Pathological study of spinal cord atrophy in multiple sclerosis suggests limited role of local lesions

N. Evangelou, G. C. DeLuca, T. Owens and M. M. Esiri

Summary
Imaging studies in multiple sclerosis have shown that spinal cord atrophy correlates with clinical disability. The pathological substrate of atrophy has not as yet been investigated adequately. In order to determine the cause of spinal cord atrophy in multiple sclerosis, five different sections of the spinal cord were examined histopathologically in 33 controls and 55 multiple sclerosis cases. In the multiple sclerosis cases in each section the total lesion load and the cross-sectional area of the cord were measured. Multiple regression models were estimated, controlling for sex, age, duration of the disease and location of the cord sections. The multiple sclerosis cords were found to be significantly smaller than the controls. The duration of the disease played the most important role in determining cord atrophy. The degree of atrophy varied in different parts of the cord. Individual lesions played a minor role in local atrophy. Our findings suggest that axonal degeneration, possibly caused by the cumulative number of lesions in the brain and cord, or an alternative atrophic process, is responsible for spinal cord atrophy in multiple sclerosis, rather than tissue loss within individual lesions.

Keywords: atrophy; multiple sclerosis; pathology; post mortem; spinal cord

Abbreviations: NAWM = normal appearing white matter

Introduction
Atrophy of the brain and spinal cord in multiple sclerosis has been recognized for many years, but recently increased interest has focused on accurately measuring and studying tissue loss. New MRI and image analysis techniques have facilitated this process, and recently atrophy measures have been used to measure treatment effects (Lin et al., 2004). The pathological basis of atrophy has not been extensively investigated. It is thought that tissue atrophy represents the consequence of destructive pathological changes within lesions and in the normal appearing white matter (NAWM) (Miller et al., 2002). The extent of axonal loss both in lesions (Barnes et al., 1991) and in the NAWM (Ganter et al., 1999; Evangelou et al., 2000a) has now been clearly documented and tissue atrophy is thought to occur predominantly due to tissue destruction in white matter lesions, or as a result of Wallerian degeneration along the fibre pathways of axons that traverse lesions. A number of other mechanisms can contribute to tissue volume alterations, such as the existence of grey matter plaques, the degree of astrocytosis in lesions and changes in tissue water content due to acute inflammation or medications.

Spinal cord atrophy is at least as important as brain atrophy in multiple sclerosis. A number of cross-sectional and longitudinal studies have shown that cord atrophy correlates with clinical disability (Losseff et al., 1996; Lin et al., 2003). Apart from a few pathological studies of small sample size most of the research on cord atrophy has relied on MRI studies. The contribution of lesions to local atrophy is difficult to assess as MRI studies have inherently limited ability to measure lesions accurately, and for technical reasons have focused
predominantly on the cervical cord (Kidd et al., 1993; Filippi et al., 1996; Stevenson et al., 1998). This study is based on one of the largest well-studied pathological series of spinal cords from patients with multiple sclerosis (DeLuca et al., 2004). By using pathological material we were in a position to measure accurately the lesions throughout the cord. Our primary aim was to investigate the contribution of lesions to local atrophy in multiple sclerosis.

**Methods**

**The data**

We studied the autopsy spinal cord material of 55 pathologically confirmed cases of multiple sclerosis (29 males and 26 females) with an age range of 25–83 years (mean 57.5). The length of disease history ranged from 2 to 43 years (mean 17.1). Most of the patients had secondary progressive disease. As control material, we studied 33 cases (15 males and 18 females) without evidence of spinal cord disease and with an age range of 31 to 81 years (mean 57.9). The post mortem material was derived from autopsy brain and spinal cord archive, from the Neuropathology Department, Oxford Radcliffe NHS Trust, and was obtained with consent from next-of-kin for use of tissue for research. The study was approved by the local research ethics committee.

For each of the multiple sclerosis and control cases, formalin-fixed, paraffin-embedded, transverse 15-mm thick sections of the spinal cord at five levels (high and low cervical, high and low thoracic and lumbar) were stained for myelin (with Luxol Fast Blue Cresyl Violet) and axons (Palmgren silver).

