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## **A DIFFUSION TENSOR MRI STUDY OF CERVICAL CORD DAMAGE IN BENIGN AND SECONDARY PROGRESSIVE MS PATIENTS**

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## Abstract

**Background.** Diffusion tensor (DT) MRI enables to quantify the severity of brain and cervical cord pathology in MS.

**Objective.** To investigate the DT MRI patterns of cervical cord damage in benign MS (BMS) and secondary progressive MS (SPMS) patients, **in order** to achieve a better understanding of the mechanisms underlying the development of irreversible disability in MS.

**Methods.** Conventional and DT MRI scans of the cervical cord and brain were acquired from 40 BMS patients, 28 SPMS patients and 18 healthy individuals. Cervical cord and brain mean diffusivity (MD) and fractional anisotropy (FA) maps were created and average MD and FA were calculated. Cross-sectional cord area (CSA) was also computed.

**Results.** Thirty-seven (92%) BMS patients and all (100%) SPMS patients had macroscopic cervical cord lesions. Compared to healthy individuals, BMS patients had higher average cord MD, while SPMS patients had higher average cord MD, lower average cord FA and lower average CSA. Compared to BMS patients, SPMS patients had lower cord average FA and lower average CSA. In MS patients, expanded disability status scale (EDSS) was correlated with CSA ( $r=-0.47$ ,  $p<0.0001$ ), average cord FA ( $r=-0.37$ ,  $p=0.002$ ) and brain T2 lesion volume (LV) ( $r=0.34$ ,  $p=0.005$ ). A multivariate regression model identified CSA, average cord FA and brain T2 LV as variables influencing independently the EDSS score ( $r=0.58$ ,  $p<0.0001$ ).

**Conclusions.** **Cervical cord damage outside focal macroscopic lesions is limited in patients with BMS.** The assessment of cord and brain pathology provides complementary information to improve the understanding of disability accumulation in MS.

## Introduction

Spinal cord damage is likely to be one of the major determinants of disability in patients with multiple sclerosis (MS).<sup>1-4</sup> However, despite the presence of macroscopic lesions in the spinal cord, some patients show a particularly favourable course of the disease, with mild or no accumulation of disability several years after the clinical onset. This condition, termed benign MS (BMS), has an estimated prevalence in cross-sectional surveys that ranges from 6 to 64%, depending on the criteria used to define it.<sup>5-7</sup> At present, the most accepted consensus definition of BMS is that of “a disease in which the patient remains fully functional in all neurological systems 15 years after disease onset”.<sup>8</sup>

During the past decade, due to the paucity of correlation between conventional MRI measures and disability,<sup>9</sup> quantitative MR-based techniques have been increasingly applied for the assessment of central nervous system (CNS) involvement in patients with MS. Among these techniques, diffusion tensor (DT) MRI has been demonstrated to be able to grade the severity of brain and cervical cord pathology in MS and other neurological conditions.<sup>10-14</sup> DT MRI enables the random diffusional motion of water molecules to be measured, and provides quantitative indices of the structural and orientational features of tissues.<sup>15</sup> Such indices include mean diffusivity (MD), which is a directionally averaged measure of the apparent diffusion coefficient, and fractional anisotropy (FA), which summarizes the orientational dependence of diffusivity. The pathological elements of MS have the potential to alter the permeability and geometry of structural barriers to water molecular diffusion in the CNS. While a few studies have applied DT MRI for the quantification of the extent and distribution of microscopic disease-related damage in the brain in patients with BMS,<sup>16-19</sup> no study has been performed to assess the extent of cervical cord involvement in these patients. Based on the criteria used for the definition of such a disease phenotype, it is tempting to speculate that the relative structural integrity of the cervical spinal cord might be among the factors contributing to limit accumulation of disability over long periods of time. To test this hypothesis, we applied DT

MRI to investigate the pattern of cervical cord damage in a relatively large sample of patients with BMS. As a control group we selected patients with secondary progressive (SP) MS with, by definition, a higher EDSS score, in whom the extent of spinal cord involvement is likely to have an important role in the accumulation of irreversible disability.

