Greater loss of axons in primary progressive multiple sclerosis plaques compared to secondary progressive disease

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The pathological substrate of progressive disability in multiple sclerosis is hypothesized to be axonal loss. Differences in the demographic, pathological and radiological features of patients with primary progressive compared with secondary progressive multiple sclerosis raise the question as to whether they actually represent separate clinical entities. So far, large pathological studies comparing axonal damage between primary progressive and secondary progressive multiple sclerosis have not been reported. In this clinico-pathological study we examined the cervical spinal cord in patients with primary and secondary progressive multiple sclerosis. Human cervical spinal cord was derived at autopsy from 54 patients (17 primary progressive, 30 secondary progressive and 7 controls). Tissue was stained immunohistochemically and examined to determine: (i) the number of surviving corticospinal tract axons; (ii) the extent of grey and white matter demyelination; (iii) the degree of inflammation inside and outside of lesions; and (iv) the relationship between demyelination and axonal loss. Associated clinical data was used to calculate expanded disability status scale for each patient preceding death. Motor disability in the primary progressive and secondary progressive groups was similar preceding death. Secondary progressive multiple sclerosis patients showed considerably more extensive demyelination of both the white and grey matter of the cervical spinal cord. The total number of corticospinal axons was equally low in primary progressive and secondary progressive multiple sclerosis groups versus controls. The reduction of axonal density in demyelinated regions compared to normal appearing white matter was significantly more extensive in primary progressive versus secondary progressive patients (33% reduction versus 16% reduction, \( P < 0.001 \)). These findings suggest axonal loss is the pathological substrate of progressive disability in both primary progressive and secondary progressive multiple sclerosis with a common plaque-centred mechanism. More extensive axonal loss within areas of demyelination in primary progressive multiple sclerosis could explain high levels of axonal loss observed in these patients despite low levels of demyelination.

Keywords: multiple sclerosis; axonal loss; primary progressive; secondary progressive

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Introduction

Inflammatory demyelination is widely accepted to account for disability experienced during a multiple sclerosis relapse but additional neuro-axonal pathology has been hypothesized to explain progressive disability in multiple sclerosis. Pathological studies have demonstrated considerable reductions of axonal number within the white matter tracts of patients with multiple sclerosis compared with controls (Ganter et al., 2000a; Lovas et al., 2000; Bergers et al., 2002; DeLuca et al., 2004). Longitudinal imaging studies support the relationship between atrophy (a presumed marker of axonal loss) and accumulation of disability (Kidd et al., 1996; Losseff et al., 1996; Fisher et al., 2000; Ge et al., 2000; Ingle et al., 2003). However, uncertainty remains as to whether patients with primary progressive and secondary progressive subtypes share common pathogenesis for the progressive accumulation of disability.

Patients with primary progressive multiple sclerosis appear to possess certain distinctive demographic, radiological and pathological features when compared with other types of multiple sclerosis. On the other hand, the similar rate of accumulation of disability observed in patients with primary progressive and secondary progressive multiple sclerosis after the onset of progression raises the possibility that these two disease subtypes share a common mechanism of neurodegeneration (Weinshenker et al., 1989; Runmarker and Andersen, 1993; Cottrell et al., 1999; Confavreux et al., 2000; Kremenchutsky et al., 2006).

It seems likely that axonal injury occurring at sites of white matter inflammatory demyelination contributes towards the progressive accumulation of disability in multiple sclerosis (Ferguson et al., 1997; Trapp et al., 1998). In primary progressive multiple sclerosis, however, the relative paucity of white matter demyelination (Thompson et al., 1990; Stevenson et al., 1999; Tortorella et al., 2000; Filippini et al., 2000b; Rovaris et al., 2001), along with low levels of blood–brain barrier (BBB) breakdown and inflammation within white matter lesions (Thompson et al., 1991; Revesz et al., 1994), makes this mechanism seem less plausible.

