Post-mortem high-resolution MRI of the spinal cord in multiple sclerosis
A correlative study with conventional MRI, histopathology and clinical phenotype


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Summary
We used high-resolution MRI to study the post-mortem appearance of spinal cord multiple sclerosis in relation to histopathology and low-resolution images. Fifty-nine 3 cm long formalin-fixed spinal cord specimens from 19 multiple sclerosis patients and three controls were studied. Clinical characteristics of each patient were reviewed. High-field MRI consisted of proton-density weighted spin-echo imaging with an in-plane resolution of 80 µm. Specimens were also imaged at 1.0 T, with 1 mm pixel resolution. After MRI, the specimens were cut at 5 mm intervals and stained for myelin (Luxol fast blue/cresyl violet) and axons (Bodian method). Two observers scored the MRIs for abnormalities and divided them into (i) well-delineated areas of high signal intensity (SI) and (ii) poorly defined areas of mildly increased SI. Abnormalities were scored semiquantitatively, white matter and grey matter separately. In 81 sections the total area of abnormalities per section was measured on both histopathology sections and on matched high-field MRIs. Abnormalities ranged from just a few abnormal areas to complete involvement of the spinal cord specimen. Patients with an aggressive disease course had more abnormalities than patients with a mild or intermediate disease course. Areas of mildly increased SI were seen in all specimens, and were often found around focal high-SI lesions. However, in six patients, areas of mildly increased SI were the predominant finding on the MRIs, correlating with a primary progressive disease course. Histopathologically, high-SI areas correlated with complete demyelination, while mildly increased SI corresponded with partial demyelination. All areas scored as abnormal by the neuropathologist were also found on the MRIs, and sizes measured using both methods correlated well (r = 0.85, P < 0.01). On conventional MRIs, abnormalities could be recognized fairly well. However, better differentiation could be made between high-SI and mildly increased SI abnormalities on the 4.7 T images. In conclusion, high-resolution MRI revealed a great range of abnormalities in spinal cord multiple sclerosis, which related to disease course during life. Furthermore, we found very good correlation between the extent of abnormalities shown by histopathology and the SI changes on proton-density MRIs, mainly relating to demyelination revealed histopathologically.

Keywords: multiple sclerosis; MRI; spinal cord; histopathology

Abbreviations: AWM = anterior white matter; LWM = lateral white matter; NAWM = normal-appearing white matter; PD = proton density; PP = primary progressive; PWM = posterior white matter; SE = spin echo; SI = signal intensity; SP = secondary progressive; TE = echo time; TR = repetition time

Introduction
MRI studies show that spinal cord abnormalities are found in 80–90% of multiple sclerosis patients (Honig and Sheremata, 1989; Kidd et al., 1993; Tartaglino et al., 1995; Thorpe et al., 1996) even in the absence of spinal cord symptoms (O’Riordan et al., 1998). On sagittal MRI, focal areas of high signal intensity (SI) are seen in most patients, while on
proton density (PD) weighted spin echo (SE) sequences the entire spinal cord may appear diffusely abnormal (Lycklama à Nijeholt et al., 1997). In the axial plane, there is a preponderance of lesions in the lateral and posterior white matter columns (Tartaglino et al., 1995; Thielen et al., 1996). Differences in the appearance of spinal cord abnormalities reflect disease type and symptomatology (Lycklama à Nijeholt et al., 1998). Furthermore, cord atrophy may occur in relation to higher disability (Losseff et al., 1996). Despite the encouraging results of spinal MRI in multiple sclerosis so far, limited spatial resolution and motion artefacts may hinder image interpretation in vivo. As a result, small multiple sclerosis lesions may be missed, and the relationship between multiple sclerosis abnormalities and cord anatomy is difficult to assess. Better knowledge of the histopathological basis of MRI findings would be helpful for the interpretation of in vivo MRI studies in multiple sclerosis. Heterogeneity in MRI findings in spinal cord multiple sclerosis could then be related to histopathology.

We used high-resolution MRI to study post-mortem spinal cord specimens of multiple sclerosis patients. The images were compared with corresponding histopathology sections and with MRIs obtained at conventional field strength. Finally, we tested for correlated between spinal MRI findings and clinical findings during life.

**Material and methods**

The spinal cords of 19 multiple sclerosis patients and three controls (known not to have CNS disease) were obtained at autopsy under the management of the Netherlands Brain Bank. All patients and controls had previously given written approval for the use of their tissue, according to the guidelines of the Netherlands Brain Bank. Specimens (3 cm long) of spinal cord tissue were excised from the cervical, thoracic and lumbar spinal cord. In five patients only two cord specimens were obtained, while in one control only one specimen was obtained. Thus, 59 specimens were acquired, and were fixed in 10% formalin. The post-mortem delay until fixation was 5.5 h at the most.