## **Patients and methods**

a) Patients. Forty **consecutive** patients with BMS and 28 patients with SPMS were studied. Criteria for a diagnosis of BMS were an EDSS score  $\leq 3.0$  and a disease duration  $\geq 15$  years.<sup>8</sup> Patients with MS, a SP course<sup>8</sup> and persistent walking limitations (i.e., a confirmed EDSS score of 4.0 or higher), were enrolled to serve as controls. To be included, patients had to be relapse- and steroid-free for at least three months **before MRI acquisition**. All patients underwent a complete neurological examination, with rating of the Expanded Disability Status Scale (EDSS) score<sup>20</sup> within 24 hours from the MRI scanning session. The main clinical and demographic characteristics of the study groups are reported in Table 1. Age did not differ between the three groups of subjects. Disease duration was longer in BMS *vs.* SPMS patients ( $p=0.002$ ) and the median EDSS score was lower in BMS *vs.* SPMS patients ( $p<0.001$ ).

Twenty-five BMS patients were not receiving any disease-modifying treatment, 8 were being treated with beta-interferon, 6 with glatiramer acetate and one with azathioprine. Eight SPMS patients were not receiving any disease-modifying treatment, 14 were being treated with beta-interferon, and 6 with azathioprine. Eighteen healthy individuals **with no previous history of neurological, major medical, and psychiatric disorders, as well as of alcohol or drug abuse and a normal neurological exam** were also studied (Table 1). All subjects signed a written informed consent prior to study entry and the study design was approved by the local Ethical Committee.

b) Image acquisition. Using a 1.5 Tesla system, the following pulse sequences were acquired from the cervical cord of all subjects, using a tailored cervical spine phased array coil for signal reception: 1) fast-short-tau inversion recovery (fast-STIR) (TR=2288, TE=60, TI=110,

echo train length=11, field of view [FOV]=280x280 mm, matrix size=264x512, number of signal averages=4; number of slices=8; sagittal plane; slice thickness=3 mm; inter-slice gap=0.3 mm); 2) pulsed-gradient, DT sensitivity encoded (SENSE) single-shot echo planar imaging (EPI) (reduction factor, R=2; TR=7000, TE=100; FOV=240x90 mm; matrix=128x48; echo train=40 ms; number of slices=5; sagittal plane; slice thickness=4 mm). This sequence collects 16 images per section, including two images with no diffusion weighting ( $b=0$  s/mm<sup>2</sup>) and 14 images with the same  $b$  factor of 900 s mm<sup>-2</sup>, but with gradients applied in different directions.<sup>10-14</sup> 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.,<sup>21</sup> which was designed to optimize DT MRI acquisition. The measurement was repeated four times to improve the signal-to-noise ratio (SNR). 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;<sup>10-14</sup> 3) sagittal T1-weighted 3D magnetization-prepared rapid acquisition gradient echo (MP-RAGE) (TR=9.7, TE=4, flip angle=12°, slab thickness=160 mm, FOV=280x280 mm, number of partitions=128).

In the same scanning session, the following pulse sequences were used to image the brain: 1) dual-echo turbo spin echo (TSE) (TR=3300, TE=16-98, echo train length = 5), and 2) pulsed-gradient DT EPI (inter-echo spacing = 0.8, TE = 123), with DT gradients applied in 8 different directions.<sup>14</sup> As for cervical cord imaging, only two  $b$  factors were used:  $b_1=0$ , and  $b_2=1044$  s mm<sup>-2</sup>. For the TSE, 24 contiguous interleaved axial slices were acquired with 5 mm slice thickness, 256x256 matrix and 250x250 mm 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 DT scans, 10 axial slices with 5 mm slice thickness, 128x128 matrix and 250x250 mm FOV were acquired, with the same orientation as the dual echo scans, and with the second-last caudal slice positioned to match exactly the central slices of the dual-echo set. This brain portion was chosen, since these central slices are less affected by the distortions due to B<sub>0</sub> field

inhomogeneity, which can affect image co-registration, and because they cover the brain regions most frequently involved by MS pathology.

c) Image analysis. Cervical cord hyperintense lesions were identified on the fast-STIR scans. After off-line reconstruction of cervical cord DT MRI data, cervical cord MD and FA maps were derived and MD and FA histograms produced, as previously described.<sup>10-14</sup> 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 errors.