In this study, we quantify axonal loss in the corticospinal tracts (CSTs) of patients with primary progressive and secondary progressive multiple sclerosis who had reached similar levels of motor disability before death. The CST at the level of the cervical spinal cord represents a relevant site of investigation given that spastic paraparesis is the typical clinical phenotype common to patients with primary progressive and secondary progressive multiple sclerosis. Furthermore, previous studies have found the cervical cord to be a location particularly susceptible to demyelination and atrophy in multiple sclerosis (Kidd et al., 1993; DeLuca et al., 2004; Gilmore et al., 2005, 2006, 2009). Determining pathologically the burden of demyelination in this region is useful, given that small white matter lesions, thought to occur more commonly in primary progressive multiple sclerosis, might be beyond the resolution of available spinal cord MRI (Thompson et al., 1990). By investigating the relationship between axonal loss and white matter demyelination, we look for evidence of a plaque-centred mechanism for neuro-axonal injury in primary progressive and secondary progressive multiple sclerosis.

Meanwhile, the extent of grey matter demyelination, a feature which is increasingly recognized as being prevalent in patients with longstanding multiple sclerosis (Bo et al., 2003a; Kutzelnigg et al., 2005; Gilmore et al., 2006), is compared for the first time in the spinal cord of patients with primary progressive and secondary progressive multiple sclerosis.

Materials and Methods

Clinical material

Human cervical spinal cord used in this study was obtained from the UK multiple sclerosis Tissue Bank, Imperial College London and the Netherlands Brain Bank, Netherlands Institute for Neuroscience, Amsterdam. Tissue from both sources was obtained at autopsy following donor or next-of-kin consent for its use in research. Consent included access to medical files after death. Cases were selected at random. Cervical cord tissue blocks from 47 patients with pathologically confirmed multiple sclerosis (17 primary progressive, 30 secondary progressive) were used in the study. Patient medical records or, in the case of the UK multiple sclerosis Tissue Bank, comprehensive summaries of the multiple sclerosis course extracted from the medical records, were reviewed by a multiple sclerosis experienced neurologist (C.H.P. or N.E.). All cases had sufficient clinical data to enable them to be classified as primary progressive or secondary progressive multiple sclerosis. Sometimes prospectively recorded expanded disability status scale (EDSS) scores were available in the medical files. In cases where EDSS scores had not been prospectively recorded, EDSS scores were constructed from available information using an EDSS questionnaire based on walking distance, need of assistance and transfer. This questionnaire was developed as the ‘EDSS by phone’ and validated as such (Lechner-Scott et al., 2003); we have found it easy to apply for patients where there is description of daily life functional (dis)abilities in the absence of formal EDSS scores. Based on the above, years that patients reached EDSS scores 6, and 8 were documented, allowing for assessment of time (from first symptoms) to these hallmarks. In this way, EDSS scores could be determined for 45 of the 47 multiple sclerosis patients.

Seven healthy control samples from patients without neurological disease were obtained from the same sources. The study was approved by the local research ethics committee.

Tissue preparation

Tissue from the Netherlands Brain bank had been fixed in 10% formalin for 30 days and embedded in paraffin. Tissue from the UK multiple sclerosis Tissue Bank had been fixed in 4% paraformaldehyde for 14 days, cryoprotected using 30% sucrose for 7 days and then frozen by immersing in isopentane pre-cooled on a bed of dry ice. Frozen tissue was later thawed and embedded in paraffin. The paraffin embedded tissue from both sources was sectioned at a thickness of 5μm and mounted on Superfrost + slides and dried overnight at 37°C.

Immunohistochemistry

The paraffin sections were deparaffinized using xylene and rehydrated in reducing concentrations of ethanol (100%, 96%, 70% and water). Endogenous peroxidase was blocked using 0.3% H2O2 in methanol. For antigen retrieval sections were heated in either Tris/EDTA (pH 9)
or sodium citrate buffer (pH 6). The type of pre-treatment depended on the primary antibody being used. Adjacent sections from each subject were stained for myelin (myelin basic protein, MBP, DAKO), T-cells (CD3, DAKO), macrophage/microglia (CD163, AbD Serotec) and axons (NE14, neurofilament, DAKO) using the sABC method as previously described (Geurts et al., 2005).