In total, 388 sections were examined at low power (12.5×) (Olympus SZH10) and digitized (Sony DKC5000 photocamera). All cross-sectional spinal cord and lesion areas were traced manually and area measurements were made in mm² using an automated computerized image analysis system running NIH Image software (Fig. 1). We used the myelin stain to quantify the lesions and the Palmgren stain to measure the cross sectional area of the cord, aiming to blind the observer as much as possible. Two operators independently measured the cord and lesion areas in 15 randomly selected sections, and their agreement was very high (r = 0.96, P < 0.001). Cases had been coded so that these measurements could be made with the observer blind to clinical information.

A shrinkage factor of 0.71 was applied to the measured areas to correct for changes in tissue block size observed before and after the fixation and embedding process.

**Statistical analysis**

To examine the cause(s) of atrophy at a specific site of the cord we controlled for the individual biological variation of each subject, the exact location of the cord and the characteristics of the disease in the multiple sclerosis cords. Natural history studies suggest that the duration of the disease is perhaps the most important determinant of disability. Accordingly, if cord atrophy is a reflection of clinical disability then we needed to take this into account in our specification. Multiple regression estimations allowed us to measure the relationship between the size of the lesion (an independent variable) and the size of the cord (our dependent variable), whilst controlling for all other characteristics that may have an effect on cord size (e.g. sex, age, location, duration of disease). The Mann–Whitney test was used for non-parametric sample comparisons.

**Results**

**Descriptive statistics**

The cross sectional area of the spinal cord was smaller in multiple sclerosis cases, controlling for age, sex and cord location (coefficient −5.96, P = 0.002; i.e. measuring the spinal cord in mm², we found that in multiple sclerosis patients the cross sectional area of the spinal cord was on average 5.96 mm² smaller than in non-multiple sclerosis patients). The cross-sectional area of the cord was smaller in multiple sclerosis compared with controls at the upper, lower cervical and the upper thoracic level. The cord in multiple sclerosis and control cases was not different at the lower thoracic and lumbar sections (Table 1).

**Table 1** Spinal cord atrophy in multiple sclerosis is only present in the upper part of the cord

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean area (mm²)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple sclerosis</td>
<td>Controls</td>
</tr>
<tr>
<td>Upper cervical</td>
<td>90.8</td>
<td>102.8</td>
</tr>
<tr>
<td>Lower cervical</td>
<td>92.3</td>
<td>105.1</td>
</tr>
<tr>
<td>Upper thoracic</td>
<td>63.5</td>
<td>69.7</td>
</tr>
<tr>
<td>Lower thoracic</td>
<td>52.4</td>
<td>52.8</td>
</tr>
<tr>
<td>Lumbar</td>
<td>70.7</td>
<td>72.8</td>
</tr>
</tbody>
</table>

Fig. 1 Cross-sectional image of spinal cord in a patient with multiple sclerosis stained with Luxol Fast Blue Cresyl Violet. The total cord (thick white line) and the demyelinated lesion (thin white line) area have been manually segmented.
Sex and age did not play a significant role in determining the size of the cord in this sample. Since multiple sclerosis and control cases were not age and sex matched (i.e. mean age and sex varied between the multiple sclerosis patients and controls) we have not reported individual statistics on these variables. However, they were always included in the regression estimations.

The duration of the disease was found to be a very important determinant of cord atrophy (coefficient $-0.76$, $P < 0.001$), controlling for age, sex and location (Fig. 2). Atrophy

**Fig. 2** Size (in mm$^2$) of the cross-sectional area of the spinal cord, in five different sections (upper and lower cervical, upper and lower thoracic and lumbar) in multiple sclerosis patients, in relation to disease duration. The effect of disease duration in atrophy does not appear to be linear, as greater tissue loss is observed during the first few years of the disease.
tended to occur in the first few years of the disease, and the relationship appeared to be non-linear. To test for this we included a squared duration variable in our regression. This variable was significant, confirming that the relationship is quadratic. With basic calculus we differentiated the curve to identify the minimum point, and hence the number of years at which the rate of atrophy slowed. Regression analysis confirmed that the impact of the disease on the atrophy was seen early, and that the rate of change appears to slow at ~3.3 years from the clinical onset of disease.