Reformatting of the original MP-RAGE data was performed using the standard, vendor-supplied multiplanar reformatting software available on the operator's console of the scanner. For each subject, a set of five contiguous, 3-mm thick axial slices (perpendicular to the spinal cord) was reconstructed using the centre of the C2-C3 disc as the caudal landmark. Then, a semi-automated technique developed by Losseff et al.<sup>22</sup> was used to segment the cord tissue and to measure the cross-sectional cord area (CSA) at the level of each slice. Values from the five slices were averaged to obtain a single value for each subject, as previously described.<sup>10, 14</sup>

Brain T2 lesion volume (LV) was measured using a computer-assisted technique, as previously described.<sup>10-14</sup> Brain MD and FA maps were derived for every pixel, as for the cord, and MD and FA histograms of the normal-appearing brain tissue (NABT) were produced, after masking of T2-visible lesions, as previously reported.<sup>10, 14</sup> As for the cord, only average MD and FA entered the analysis.

d) Statistical analysis. An analysis of variance model was used to compare conventional and DT MRI-derived measures from healthy individuals, BMS and SPMS patients. Univariate correlations were assessed using the Spearman Rank Correlation Coefficient (SRCC). **To address group membership (BMS vs. SPMS), logistic regression analysis was run.** A multivariate linear regression model was used to identify MRI variables independently correlated with EDSS. To this end, all the MRI variables significantly different between groups were included in the model. The variables retained in the final model were correlated with the

EDSS using the SRCC. Considering that the definition of BMS is also based on disease duration, a multivariate regression model was also used to identify MRI variables independently correlated with the rate of disability accrual defined as EDSS/disease duration. All statistical analyses were performed using the SPSS software.

## Results

In Table 2, cervical cord MRI-derived metrics from patients and healthy volunteers are reported. No macroscopic abnormalities were seen on the cervical cord MR images from healthy individuals. Cord lesions were identified on fast-STIR images in 37 (92%) BMS patients (median number of lesions=2, range=1-6) and in all (100%) SPMS patients (median number of lesions=2, range=1-5). Compared to healthy individuals, BMS patients had higher average cord MD ( $p=0.009$ ), and SPMS patients higher average cord MD, lower average cord FA and lower average CSA ( $p<0.0001$ , for all the comparisons). Finally, compared to BMS patients, SPMS patients had lower cord average FA ( $p=0.01$ ) and lower average CSA ( $p<0.0001$ ).

No macroscopic T2-visible lesions were identified on brain T2-weighted scans from healthy individuals. Brain T2 LV was significantly higher in SPMS than BMS patients. MD and FA metrics of the NABT were significantly heterogeneous between the three study groups (Table 2). At post-hoc analysis, NABT average MD was significantly higher in SPMS patients in comparison to both healthy individuals ( $p=0.002$ ) and BMS patients ( $p=0.04$ ). Similarly, NABT average FA was lower in SPMS patients in comparison to healthy individuals ( $p<0.0001$ ) and BMS patients ( $p=0.02$ ). BMS patients had lower NABT average FA in comparison to healthy individuals ( $p<0.0001$ ).

The logistic regression analysis identified CSA ( $p=0.007$ , odds ratio=0.91) and T2LV ( $p=0.01$ , odds ratio=1.05) as variables associated with the risk of having SPMS vs. BMS.