Image analysis

Stained sections were used to create digital images of the entire cord cross section which could be viewed electronically with up to 40× magnification (using Nanozoomer NDP, Hamamatsu, Japan). Cases were coded so observers were blinded to disease category. Using these images the spinal cord level was classified as upper (C1–C4), middle (C5–C6) or lower (C7–C8) cervical according to published descriptions of typical spinal cord morphology (Kameyama et al., 1994; Altman and Bayer, 2001). This method showed good interobserver reproducibility (κ 0.9, P < 0.0001).

Characterization of demyelination

Manual outlining using MBP-stained sections was used to determine the spinal cord cross sectional area, the grey and white matter area and the proportion of grey and white matter that was demyelinated (white matter and grey matter demyelination fraction). Each demyelinated lesion was classified as being purely white matter, purely grey matter or mixed white matter/grey matter. The location of each white matter lesion was also classified according to its involvement of the posterior, lateral or anterior spinal cord columns.

Characterization of inflammation

Sections stained with antibodies against CD3 and CD163 were used to quantify the degree of inflammation within each lesion and within normal appearing (non-demyelinated) tissues (NAWM and NAGM). Semi-quantitative measurements of lesion inflammation were made by ranking the densities of T cells and activated microglia/macrophages between 0 and 4. The ranking scale was established by first determining the range of inflammation observed within the entire tissue sample. Then regions were identified which appeared representative of points distributed at regular intervals along this continuum. Digital images were created for these reference regions. Each area of interest in the tissue was subsequently compared visually with the reference images to determine the appropriate score. This method was applied to both the parenchymal and perivascular compartments at both the centre and border of each lesion giving rise to a score of inflammation for each white matter lesion out of 32. Using the same sections, white matter lesions were also classified as either active, chronic active or chronic inactive based on previously described (Ferguson et al., 1997). Accuracy of lesion classification was verified by ensuring agreement between the primary observer (E.C.T.) and an experienced neuropathologist (J.L.) for 20 lesions located in spinal cords of 10 subjects. Inflammation in the non-demyelinated white and grey matter was measured by scoring the density of perivascular and parenchymal T-cells and activated microglia/macrophages as 0–4 using the same scale as before (maximum overall score 16).

Axonal measurements

Digital images of the NE14-stained spinal cord sections were used to measure axonal number in the CSTs. The borders of the lateral CSTs were manually outlined and their areas measured using image analysis software (NDP View, Hamamatsu). The borders of the lateral CSTs were defined according to previously published criteria (DeLuca et al., 2004): anterior—a transverse line extending from the posterior border of the grey matter commissure; medial—the lateral boundary of the dorsal horn; posterolateral—the outer border of the spinal cord (Fig. 1A). Anatomical landmarks were used to determine the location of 10 regions of interest (ROIs; each measuring 0.015 mm² in area) within the CST which would be studied in each individual. The regions of interest locations were chosen in such a way that they constituted a representative sample of the bilateral CST area (Fig. 1B). A digital image of each region of interest was created using 40× magnification. Each region of interest was documented to be either myelinated or demyelinated using corresponding MBP stained sections. When a region of interest was partially demyelinated, the category was assigned that occupied the majority of the region of interest.

Validation of semi-automated counting technique

Using image analysis software (Image J, 1.39, National Institutes of Health, USA), axons within 20 regions of interest, derived from 10 subjects, were manually counted. The same 20 regions of interest were subjected to image segmentation techniques (image brightness threshold and watershed tools) to generate a binary map. Particles of the binary maps were counted automatically. Segmentation methods were chosen that generated the strongest correlation between automated axonal counts and manual counts (Pearson correlation coefficient r = 0.96, P < 0.05).

Axonal counting technique

Automated counting, using the validated settings, was performed on each region of interest image from each individual. Using these values, the mean CST axonal density was calculated for each individual. This value was multiplied by the cross-sectional area of the bilateral CSTs to generate an estimate of the total number of corticospinal axons at the level of the cervical cord. Axonal densities within myelinated and demyelinated CST regions of interest were measured within each individual and the magnitude of difference between these axonal densities was calculated in each subgroup.

