>
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<tr>
<td>P</td>
<td>0.03</td>
<td>0.26</td>
<td>0.35</td>
<td>0.26</td>
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<tr>
<td>GM average MD—baseline (×10⁻³ mm²/s) Mean (SD)</td>
<td>1.11 (0.11)</td>
<td>1.09 (0.10)</td>
<td>1.13 (0.15)</td>
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<td>GM average MD—FU (×10⁻³ mm²/s) Mean (SD)</td>
<td>1.10 (0.11)</td>
<td>1.08 (0.09)</td>
<td>1.13 (0.16)</td>
<td>1.08 (0.04)</td>
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<tr>
<td>P</td>
<td>0.10</td>
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<td>0.26</td>
<td>0.26</td>
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<td>NAWM average FA—baseline Mean (SD)</td>
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<td>0.28 (0.02)</td>
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<td>Range</td>
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<td>P</td>
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Abbreviations: RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; NAWM = normal-appearing white matter; MD = mean diffusivity; SD = standard deviation; FU = follow-up; GM = grey matter; FA = fractional anisotropy.

Fig. 2  Forest plot of the ‘time × group’ interaction analysis of brain diffusivity changes over time, for the overall sample of multiple sclerosis patients and for each of the three disease phenotypes. The x-axis reports magnetic resonance imaging metrics percentage changes. The bars represent the 95% confidence intervals of measurements. Note that when the 'time × group' interaction effect was not significant, we cannot exclude that the diffusivity differences between groups are just a chance finding. Abbreviations: RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; NAWM = normal appearing white matter; MD = mean diffusivity; SD = standard deviation; FU = follow-up; GM = grey matter; FA = fractional anisotropy.

Discussion

Despite post-mortem studies have convincingly shown that cord atrophy does not reflect the actual extent of irreversible tissue damage of this structure (Ganter et al., 1999; Bergers et al., 2002; Bot et al., 2004; DeLuca et al., 2004, 2006), possibly because of the ‘counterbalancing’ effect of inflammatory and gliotic changes in the vicinity of axonal pathology (Lycklama à Nijeholt et al., 2001; Mottershead et al., 2003), most of the available in vivo MRI studies of MS-related cord injury are still based only on measures of cord atrophy (Filippi et al., 1996; Lossieff et al., 1996; Lycklama à Nijeholt et al., 1998; Stevenson et al., 1998a, b, 2000; Rovaris et al., 2001; Ingle et al., 2003; Lin et al., 2003; Sastre-Garriga et al., 2005). Only a few MRI studies have attempted to assess the extent of intrinsic cord damage, using either MT (Bozzali et al., 1999; Filippi et al., 2000; Rovaris et al., 2000) or DT (Agosta et al., 2005; Valsasina et al., 2005; Benedetti et al., 2006; Hesselink et al., 2006) MRI. None of these latter studies was,
however, longitudinal and thus did not provide any clue on the dynamics of intrinsic cord damage associated to MS and how this relates to the development of cord atrophy and disability. This study shows that MS patients not only experience a progressive cord atrophy, a finding which is consistent with those of previous longitudinal studies (Stevenson et al., 1998b, 2000; Lin et al., 2003; Ingle et al., 2003; Sastre-Garriga et al., 2005), but also progressive structural changes in the residual cervical cord tissue. It also shows that such changes can be monitored by the use of serial DT MRI scans and that their assessment may be important to achieve a more complete picture of the mechanisms associated to the development of irreversible tissue loss and disability in patients with MS.