For the whole group, we found an average volume reduction of 0.75% per year. When the rate of atrophy was calculated for patients that died within 10 years from symptom onset it was found to be much faster, at 3.5% per year.

The contribution of the duration of the disease to local atrophy decreased in importance monotonically as we examined more caudal parts of the cord, becoming insignificant by the lumbar area (Table 2).

Lesion size did not correlate with the degree of local atrophy of the cord controlling for age, sex, location and duration of disease ($P = 0.94$). The lack of significant contribution of lesions to atrophy applied to both sexes.

As it is not an uncommon finding for cervical lesions on MRI to appear to cause local atrophy, the effect of lesions on atrophy was examined at different locations of the cord. Although it did not reach 5% significance, a trend appeared with lesions causing atrophy (negative correlation) in the upper cervical (coefficient $-0.22$, not significant), lower cervical (coefficient $-0.06$, not significant) and lumbar sections (coefficient $-0.21$, not significant) and expansion (positive correlation) in the upper thoracic (coefficient $0.36$, $P = 0.08$) and lower thoracic cord (coefficient $0.64$, $P = 0.08$), taking into account age, sex and duration of disease.

If the area measurements were grouped into cervical, thoracic and lumbar levels, similar results were obtained with lesions tending to cause atrophy at cervical (coefficient $-0.12$, not significant) and lumbar (coefficient $-0.21$, not significant) levels and causing significant expansion at the thoracic levels of the cord (coefficient $0.45$, $P = 0.02$).

Males and females were examined separately and a similar trend was apparent with correlations between lesion load and cord expansion in the thoracic cord reaching significance below 5% (males in upper thoracic area, coefficient 0.56, $P = 0.04$; females in lower thoracic area, coefficient 1.2, $P = 0.01$).

**Discussion**

Brain and spinal cord atrophy is increasingly used as a marker of disease progression in multiple sclerosis, and considerable debate exists as to the optimal image analysis methods used to measure atrophy. Yet the pathological basis of atrophy in multiple sclerosis has not been clearly defined. Broadly speaking, atrophy in multiple sclerosis has been attributed to a combination of local tissue loss within demyelinated lesions and Wallerian degeneration of fibre pathways (Miller *et al.*, 2002). Our study, the largest pathology study of atrophy of the spinal cord in multiple sclerosis, shows that tissue loss within lesions does not play a significant role in the size of the cord at the level of the lesion.

We should not confuse the minimal contribution of lesions to local atrophy with lack of contribution to the overall tissue loss. In brain tissue, axonal transection has been documented at the centre and the periphery of demyelinating lesions (Ferguson *et al.*, 1997; Trapp *et al.*, 1998), leading to axonal and tissue volume loss in the adjacent white matter (Evangelou *et al.*, 2000b), presumably by Wallerian degeneration. Similar mechanisms might be expected to operate in the cord, and indeed in a pathological study of the cervical cord, the axonal density was reduced both in the plaques and in the NAWM (Lovas *et al.*, 2000). However, surprisingly, in a large cross-sectional MRI study (Nijeholt *et al.*, 1998) and in a post mortem study (Ganter *et al.*, 1999), no relation between spinal cord atrophy and brain lesions was found. Although axonal loss was found in the cervical and upper thoracic cord, the axonal density did not correlate with the size of the lateral columns in the cord. Hence, axonal loss secondary to Wallerian degeneration may play a role in tissue atrophy but it seems that other pathophysiological mechanisms contribute to the loss of tissue volume in multiple sclerosis.