In MS patients, EDSS was correlated with cord CSA ( $r=-0.47$ ,  $p<0.0001$ ), average cord FA ( $r=-0.37$ ,  $p=0.002$ ), and brain T2 LV ( $r=0.34$ ,  $p=0.005$ ) (Figure). No correlation was found

between the number of cervical cord lesions and EDSS. Cord CSA was correlated with brain T2 LV ( $r=-0.32$ ,  $p=0.007$ ), NABT average FA ( $r=0.27$ ,  $p=0.03$ ), and NABT average MD ( $r=-0.29$ ,  $p=0.02$ ).

A multivariate regression model with EDSS as the dependent variable was run, including cord CSA, cord average MD, cord average FA, brain T2 LV, NABT average MD and NABT average FA, as independent variables. The final model retained CSA ( $p=0.001$ ,  $\beta=-0.38$ , standard error [SE]=0.02, partial correlation=-0.39), average cord FA ( $p=0.05$ ,  $\beta=-0.21$ , SE=0.004, partial correlation=-0.23), and brain T2 LV ( $p=0.04$ ,  $\beta=0.21$ , SE=0.01, partial correlation=0.24) as variables influencing independently the EDSS score. The  $r$  value of the correlation between the EDSS score and the composite score obtained combining these three variables was=0.58 ( $p<0.0001$ ). When the multivariate regression model was run with EDSS/disease duration as the dependent variable, only CSA ( $p=0.03$ ,  $\beta=-0.25$ , SE=0.003) was retained in the final model.

## Discussion

MS is a highly variable disease in its clinical presentation, course and prognosis. While some patients remain clinically stable for relatively long periods of time, others tend to accumulate irreversible disability. Although clinical and epidemiological studies have defined several factors which might contribute to identify patients with a more favourable disease evolution, including gender, age at onset, time to second relapse, type and duration of initial symptom,<sup>6, 23</sup> the definition of BMS patients still remains a challenge. Similarly, MRI studies tailored towards the assessment of the extent of focal<sup>16-19, 24, 25</sup> and diffuse<sup>2, 16, 17, 24, 26</sup> brain damage in these patients have yielded conflicting results. Considering that EDSS, the most common scale used for grading disability in MS, is heavily weighted towards locomotor disability, we argued that the relative sparing of cervical cord in BMS is likely to be a factor explaining the favourable clinical status of these patients.

In line with previous studies, which showed only a mild correlation between the extent of cord MRI abnormalities and clinical disability in MS patients,<sup>26,27</sup> the quantification of cervical cord macroscopic lesions did not demonstrate a significant difference between SPMS and BMS patients. Such a finding might be related to the fact that this measure is only semiquantitative. However, a quantitative assessment of lesion load in the cervical cord is usually not performed, due to methodological limitations which include contaminations from partial volume effects and movement artefacts. To achieve a comprehensive estimate of cervical cord damage in our patients, we did not limit the analysis to the assessment of focal lesions, but used additional MRI techniques, which have been proven to provide a measure of irreversible tissue loss (i.e., atrophy), and “diffuse” tissue abnormalities (i.e., damage possibly associated to axon and myelin loss).<sup>28-30</sup> *Post-mortem* studies have demonstrated that demyelination and axonal injury occur not only inside T2-visible cord lesions, but also in the normal-appearing cord tissues in patients with MS.<sup>28-30</sup> Demyelination and axonal loss might lead to increased extracellular spaces between surviving fibers, which can harbour cell debris, inflammatory infiltrates, fibrillary gliosis and astrocytic proliferation.<sup>31</sup> Moreover, similarly to what occurs in the brain, the loss of neurons secondary to lesions in the grey matter or to axonal transection in the white matter can also occur in the cord.<sup>32</sup> All these pathological changes have the potential to alter the diffusivity and volumetric characteristics of the cervical cord.