One neurologist reviewed the clinical history of each patient, using all available hospital charts. Disease duration, the severity of disease and the occurrence of relapses were determined. In addition, the character of the disease course was judged to be aggressive (defined as severe disease resulting in death due to multiple sclerosis in <10 years), intermediate (disease course 10–20 years) or mild (a more prolonged disease course, the patient having died of old age and not from multiple sclerosis). Finally, the type of disease was classified as either secondary progressive (SP) or primary progressive (PP) (Lublin and Reingold, 1996).

All specimens were imaged at 4.7 T. In addition, one specimen from each subject was also studied at conventional field strength (1.0 T), using the same acquisition technique and receiver coil as used for conventional in vivo spinal MRI. As formalin fixationshortens MR relaxation times, PD remaining similar (Carvlin et al., 1989), we optimized our imaging sequence at 4.7 T by varying repetition time (TR) and echo time (TE). Based on these experiments, a combination of long TR and short TE was chosen, resulting in a PD-

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**Table 1 Clinical and spinal cord MRI findings in 19 multiple sclerosis patients studied post-mortem**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical characteristics</th>
<th>Type of abnormality (MRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex</td>
<td>Duration of disease (years)</td>
</tr>
<tr>
<td>97–189</td>
<td>F</td>
<td>15</td>
</tr>
<tr>
<td>96–115</td>
<td>F</td>
<td>19</td>
</tr>
<tr>
<td>97–338</td>
<td>M</td>
<td>19</td>
</tr>
<tr>
<td>96–102</td>
<td>F</td>
<td>25</td>
</tr>
<tr>
<td>96–194</td>
<td>F</td>
<td>43</td>
</tr>
<tr>
<td>96–234</td>
<td>F</td>
<td>49</td>
</tr>
<tr>
<td>97–155</td>
<td>F</td>
<td>54</td>
</tr>
<tr>
<td>96–232</td>
<td>F</td>
<td>4</td>
</tr>
<tr>
<td>96–059</td>
<td>F</td>
<td>6</td>
</tr>
<tr>
<td>96–307</td>
<td>M</td>
<td>22</td>
</tr>
<tr>
<td>95–161</td>
<td>F</td>
<td>7</td>
</tr>
<tr>
<td>97–283</td>
<td>M</td>
<td>23</td>
</tr>
<tr>
<td>96–116</td>
<td>F</td>
<td>11</td>
</tr>
<tr>
<td>97–024</td>
<td>F</td>
<td>15</td>
</tr>
<tr>
<td>97–192</td>
<td>F</td>
<td>14</td>
</tr>
<tr>
<td>96–352</td>
<td>F</td>
<td>16</td>
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<tr>
<td>96–060</td>
<td>F</td>
<td>19</td>
</tr>
<tr>
<td>97–202</td>
<td>M</td>
<td>7</td>
</tr>
<tr>
<td>95–276</td>
<td>M</td>
<td>12</td>
</tr>
</tbody>
</table>

SI = signal intensity; SP = secondary progressive; PP = primary progressive; SP/PP = progressive disease; presence of relapses not reliably assessable. *MRI findings for the spinal cord are expressed as the total score of abnormalities (high SI, mild SI increase and grey matter involvement) divided by the maximal score possible per type of abnormality (%).
Fig. 1 High-resolution MRIs (A) and corresponding conventional MRI (B) of a control specimen. Compared with the conventional MRI, the high-resolution MRI shows much more anatomical detail because of better spatial resolution (0.08 mm) than the conventional MRI (in-plane resolution 1 mm). High-resolution MRI (C) and corresponding histopathology sections (D and E). The sections were stained by the Bodian method (D, F and H) for axons and with Luxol fast blue/cresyl violet for myelin (E, G and I). The magnifications (×400 in F–I) can be compared with those of multiple sclerosis patients (Figs 2–5) to appreciate the axonal density and degree of myelin staining.

Contiguous axial slices (1 mm thick) were obtained with an SE sequence [3000/15/10 (TR/TE/NEX) (where NEX = number of excitations)] using a small solenoid receiver coil. In-plane resolution was 80 µm and acquisition time was 1 h. At 1.0 T, axial slices (3 mm) with an interslice gap of 0.3 mm
were obtained with a standard SE sequence (2200/14/3), using a spinal phased-array surface coil. In-plane resolution was 1 mm. The specimens were submerged in formalin solution to avoid truncation artefacts in the phase-encoding direction (Curtin et al., 1989). Acquisition time was 10 min.