Statistical analysis

Given that different methods of tissue fixation had been employed by the UK and Amsterdam tissue banks, different tissue shrinkage is anticipated between the groups. For this reason, actual area measurements were not compared between subgroups. Total CST axonal number as well as white matter and grey matter demyelination fractions are not expected to be influenced by the effects of tissue shrinkage as they are independent of the cord area.

Chi-squared test was used to compare disability between primary progressive and secondary progressive subgroups. Mann–Whitney tests were used to compare the CST axonal number and degree of demyelination and inflammation between subgroups. Pearson’s two-tailed correlation testing was used to compare the white matter and grey matter demyelination fractions in multiple sclerosis subjects. Unpaired t-tests were used to compare the degree of inflammation between different tissue compartments. As the degree of inflammation was quantified both at the centre and the periphery of each lesion, inflammation in normal appearing tissues were compared with lesions by halving lesion inflammation scores to give equivalent scores out of 16. Chi-squared test was used to compare the number of active versus inactive white matter lesions in primary progressive and secondary progressive multiple sclerosis groups.
The effect of multiple sclerosis disease course on CST axonal number was investigated using linear regression analysis (STATA 10). Independent variables were disease course, age, gender, disease duration, post-mortem interval (PMI), white matter demyelination fraction, degree of disability and cord level. Similarly, the effect of demyelination on axonal density was tested using linear regression. Independent variables were region of interest myelination, disease course, age, gender, post-mortem interval and disease duration.

## Results

### Clinical information

Demographic and clinical information of cases are summarized in Table 1. Age was significantly higher in primary progressive than secondary progressive patients as might be expected with groups...
of similar disease duration. Post mortem interval was significantly higher in controls than multiple sclerosis patients. EDSS was not significantly different between the primary progressive and secondary progressive groups \((P = 0.37)\).

### Pathological observations

In our sample, 14 blocks were from the upper-cervical cord, 24 middle-cervical and 16 lower-cervical.

### Characterization of demyelination

Patients with secondary progressive multiple sclerosis showed significantly higher proportion of the cord cross sectional area to be demyelinated compared with patients with primary progressive multiple sclerosis. The same was true when white matter and grey matter demyelination analysed separately (Fig. 2). There was a strong correlation across individuals with multiple sclerosis between the proportion of white and grey matter demyelination \((r = 0.84, P < 0.0001)\).

Overall, 62 demyelinating lesions were found in the spinal cords of 36 patients (9 primary progressive; 27 secondary progressive). There were 28 purely white matter lesions, 32 mixed white matter/grey matter lesions and only two pure grey matter lesions. Patients with secondary progressive multiple sclerosis showed a higher proportion of lesions to be mixed white matter/grey matter than primary progressive multiple sclerosis patients (55% versus 40%) but this did not reach statistical significance.

Lesion location was similar in the two disease subtypes and complete demyelination of the entire cord area occurred occasionally in both subgroups. In several of the grey matter lesions in both primary progressive and secondary progressive multiple sclerosis, a proportion of the border of the lesion maintained a strict respect for the white matter/grey matter boundary (Fig. 3). However, few white matter lesions in either group seemed to respect the white matter/grey matter border.

![Figure 3](https://example.com/figure3.png)

**Figure 3** Cervical spinal cord section from a patient with primary progressive multiple sclerosis stained for MBP. A large mixed grey matter/white matter lesion is evident. Arrows indicate several regions where the grey matter component of the lesion respects the grey matter/white matter boundary.
Characterization of inflammation

Across the multiple sclerosis group, lesions demonstrated little in the way of inflammation (mean inflammation score 6.5 out of 32). The majority of lesions appeared chronically inactive in both multiple sclerosis subgroups. Although the secondary progressive group had a higher number of chronic active white matter lesions than primary progressive patients (Fig. 4), this did not reach statistical significance. There was a trend towards higher average lesion inflammatory scores in secondary progressive multiple sclerosis (secondary progressive 7.1; primary progressive 4.4; \( P = 0.087 \)). Mixed white matter/grey matter lesions showed equal levels of inflammation in the grey matter and white matter components (WM 7.0 versus GM 5.9; \( P = 0.38 \)). There was no significant difference between primary progressive and secondary progressive patients for inflammatory scores in normal appearing white and grey matter (Table 2).