In line with the results of two seminal studies,<sup>26,33</sup> the analysis of cervical cord atrophy demonstrated significant loss of cervical cord area in SPMS patients, but not in BMS patients. For DT MRI, we used a sequence tailored for imaging the cervical cord, which has already been applied in patients with different neurological conditions, including MS,<sup>10, 11, 14</sup> neuromyelitis optica,<sup>12</sup> and neuropsychiatric systemic lupus erythematosus.<sup>13</sup> The analysis of DT MRI characteristics of the individuals enrolled in this study revealed some interesting differences between BMS and SPMS patients. The two diseased groups showed a significant increase of cord MD values in comparison with healthy individuals, without significant difference between

them. Remarkably, SPMS patients also had a significant decrease of cord average FA values in comparison with both healthy individuals and BMS patients. Admittedly, we studied a cohort of SPMS patients with a disease duration shorter than that of BMS cases. However, such a difference is expected to reduce the magnitude of MRI abnormalities we found in the SPMS group. We can also reasonably exclude that the differences in diffusivity of the cord are secondary to partial volume effect from the surrounding CSF. Nevertheless, contamination from the CSF was minimized by considering in the analysis only pixels located in the central slice of the sagittal slab and by including only those from C1 to C5. This was at the price of restricting the portion of the cervical cord studied, but we believe this made our observations more robust and reliable. As a consequence, these results indicate that cervical cord in BMS patients without locomotor disability is relatively spared by the most destructive components of MS pathology **outside focal macroscopic lesions** and, hence, suggest that this might **contribute to explain** the favourable clinical status of these patients. **Such a finding is not unexpected, considering that the clinical definition of BMS, which is based on the EDSS, assumes implicitly that these patients would have a relative spinal cord sparing.** The discrepancy between FA and MD measures in BMS patients also suggests a relative preservation of the orientation of the major white matter fiber bundles in the cervical cord, despite the reduction of structural barriers to water molecular motions. **Admittedly, we limited our analysis to the cervical spinal cord, which is the most commonly affected by the disease, and, due to technical challenges, we did not investigate if similar differences are also found in the thoracic and lumbar segments. Furthermore, we used as a control group patients with SPMS, who had a similar disease duration and higher disability compared to those with BMS. An additional helpful control group would have been RRMS patients. Future studies are now warranted to assess whether spinal cord diffusivity is different between patients with different disease duration, but similar levels of disability.**

Another important issue to be addressed is how much of cord pathology of patients with MS is due to local damage and how much is due to retrograde and anterograde degeneration of

neurons secondary to the injury of fibers passing through T2-visible lesions of the brain. In line with previous pathological<sup>29</sup> and MRI<sup>10,11,14,27</sup> studies, we found only of a relatively modest correlation between cord CSA and brain T2 lesion load **and NABT damage**, suggesting that distant pathology in the brain results in only a marginal contribution to the observed cord MRI changes.

Since our main objective was to define the factors associated to the accumulation of irreversible disability in long lasting MS, we analyzed the contribution of cord and brain measures of macro- and microscopic tissue abnormalities, separately, in determining EDSS scores in the entire sample of patients. The univariate analysis of correlation showed that EDSS was significantly correlated with cord CSA, cord average FA and brain T2 lesion volume. Several studies have convincingly demonstrated that cord area has a role in predicting the accumulation of disability in patients with MS.<sup>22, 27, 33</sup> To this regard, our study provides an additional piece of information by showing that the assessment of intrinsic cord pathology is an useful tool to further improve the understanding of the mechanisms responsible for the development of “fixed” disability in this disease. It is worth noting that, contrary to previous studies,<sup>11, 14</sup> also brain T2 lesion volume was significantly correlated with EDSS in our sample. Such a finding is in agreement with the results of a seminal study<sup>34</sup> in patients with progressive MS, which demonstrated that brain T2 lesion burden can contribute to differentiate patients with and without locomotor disability (defined, as it is the case for the present study, by an EDSS  $\geq$  4.0).