After completing MRI, all spinal cord specimens were cut transaxially into six pieces, 0.5 cm thick, evenly spaced through the sample. From each tissue block, 8 µm thick histological sections were stained to assess general cellularity (haematoxylin and eosin), myelin (Luxol fast blue/cresyl violet) and axons (Bodian method). They were matched with high-resolution MRIs. A complete autopsy of the brain and spinal cord was performed in all cases, and the clinical diagnosis of multiple sclerosis was confirmed for the multiple sclerosis patients. No abnormalities were found in the control patients.

All high-resolution MRIs of patients and controls were printed out and scored for abnormalities by mutual agreement between two observers. Abnormalities were divided into well-delineated areas of high SI (higher than grey matter) and more poorly defined areas of mildly increased SI [lower than grey matter but higher than normal-appearing white matter (NAWM)]. We scored the anterior (AWM), lateral (LWM) and posterior (PWM) white matter columns separately for both the left and the right side. A semiquantitative scoring system was used: a score of 1 was given if <25% of a particular white matter column was affected; 2 for 25–50% involvement; 3 for >50%; and 4 if the entire column was affected on a particular slice. The grey matter was scored separately (1, <25% affected; 2, 25–50% affected; 3, 50% affected but recognizable; 4, if the grey matter was not recognizable as such). Lesion load per white matter column was calculated as the total score over all slices divided by the highest score possible.

For the 1.0 T MRIs, the number of slices showing increased signal intensity, described for the different white matter columns of the spinal cord, was scored with similar differentiation in high-SI abnormalities and mildly increased SI. However, due to lower spatial resolution, no semiquantitative scale was applied.

In order to study quantitative correlations between high-resolution MRIs and histopathology, a subset of 81 sections, selected from 10 patients to cover a wide range of abnormalities, was analysed as follows. The neuropathologist indicated the borders of abnormal areas on photographs of low-power magnifications (125%) of Luxol fast blue/cresyl violet-stained sections. Abnormalities were categorized as sharply defined areas with no residual staining, or more vaguely delineated areas representing decreased myelin staining. A different observer further quantified the area of all abnormal regions (as indicated on the photographs), using dedicated computer-based, home-developed quantitative pathology software. The matched high-resolution MRIs were quantified by another reader, who was unaware of the histopathological findings, using home-developed local thresholding software. Furthermore, SI was measured in areas of focal high SI, areas of mild SI increase and in NAWM, and the surrounding air (noise). SI measurements were performed in six patients with a large range of abnormalities and in two controls. Measurements were performed on four slices from each subject.

For all comparisons, a paired test for unevenly distributed data was used (Wilcoxon test), while correlations were assessed using Spearman’s rank correlation test (r).

**Results**

Clinical, MRI and histopathological data are summarized in Table 1. Median age at diagnosis was 37 years (range 23–67 years). Disease duration ranged from 7 to 54 years (median 19 years). In nine patients, the disease course was SP and in seven patients it was PP. In another three patients, a reliable classification could not be made retrospectively.

None of the control samples showed abnormalities (Fig. 1). The PD-weighted MRIs showed good contrast between grey and white matter, and the high spatial resolution enabled visualization of separate white matter columns (Fig. 1). In all multiple sclerosis patients, areas of increased SI were found. Regarding areas of high SI, there was considerable variation in the number of abnormalities between patients (Table 1). In eight patients, only a few high-SI abnormalities were seen, which extended over only a few slices and involved <25% of the cross-sectional area (Fig. 2). In contrast, in seven patients all slices showed severe high-SI abnormalities, involving the whole cross-sectional area of the cord, rendering the grey matter unrecognizable and deforming the spinal cord contour (Figs 3 and 4). A total of 192 separate lesions were counted in 54 specimens. High-SI lesions were found more frequently in the LWM and PWM than in the AWM (Table 2). The cranio-caudal length of the high-SI abnormalities was difficult to assess because in most cases the abnormalities encompassed the entire spinal cord sample. However, 27 of 192 (14%) lesions began and ended within the specimen, thus being <3 cm long.

Areas of mildly increased SI were seen in all patients, often being found around focal high-SI lesions (Figs 2 and 4). However, in six patients, areas of mildly increased SI were the predominant finding on MRIs, with only few focal high-SI abnormalities and the central grey matter remaining clearly recognizable (Fig. 5). In the patients, the signal-to-noise ratio in areas of focal high SI was higher (median 57, range 46–141) than in areas of mild SI increase (median 49, range 35–114; P = 0.005).