The ideal method of assessing evolution of atrophy is undoubtedly longitudinal imaging studies of both the spinal cord and the brain. MRI measurements of atrophy are accurate and can be reproducible. Unfortunately, because of technical limitations (Mikulis *et al.*, 1994) in achieving high spatial resolution the scanning time is prolonged, resulting in most studies measuring atrophy from individual cross-sectional slices of the cord, usually in the cervical region. In addition, the small size of some lesions does not allow all multiple sclerosis pathology to be visualized with MRI (Bergers *et al.*, 1998), leading to axonal loss. Similar mechanisms might be expected to operate in the cord, and indeed in a pathological study of the cervical cord, the axonal density was reduced both in the plaques and in the NAWM (Lovas *et al.*, 2000). However, surprisingly, in a large cross-sectional MRI study (Nijeholt *et al.*, 1998) and in a post mortem study (Ganter *et al.*, 1999), no relation between spinal cord atrophy and brain lesions was found. Although axonal loss was found in the cervical and upper thoracic cord, the axonal density did not correlate with the size of the lateral columns in the cord. Hence, axonal loss secondary to Wallerian degeneration may play a role in tissue atrophy but it seems that other pathophysiological mechanisms contribute to the loss of tissue volume in multiple sclerosis.

The duration of the disease is the most important determinant of cord atrophy in multiple sclerosis. This has been found in previous MRI studies of the spinal cord (Edwards *et al.*, 1998). Hence pathological studies are still invaluable in understanding multiple sclerosis, not only in defining the basic mechanisms of the disease, but also in overcoming the technical limitations of the MRI.

The duration of the disease was apparent with correlations between lesion load and thoracic levels of the cord (coefficient 0.45, $P = 0.08$). Similar results were obtained with age, sex and duration of disease. Lower thoracic cord (coefficient 0.64, $P = 0.08$), taking into account age, sex and duration of disease.

If the area measurements were grouped into cervical, thoracic and lumbar levels, similar results were obtained with lesions tending to cause atrophy at cervical (coefficient $-0.12$, not significant) and lumbar (coefficient $-0.21$, not significant) levels and causing significant expansion at the thoracic levels of the cord (coefficient 0.45, $P = 0.02$).

Males and females were examined separately and a similar trend was apparent with correlations between lesion load and cord expansion in the thoracic cord reaching significance below 5% (males in upper thoracic area, coefficient 0.56, $P = 0.04$; females in lower thoracic area, coefficient 1.2, $P = 0.01$).

**Table 2** Effect of duration of the disease on the cross-sectional area of the spinal cord

<table>
<thead>
<tr>
<th>Location</th>
<th>Coefficient</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper cervical</td>
<td>$-1.63$</td>
<td>0.001</td>
</tr>
<tr>
<td>Lower cervical</td>
<td>$-1.11$</td>
<td>0.013</td>
</tr>
<tr>
<td>Upper thoracic</td>
<td>$-0.79$</td>
<td>0.010</td>
</tr>
<tr>
<td>Lower thoracic</td>
<td>$-0.38$</td>
<td>0.050</td>
</tr>
<tr>
<td>Lumbar</td>
<td>$-0.36$</td>
<td>0.263 (NS)</td>
</tr>
</tbody>
</table>

As the absolute value of the coefficient of the duration decreases, the impact of the duration on the size of the cord decreases. Controlling for age and sex, for every year of having multiple sclerosis the cross-sectional upper cervical area decreases by 1.63 mm$^2$, the lower cervical area decreases by 1.11 mm$^2$, etc. NS = not significant.
rates in MRI studies (Fox et al., 1999) appeared similar in our study to that in MRI studies. We found different in younger and older patients (Kalkers et al., 1999) that atrophy occurs more rapidly in the early stages of multiple sclerosis. Thus, the present pathological study, as well as lower rather than at higher EDSS scores, suggesting that cer-