All the previously reported MRI variables were retained in the final multivariate model, as independent factors influencing the disability status. This suggests that brain and cord pathology accumulates, at least partially, independently one from the other and both may contribute to the development of disability. In addition, it seems that different factors in these two CNS compartments are likely to play a role in disability accumulation over relatively long period of time, since we found that in the brain, T2 lesion burden rather than microscopic NABT changes

may be relevant, whereas, in the cord, “diffuse” abnormalities and tissue loss more than focal lesions are likely to be the central aspects of the disease. **Assessing a potential treatment effect on the observed findings was beyond the scope of this study. In addition, the cohort of subjects studied was too small and the treatment regimen too heterogeneous to address this issue.**

The finding of an independent contribution of brain and cord damage to disability status in MS confirms previous reports,<sup>15,34</sup> and points to the use of multicomposite MRI scores as potential useful measures of outcome in studies aimed at monitoring disease evolution. Admittedly, since this is a cross sectional study, caution must be exercised before drawing firm conclusions on MS dynamics. Longitudinal studies are indeed warranted to confirm or reject our speculation.

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**Table 1.** Main clinical and demographic characteristic of the study groups.

	<b>Healthy subjects</b>	<b>BMS patients</b>	<b>SPMS patients</b>	<b>p</b>
Men/Women	7/11	12/28	10/18	<b>n.s.</b>
Mean age (range) [years]	42.0 (28-60)	45.2 (27-62)	46.5 (32-68)	<b>n.s.</b>
Mean disease duration (range) [years]	-	22.1 (15-32)	17.0 (5-41)	<b>0.002</b>
Median EDSS score (range)	-	2.0 (0.0-3.0)	6.0 (4.0-8.5)	<b>&lt;0.001</b>

Abbreviations: BMS = benign multiple sclerosis, SPMS = secondary progressive multiple sclerosis patients, EDSS = expanded disability status scale.

**Table 2.** Cervical cord and brain conventional and DT MRI derived metrics from the study groups.

	<b>Healthy subjects</b>	<b>BMS patients</b>	<b>SPMS patients</b>	<b>p*</b>
<b>**Median number cervical cord lesions (range)</b>	<b>0</b>	<b>2 (1-6)</b>	<b>2 (1-5)</b>	<b>n.s.</b>
Cord average MD (SD)	1.24 (0.1)	1.38 (0.15)	1.44 (0.21)	0.001
Cord average FA (SD)	0.41 (0.05)	0.37 (0.07)	0.33 (0.05)	<0.0001
Cord CSA (SD) [mm <sup>2</sup> ]	83.1 (8.5)	78.0 (7.4)	69.0 (12.0)	<0.0001
Brain T2 LV (SD) [ml]	-	12.4 (9.8)	24.7 (17.8)	0.001
Brain NABT average MD (SD)	0.91 (0.06)	0.98 (0.07)	1.04 (0.12)	0.001
Brain NABT average FA (SD)	0.22 (0.009)	0.20 (0.02)	0.18 (0.01)	<0.0001

Abbreviations: LV = lesion volume, SD = standard deviation, MD = mean diffusivity, FA = fractional anisotropy, BMS = benign multiple sclerosis, SPMS = secondary progressive multiple sclerosis patients, NABT = normal-appearing brain tissue, CSA = cross-sectional cord area.

MD is expressed in  $\text{mm}^2\text{s}^{-1}\times 10^{-3}$ ; FA is a dimensionless index.