Axonal measurements

The total numbers of axons calculated to exist in the bilateral CST are shown in Fig. 5. Axonal number was significantly lower in multiple sclerosis subjects \((2.2 \times 10^5)\) than controls \((6.9 \times 10^5)\) but no significant difference was found between primary progressive and secondary progressive multiple sclerosis patients using either non-parametric testing or regression analysis which controlled for other potential contributing factors.

Correlation between axonal number and demyelination

Overall 32 patients (6 primary progressive, 26 secondary progressive) had demyelination affecting their CSTs. Mean axonal density was significantly lower in demyelinated versus myelinated regions of interest in the entire multiple sclerosis group and in both primary progressive and secondary progressive subgroups (Fig. 6). This effect persisted even when axonal density in myelinated and demyelinated regions was compared only in primary progressive and secondary progressive patients who had CST demyelination. Using multiple regression, controlling for disease course, the reduction of axonal density in demyelinated regions compared to normal appearing white matter was more extensive in primary progressive versus secondary progressive patients (33% reduction versus 16% reduction, \( P < 0.001 \)).

Discussion

We have performed a histological comparison of pathology in the CST of patients with primary progressive and secondary progressive multiple sclerosis and have related findings to patients’ clinical status prior to death. Similar levels of axonal loss were seen to exist in the motor pathway of primary progressive and secondary progressive patients who had reached similar levels of motor disability before death. The new finding of this study is that primary progressive subjects have a similar degree of axonal loss as secondary progressive subjects, despite having significantly lower levels of white matter demyelination; this is explained by more extensive axonal loss within demyelinated areas in primary progressive multiple sclerosis.

The degree of axonal loss seen in the white matter tracts of multiple sclerosis patients compared to controls in this study is in
keeping with previous studies (Table 3). The similarly low axonal numbers in the spinal cords of patients with primary progressive and secondary progressive multiple sclerosis is in line with imaging studies showing no significant difference in spinal cord cross-sectional area between primary progressive and secondary progressive patients matched for disability (Nijeholt et al., 1998; Rovaris et al., 2001; Agosta et al., 2007).

The finding of reduced axonal density within white matter lesions in the spinal cord of multiple sclerosis patients has also been reported by other authors. In their combined MRI-histology study, Bergers et al. found a 26% reduction in axonal density when white matter areas with high T2 MRI signal were compared with normal appearing white matter of post mortem spinal cord (Bergers et al., 2002). Another study of post-mortem spinal cord tissue by Lovas et al. revealed significantly lower axonal density in 10 multiple sclerosis lesions compared with equivalent cord regions of non-neurological controls. However, no significant difference was detected between the axonal density of lesions and normal appearing white matter within the multiple sclerosis group (Lovas et al., 2000).

Imaging studies have shown lower lesion loads in the central nervous system of patients with primary progressive versus secondary progressive multiple sclerosis (Nijeholt et al., 1998; Stevenson et al., 1999; Filippi et al., 2000a; Rovaris et al., 2000). However, this effect seems to be much more prominent in the brain; some of these studies failed to detect any significant differences in lesion load between primary progressive and secondary progressive patients in the spinal cord (Nijeholt et al., 1998; Stevenson et al., 1999). In contrast, our findings suggest that the extent of white matter demyelination of the spinal cord in primary progressive multiple sclerosis is likely to be lower than in secondary progressive multiple sclerosis. The extent of grey matter demyelination, which is difficult to visualize using MRI, was also found be significantly lower in primary progressive versus