Histopathological examination of specimens from multiple sclerosis patients revealed changes compatible with multiple sclerosis in all patients. In accordance with the high-resolution MRI findings, histopathological abnormalities ranged from only a few abnormalities to complete involvement of all sections examined. Focal areas of high SI, as seen on MRIs, displayed complete demyelination, while on corresponding axon stains a variable degree of axonal loss was found; in some cases no axonal loss was apparent in relation to NAWM.
in the same section (Figs 2–5). Areas of mildly increased SI corresponded with areas of partial demyelination. All areas scored as abnormal by the neuropathologist on Luxol fast blue/cresyl violet stains were found as areas of abnormal signal intensity on 4.7 T images, suggesting 100% sensitivity for abnormalities. Also, the total area of abnormalities, as measured on histopathology sections, correlated well with the total area of high-SI abnormalities on 4.7 T images (r = 0.85, P = 0.000; Fig. 6). Nevertheless, the total area of abnormalities on the MRIs (median 52 mm², range 28–93 mm²) was higher than scored by the neuropathologist (median 44 mm², range 23–77 mm²; P = 0.000). A review of the discrepancies revealed that this difference was mainly caused by areas of mildly increased SI on MRIs. Such areas were initially not always judged as abnormal by the neuropathologist, and exhibited intermediate staining density on the Luxol fast blue/cresyl violet stains, suggestive of partial demyelination rather than typical, completely demyelinated multiple sclerosis plaques. Correlation of the size of abnormalities seen on MRIs and on neuropathology sections improved when only high-SI MRI abnormalities and typical, completely demyelinated multiple sclerosis plaques were compared (r = 0.94, P = 0.000). While most high-SI plaques were inactive pathologically, some had evidence of active disease, as suggested by the presence of foam cells. On MRIs, no distinction could be made between inactive lesions and lesions showing partial activity. Furthermore, the degree of gliosis was not reflected in differences in MRI appearance. In six patients, mildly increased SI abnormalities were the predominant abnormality (Table 1 and Fig. 5). In these patients, histopathological findings consisted mainly of areas of partial demyelination, while gliosis was scarce. Also, the
grey matter remained clearly recognizable. Axonal density in such areas of mild SI increase did not appear different from that in NAWM in the same section (Fig. 5). However, compared with control specimens, there seemed to be some axonal thinning (compare with Fig. 1).

MRI examination of patients who suffered an aggressive disease course showed more abnormalities (both high-SI and mildly increased SI) than patients with a mild or intermediate disease course (Fig. 7). In patients with a disease course suggestive of PP multiple sclerosis, extensive involvement of the spinal cord was seen, but mainly with a mild SI increase and little grey matter involvement, compared with SP patients (Fig. 8). Furthermore, in PP multiple sclerosis patients, the proportion of areas with mildly increased SI in the total amount of abnormalities was higher. In SP patients, a reverse pattern was seen: most abnormal areas consisted of high-SI changes (Table 1).

No abnormalities were detected in control specimens using 1.0 T images, whereas all spinal cord specimens of multiple sclerosis patients showed abnormalities at 1.0 T. Cross-sectional anatomy could be recognized fairly well on the 1.0 T images in most cases (Figs 1–5), enabling localization of the abnormalities within white matter columns and/or grey matter. High-SI abnormalities, as seen on 4.7 T images, were also identified on 1.0 T images. However, on 4.7 T images, high-SI and mildly increased SI abnormalities could be better differentiated (Table 3), and larger numbers of the latter type of abnormalities were seen at 4.7 T.

**Discussion**

Our study involved a large series of patients and a wide range of abnormalities. The results are in concordance with a previous study concerning the post-mortem MRI appearance of the spinal cord in one patient (Nagao et al., 1994). We found that the sensitivity of high-resolution MRI was very high when compared with histopathology. In addition, we saw that the amount and type of MRI abnormalities correlated with the clinical course during life, even though they were established in retrospect.