Our study was a cross sectional pathological investigation, which found a greater rate of atrophy of the spinal cord in the early years of multiple sclerosis. A major difficulty interpreting post mortem/cross-sectional studies in diseases with variable rate of progression is that most patients who die soon after diagnosis have a more aggressive disease. This can lead to false interpretation of results of variables such as atrophy that correlate with survival. In our study there were a number of patients that died soon after diagnosis, and although most early deaths were not from multiple sclerosis-related compli-
cations, we still have to interpret the rate of atrophy result with caution. In an MRI study that estimated rates of atrophy, particularly for infratentorial structures, higher rates of atrophy were found in relapsing–remitting than in secondary progressive patients (Liu et al., 1999). The rate of atrophy appeared similar in our study to that in MRI studies. We found an average of 0.75% atrophy per year, similar to brain atrophy rates in MRI studies (Fox et al., 2000; Kalkers et al., 2002). Using magnetic resonance spectroscopy (MRS) N-acetyl-
aspartate (NAA), an index of axonal integrity, was measured relative to creatine (Cr) in a large central brain volume in a study with 88 patients with a wide range of clinical disability and disease duration. The NAA/Cr ratio declined more rapidly with respect to Expanded Disability Status Scale (EDSS) at lower rather than at higher EDSS scores, suggesting that cerebral axonal damage might be faster in the earliest stages of the disease. Thus, the present pathological study, as well as previous MRI and MRS studies, are consistent in suggesting that atrophy occurs more rapidly in the early stages of multiple sclerosis.

A number of studies reported that the rate of atrophy is different in younger and older patients (Kalkers et al., 2002). In our study age did not play an independent role. Duration of disease correlated positively with age. Hence, it is not surpris-

Atrophy is not the same in all parts of the cord. It has been recognized previously that different parts of the CNS show different degrees of atrophy (Edwards et al., 1999), but to our knowledge no study has compared atrophy in different regions of the spinal cord. We found atrophy in multiple sclero-
sis to be more prominent in the upper part compared with the lower part of the cord. This was not due to measurement error, as the cord becomes smaller below the cervical expansion. The cross-sectional area of the cord is significantly larger in the lumbar cord compared with the upper thoracic section (Mann–Whitney, P = 0.01), but still showed no evidence of atrophy as opposed to the upper thoracic cord. It might simply be that the lower parts of the cord have less white matter than the upper, which would suggest that cord atrophy is mainly a function of the white matter volume loss, despite evidence that, at least in the brain, grey matter volume loss is substantial (De Stefano et al., 2003; Sailer et al., 2003). The white matter atrophy could of course be either due to Wallerian degenera-
tion, loss of myelin or to a combination of these mechanisms. The cervical cord is also affected by a higher lesion load than other parts of the cord, but overall we have found that lesions do not play a major role in local atrophy.

Although some differences were found between sexes, overall, sex did not have a major effect on the size of the cord. Ganter et al. (1999) found significantly more axonal loss in males in the cervical and upper thoracic cord compared with females, but again, interestingly, the axonal density did not correlate with the size of the lateral columns in the cord. We controlled for other variables known to have an effect on the size of the cord, namely age and sex. We did not observe a strong and consistent correlation between age and sex of our cases and size of the cord. This can possibly be explained by not taking into account the height of our subjects. Unfortu-

The value of multiple regressions as opposed to simple correlations is evident in examining the role of lesions in local atrophy. If location of the cord and duration were not taken into account, lesions seemed to cause swelling of the cord (coefficient 0.22, P = 0.03). This is not surprising, as most lesions were in the cervical cord, which is larger than the rest of the cord. When the location and the duration were considered, the effect of individual lesion became non-
significant (P = 0.94).

In conclusion, this study shows that significant atrophy of the spinal cord occurs in multiple sclerosis and that the degree of atrophy varies in different parts of the cord. These observa-
tions have important implications as to the pathophysiology of atrophy and to the use of atrophy measures in detecting disease progression. Our pathological study is in agreement with previous MRI studies, which found very early atrophy in multiple sclerosis. In our study, for the average multiple sclerosis patient, most atrophy had occurred by approximately the third year of the onset of the disease. This raises the possibility that this is the critical time for therapeutic interventions.

References
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