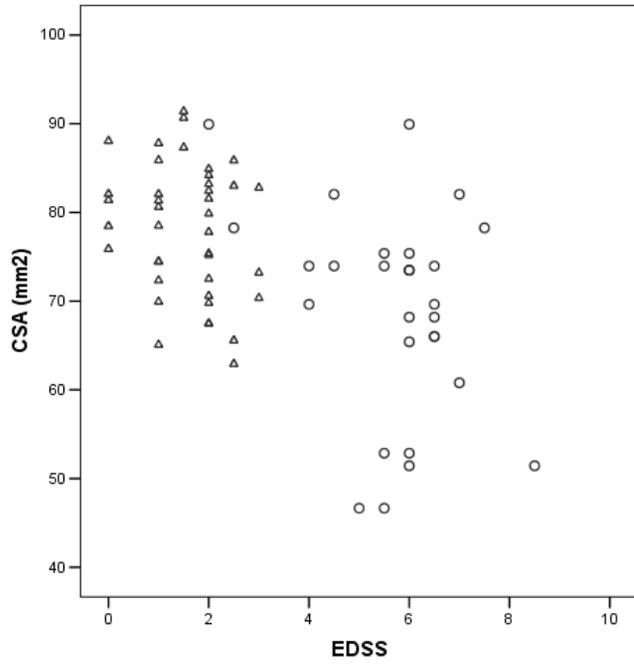
\***Analysis of variance model.**

\*\***Independent-sample t test between BMS and SPMS patients.**

**Figure legend.**

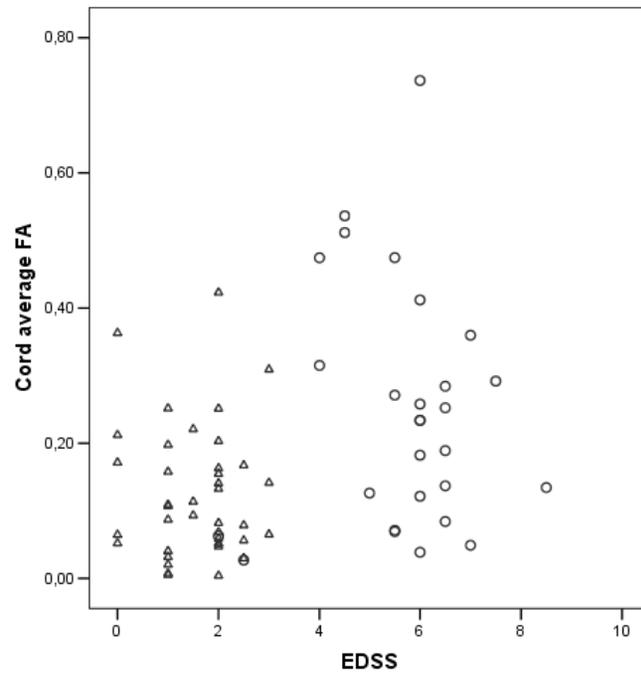
**Figure.** Scatter plots showing the correlation between Expanded Disability Status Scale (EDSS) scores and cervical cord cross sectional area (CSA) (A), cord average fractional anisotropy (FA) (B), and brain T2 lesion volume (LV) (C) in patients with benign (triangle) and secondary progressive (circle) MS. See text for further details and statistical analysis.

$r=-0.47, p<0.0001$



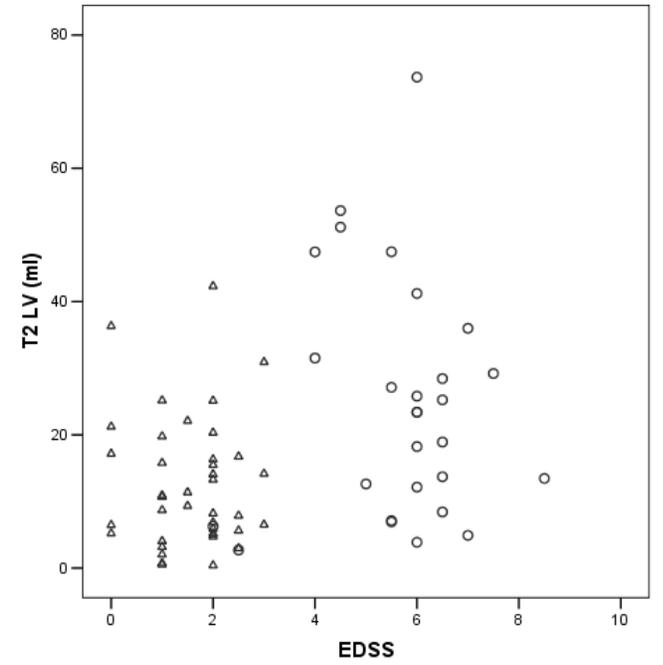
a

$r=-0.37, p=0.002$



b

$r=0.34, p=0.005$



c