With this in mind, a major issue to be addressed is to attempt to define the possible pathological substrates of the observed progressive change of cervical cord water diffusivity in MS. There is now compelling evidence, based on post-mortem studies, that in addition to demyelination (Lycklama à Nijeholt et al., 2001; Bergers et al., 2002; Mottershead et al., 2003; Bot et al., 2004; De Luca et al., 2006), axonal loss is a major feature of cord T2-visible lesions and normal-appearing tissue (Ganter et al., 1999; Bjartmar et al., 2000; Lovas et al., 2000; Bergers et al., 2002; De Luca et al., 2004; Evangelou et al., 2005; De Luca et al., 2006). Post-mortem assessment also showed that axonal loss in patients with MS is more pronounced in the cervical than in other portions of the cord (De Luca et al., 2004) and that small diameter nerve fibres are more severely injured by the pathological process, with large fibres being relatively preserved (Ganter et al., 1999; DeLuca et al., 2004). Demyelination and axonal loss may result in an increased extracellular space between surviving fibres, which, however, can harbour cell debris, inflammatory infiltrates, fibrillary gliosis and astrocytic proliferation (Lycklama à Nijeholt et al., 2001). Finally, although only a few post-mortem studies assessed cord GM damage in MS (Lycklama à Nijeholt et al. 2004; Bot et al., 2004; Gilmore et al., 2005, 2006), it is conceivable, as it has been shown for the brain (Kidd et al., 1999; Evangelou et al., 2000; Peterson et al., 2001; Geurts et al., 2005), that loss of neurons, secondary to GM discrete lesions or to axonal transection in the WM, may also occur. All these pathological changes have the potential to alter the diffusivity properties of the cervical cord. Increased extracellular spaces (due to axonal and neuronal loss), as well as an increased proportion of large diameter fibres can indeed encourage water diffusion and lead to an increased average MD. Intracellular abnormalities of the surviving axons with a consequent reduced anisotropy of intracellular diffusion (Pierpaoli et al., 1996), and the formation of ‘new’ barriers, due to cell debris, inflammatory infiltrates and gliosis, which restrict water molecular motion isotropically, can result in a reduced average FA. Our findings, therefore, suggest that the progressive increase of MD and decrease of FA in the cervical cord of MS patients during follow-up might reflect a net loss of structural barriers to water molecular motion and fibre bundle coherence, which in turn are likely to be the result of axonal pathology. This notion is also supported by a high-field study of post-mortem MS spinal cords (Mottershead et al., 2003), which demonstrated that axonal density correlated with decreased diffusion anisotropy and, although weakly, increased apparent diffusion coefficient.

Although the development of cord atrophy, which occurred in our sample during the follow-up, would increase the likelihood of partial volume effect from the CSF and hence result in increased diffusivity changes at follow-up compared to baseline, we do not believe that the observed progressive alteration of DT MRI metrics is influenced a great deal by contamination of pixels at the edge of the cord. This is because of at least four reasons. First, cord diffusivity changes over follow-up were not significantly associated to the concomitant decrease of cord area. Second, since atrophy developed at a similar rate in all the three patient groups, one would have expected, in case of a significant partial volume effect, to see a similar rate of diffusivity changes in all three groups, but this was not the case at least for FA. Third, we ran the analysis of DT MRI changes over time with correction for cord area changes. Finally, contamination from the CSF was further minimized by considering in the analysis only pixels away from the edge of the cord (Valsasina et al., 2005).

Another important issue to be addressed is how much of the axonal pathology in the cervical cord of MS patients is due to local damage and how much is due to retrograde and anterograde degeneration of neurons secondary to the injury of fibres traversing WM lesions of the brain (Evangelou et al., 2000). Although this study can only give a partial answer to this question, it is likely that ‘distant’ pathology (i.e. axonal damage followed by anterograde/retrograde degeneration) is not a major contributor of the observed cord diffusivity changes. This notion is supported by the lack of a correlation between cord and brain MRI metrics found in the present study, which agrees with previous pathological (DeLuca et al., 2004, 2006) and cross-sectional MRI (Lycklama à Nijeholt et al., 1998; Rovaris et al., 2000; Agosta et al., 2005; Valsasina et al. 2005) reports. We also did not find a significant correlation between the extent of cervical cord T2 lesions and diffusivity alterations in these patients. This agrees with the results of post-mortem studies, showing that axonal damage and cord atrophy occur largely independent of local areas of inflammatory demyelination (Bjartmar et al., 2000; Bergers et al., 2002; DeLuca et al., 2006). Therefore, our data support the notion that inflammatory demyelination (local and distant) may not be the only causative factor of intrinsic diffusivity changes of the cervical cord seen in patients with MS and that the nature of the process may be one of neurodegeneration wherein an underlying diffuse axonopathy, independent of inflammatory demyelination, contributes significantly to axonal loss in the cord and
hence to diffusivity changes. Clearly, since post-mortem studies showed variable degrees of correlation between plaque load and axonal loss in different brain and cord tracts (Evangelou et al., 2000; DeLuca et al., 2006) and since it is at present not possible to obtain reliable in vivo MRI estimates of specific tract pathology in the cord, we cannot exclude that both processes (i.e. inflammatory demyelination and diffuse axonopathy) may operate to varying extent in different patients and in different tracts.