### Table 3 Summary of histopathology studies quantifying axonal loss in multiple sclerosis

<table>
<thead>
<tr>
<th>Study</th>
<th>MS cases (n)</th>
<th>Control cases (n)</th>
<th>Site</th>
<th>Findings MS versus controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganter et al. (1999)</td>
<td>43</td>
<td>31</td>
<td>Cervical cord (lateral column)</td>
<td>13–21% reduction in cord area</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11–42% reduction in axonal density</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>53% reduction in axonal number</td>
</tr>
<tr>
<td>Evangelou et al. (2000b)</td>
<td>8</td>
<td>8</td>
<td>Corpus callosum</td>
<td>~55% reduction in axonal density</td>
</tr>
<tr>
<td>Lovas et al. (2000)</td>
<td>10</td>
<td>11</td>
<td>Cervical cord (lateral and posterior column)</td>
<td>56% reduction in axonal density</td>
</tr>
<tr>
<td>Bergers et al. (2002)</td>
<td>9</td>
<td>4</td>
<td>Cervical and thoracic cord (anterior, lateral and posterior columns)</td>
<td>5–44% reduction in axonal number</td>
</tr>
<tr>
<td>De Luca et al. (2004)</td>
<td>55</td>
<td>32</td>
<td>Whole cord (lateral and posterior columns)</td>
<td>68% reduction in axonal number</td>
</tr>
<tr>
<td>Tallantyre et al. (this article)</td>
<td>54</td>
<td>7</td>
<td>Cervical cord (lateral column)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6 Mean density of CST axons within myelinated and demyelinated regions of interest according to multiple sclerosis subgroup. Axonal density was reduced in the demyelinated versus myelinated CST of both primary progressive and secondary progressive patients. The magnitude of this effect appeared higher in primary progressive multiple sclerosis patients. This effect persisted when regions of interest only from those individuals with any CST demyelination were included (PP Dem and SP Dem).
secondary progressive patients in this study. This is interesting given that previous studies have shown the degree of cortical grey matter demyelination to be similar in primary progressive and secondary progressive patients or even to be higher in primary progressive patients (Bo et al., 2003b; Kutzelnigg et al., 2005). In keeping with previous work in the spinal cord and in the cortex (Kutzelnigg et al., 2005; Gilmore et al., 2006), patients with chronic multiple sclerosis in this study showed significantly higher proportions of grey matter demyelination than white matter demyelination and there was a strong correlation between these variables in the spinal cord (Gilmore et al., 2006).

Axonal injury has been shown to be caused, at least in part, by damage of axons as they traverse areas of focal inflammatory demyelination (Ferguson et al., 1997; Trapp et al., 1998; Bitsch et al., 2000; Evangelou et al., 2000b; Kornek et al., 2000; Lovas et al., 2000). The paradoxical finding of extensive CNS atrophy, despite moderately low levels of demyelination and inflammation in primary progressive multiple sclerosis, has long been recognized on MRI (Kidd et al., 1996; Nijeholt et al., 1998; Stevenson et al., 1999), raising the possibility that axonal loss occurs independently of demyelination in primary progressive multiple sclerosis. We found the presence of demyelination to contribute significantly to axonal density with CST regions of interest in both disease subtypes. This effect was of greater magnitude in primary progressive multiple sclerosis patients suggesting that primary progressive multiple sclerosis axons are more vulnerable to white matter inflammation/demyelination compared to secondary progressive multiple sclerosis axons. An imaging study of 91 primary progressive and 36 secondary progressive multiple sclerosis patients matched for EDSS showed a non-significant difference in brain volume between primary progressive and secondary progressive groups (1097 ml versus 1095 ml; $P = 0.95$) despite significantly lower $T_2$ lesion volumes in the brain of primary progressive patients (19.8 ml versus 31.2 ml; $P = 0.004$). Our findings of a reduction in axonal density in demyelinated regions of primary progressive multiple sclerosis patients, which is twice that seen in secondary progressive patients, could explain this imaging paradox.