Another novel finding of this study was the demonstration that intrinsic cord diffusivity abnormalities at baseline correlate moderately with the severity of cord atrophy at follow-up. This suggests that intrinsic cord abnormalities precede the development of cord atrophy. Demyelinated and partially degenerated axons, as well as sublethally injured and functionally impaired neurons may cause diffusivity changes that would not necessarily result in cord atrophy. This is also supported by the observation that, during the follow-up, DT MRI changes over time did not correlate with the concomitant changes of cord atrophy, which indicates that a relatively long period of time may be needed for intrinsic cord changes to result in irreversible tissue loss or, alternatively but not mutually exclusively, for irreversible tissue loss to develop at a magnitude enough to overcome the ‘masking’ effect of inflammatory infiltrates and gliosis on MRI measures of cord atrophy. Although additional longitudinal multiparametric MRI studies of early RRMS patients with longer follow-up periods are needed to better elucidate the dynamics of cord atrophy evolution in MS, the possibility of detecting with DT MRI the presence of incipient and potentially reversible intrinsic cord changes opens up new venues for treatment monitoring in MS.

We also investigated whether the dynamics of cord damage vary among the three major clinical phenotypes of the disease. We found that, whereas cord cross-sectional area and average MD evolve at similar rates in all patient groups, average cord FA declines more rapidly in patients with PPMS than in those with RRMS and SPMS, thus suggesting a more pronounced loss of cord fibres integrity in the former group. PPMS patients are typically characterized by severe locomotor disability despite the paucity of brain abnormalities (Thompson et al., 2000). As a consequence, our findings strengthen the notion that the ‘preferential’ involvement of the cervical cord in PPMS patients (Lycklama à Nijeholt et al., 1998; Filippi et al., 2000) may, at least partially, explain the discrepancy between brain MRI and clinical findings and the disproportion between the severity of locomotor disability and the less-pronounced impairment of other functional systems (Thompson et al., 2000). The observed faster rate of cervical cord damage development in PPMS patients may also contribute to explain why progressive patients from onset reach disability milestones in a fraction of the time needed to patients with an exacerbating-remitting initial MS course to accrual similar levels of disability (Confavreux and Vukusic, 2006).

This study also showed that cross-sectional area and FA of the cervical cord at baseline were associated not only to the baseline EDSS score, but also to the accrual of disability over the follow-up. Interestingly, this was not found to be the case for any of the brain MRI-derived quantities measured in the present study. The role of cord area in predicting the accumulation of disability in MS patients has been already highlighted by previous cross-sectional (Filippi et al., 1996; Losseff et al., 1996; Lycklama à Nijeholt et al., 1998; Stevenson et al., 1998a; Rovaris et al., 2001) and longitudinal (Ingle et al., 2003; Lin et al., 2003; Sastre-Garriga et al., 2005) MRI studies. This study adds to this by showing that the assessment of intrinsic cord pathology may be a rewarding exercise to improve our ability to predict medium-term evolution of disability. This is particularly true when considering that the magnitude of the correlation between cord cross-sectional area and FA is moderate, thus suggesting that these two metrics convey pieces of information that only partially overlap. The fact that cord FA and not cord MD is associated to the subsequent clinical evolution of the disease may be due to the fact that all possible MS pathological substrates of cord damage (i.e. inflammatory infiltrates and oedema, cell debris, demyelination, axonal loss and injury, neuronal loss and astrocytic proliferation) do concur to FA reduction, whereas their impact on MD is likely to be variable, with some of them leading to decreased MD values (i.e. inflammatory oedema, demyelination, axonal loss and injury and neuronal loss) and others leading to MD ‘pseudonormalization’ (i.e. inflammatory infiltrates, cell debris, astrocytic proliferation, fibrillary gliosis and shrinkage of the tissue).

The absence of a significant correlation between cord MRI and disability changes over time, as well as the finding that patients with and without a significant EDSS score increase during the follow-up did not differ in terms of concomitant changes of cord DT MRI metrics, albeit possibly disappointing, were not unexpected for a number of reasons. First, at least in the less-disabled patients, an increase in the EDSS score might just reflect a worsening of clinical signs, which may not be associated to an accumulation of ‘fixed’ disability. Second, EDSS score has several limitations, including a relatively poor sensitivity to disease-related changes (Hobart et al., 2004). Third, some of our patients (e.g. two patients with SPMS and a relatively low EDSS at study entry) might not have been fully representative of the ‘classical’ course of the disease. Finally and most importantly, it is conceivable that pathological changes occurring at one stage of the disease may give rise to an immediate change of the MR properties of the injured tissues, but result in a measurable clinical impact at a later stage. These considerations would call for future longitudinal studies with a longer follow-up duration on patients with a more homogeneous
clinical phenotype, possibly recruited from the clinical onset of the disease.

References