Magnetic resonance spectroscopy has been used to show significant reductions in NAA concentration in the NAWM of patients with progressive multiple sclerosis versus controls. In keeping with our results, no significant differences were found in the NAA values in the NAWM between primary progressive and secondary progressive patients matched for EDSS (Cucurella et al., 2000). In contrast to our results, this group did not find a significant difference between lesion NAA concentration in primary progressive and secondary progressive patients. However, Cucurella et al. did not measure the degree of neuro-axonal loss within lesions (compared with surrounding NAWM) because data for lesions and NAWM were derived from different groups.

In a biopsy study investigating acute axonal pathology, Bitsch et al. found the degree of axonal pathology to be highly variable between individuals (including four primary progressive and four secondary progressive cases). Variance was not attributable uniformly to any clinical feature (Bitsch et al., 2000). However, APP is a marker of acute axonal injury whereas the axonal density we measured reflects the cumulative effects of axonal loss over time. These differences could therefore be explained if destructive processes within multiple sclerosis lesions are slowly progressive over time as has been hypothesized by other groups (Prineas et al., 2001).

This study showed a trend towards higher levels of inflammation in the lesions of patients with secondary progressive versus primary progressive multiple sclerosis. This is in line with results of a similar study in which significantly higher numbers of perivascular lymphocytic cuffs were observed in spinal cord white matter lesions in secondary progressive patients versus primary progressive patients (Revesz et al., 1994). However, it must be noted that in both studies lesions were predominantly chronic inactive and in general they showed low levels of inflammatory activity. We cannot draw conclusions about the contribution of inflammation to disability early in the disease using data derived from patients who had reached a very late stage of multiple sclerosis.

We did not find evidence of diffuse parenchymal inflammatory activity in the normal appearing spinal cord tissue of the primary progressive or the secondary progressive group. This contrasts with the findings of Kuzelnigg et al. who found patients with primary progressive and secondary progressive multiple sclerosis to demonstrate profound microglial activation/macrophage infiltration in the NAWM of the brain (Kutzelnigg et al., 2005). Several authors have shown that inflammatory responses to injury differ between brain and spinal cord (Schnell et al., 1999a, b; Batchelor et al., 2008). In patients with long standing multiple sclerosis we are likely to be observing a chronic inflammatory response. Macrophages which persist chronically in areas of CNS injury appear to possess less harmful phenotypes than those seen in acute inflammation and may even contribute to tissue repair by mechanisms such as augmentation of neuronal sprouting (David et al., 1990; Hirschberg et al., 1994; Fleming et al., 2006). Thus the appearance of a high density of activated microglia/macrophages in the NAWM of the brain but not the spinal cord in progressive multiple sclerosis, may suggest that the spinal cord is a less favourable environment for regeneration and repair, possibly explaining the propensity of progressive multiple sclerosis patients to develop a spinal cord syndrome.

Our data is derived using density measurements so several potential contributory factors must be considered. Oedema at sites of inflammatory demyelination could reduce local axonal density without affecting actual axonal number. However, this seems unlikely in this study, especially in primary progressive cases, given the low levels of active inflammation observed within white matter lesions. Similarly axonal densities could vary between individuals according to variations in tissue shrinkage. Relative axonal densities are dimensionless and therefore should be independent of tissue shrinkage. However, it is possible that differential shrinkage may occur in normal versus diseased tissue. While this could introduce systematic error, we do not believe that source bias accounts for the differences observed between primary progressive and secondary progressive multiple sclerosis cases.

The strength of our pathological study is the good clinical history accompanying the pathological specimens and the large number of primary progressive multiple sclerosis cases. As primary progressive multiple sclerosis patients show a relative paucity of demyelination, our findings of reduced axonal density in
Hirschberg DL, Yoles E, Belkin M, Schwartz M. Inflammation after axonal injury has conflicting consequences for recovery of function: rescue of spared axons is impaired but regeneration is supported. J Neuroimmunol 1994; 50: 9–16.
Kremenchutzky M, Rice GP, Baskerville J, Wingerchuk DM, Ebers GC.