We found typical focal areas of high SI, which represented complete demyelination on histopathology. Such abnormalities extended into the grey matter in many cases, especially in patients with aggressive disease. The morphology, location

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**Fig. 2** High-resolution MRIs, conventional MRI and corresponding histology sections of multiple sclerosis case 95–276. This male patient had died at age 56 years having had progressive multiple sclerosis for 12 years. He had a progressive disease course, probably with some relapses, although this could not be confirmed from the medical records. Contiguous high-resolution MRIs from a high thoracic cord specimen (A) show areas of well-demarcated high-SI abnormalities (straight arrow) extending over several contiguous 1 mm thick slices. Some areas of mild SI increase are indicated by the curved arrow. Correlation between MRI findings at 4.7 and 1.0 T was studied at a cervical spinal cord level (B and C). This level was also used for comparison with histopathology (D–L). Note that for the 4.7/1.0 T comparison, the 4.7 T image is rotated to facilitate interpretation (B). The histology sections were stained by the Bodian method (E, G, I and J) for axons and with Luxol fast blue/cresyl violet for myelin (F, H, K and L). The microscopic images show areas of complete demyelination (K), corresponding to high-SI changes on the MRIs. The wavy fibres in the corresponding Bodian-stained image (I) correspond to intense gliosis. Areas of NAWM (G and H) are seen. Areas of partial demyelination are noted around the areas of complete demyelination (J and L), corresponding with mild increases in SI on the MRIs (D). The presence of partial demyelination was found by the neuropathologist independently of the MRI findings. In these areas, axonal density appears to be normal compared with NAWM (H), although there appears to be some axonal thinning (J versus G; also compare with Fig. 1).
Fig. 3 High-resolution MRIs, conventional MRI and corresponding histology sections of multiple sclerosis case 96–116. This woman had died from complications of multiple sclerosis at age 36 years after having had an aggressive secondary progressive disease course for 11 years. High-resolution MRIs (A) and corresponding conventional MRI (B) of cervical spinal cord. The high-resolution MRIs show severe high-SI changes, sparing only a small area in the AWM, which shows a mild increase in SI. The 1.0 T image shows good correlation with the 4.7 T images, depicting the relatively normal area in the AWM (arrow). Comparison with histopathology was performed at another cervical level (C). The histology sections (D–I) were stained by the Bodian method (D, F and H) for axons and with Luxol fast blue/cresyl violet for myelin (E, G and I). The microscopic images show that the high-SI changes represent areas of complete demyelination (I) and intense gliosis (H). The residual blue staining in the Luxol fast blue/cresyl violet-stained images (E, arrow) represents gliotic tissue, not myelinated axons. The mild increase in SI in the AWM corresponds with partial demyelination (G).
Post-mortem MRI in spinal cord multiple sclerosis

Fig. 4 High-resolution MRIs, conventional MRI and corresponding histology sections of multiple sclerosis case 95–161. This woman had died at age 41 years after having had an aggressive secondary progressive disease course for 7 years. High-resolution MRIs (A) and corresponding conventional MRI (B). The high-resolution MRIs show high-SI abnormality in the LWM, partially involving the central grey matter. In the PWM a mild increase in SI is noted. The corresponding histology sections (D–K) were stained by the Bodian method (D, F, H and I) for axons and with Luxol fast blue/cresyl violet for myelin (E, G, J and K). The microscopic images show that the high-SI changes represent areas of complete demyelination (K) and intense gliosis (I). The mild increase in SI in the PWM corresponds with partial demyelination (J and H) compared with NAWM in the AWM (F and G). Note that axonal density in the area with partial demyelination (F) is comparable to that of NAWM (E).

and shape of such isolated high-SI lesions were comparable to those found in histopathology studies (Fog, 1950; Oppenheimer, 1978); the lesions appeared wedge-shaped and were mostly located in lateral and posterior columns. Apart from well-demarcated, focal high-SI abnormalities, we encountered areas of relatively mild SI increase, corresponding histopathologically with partial demyelination. Such areas were not typically demyelinated multiple sclerosis
Fig. 5 High-resolution MRIs, conventional MRI and corresponding histology sections of multiple sclerosis case 96–307. This man had died at age 72 years after having had a slowly progressive disease course for 22 years, with only a few relapses. High-resolution MRIs (A) show mainly mild SI changes (white stars), while a few high-SI changes are also found (open arrows). The corresponding conventional 1.0 T MRI (C) and histopathology sections (E–L) were selected at a different cervical level. The conventional MRIs show the same abnormalities in the LWM as the 4.7 T image (B and C), although differentiation between high-SI changes (C, arrow) and mildly increased SI is not possible on the 1.0 T image. The corresponding histopathology sections were stained by the Bodian method (E, G, I and J) for axons and with Luxol fast blue/cresyl violet for myelin (F, H, K and L). The microscopic images show that the mild SI changes represent areas of partial demyelination (K). Note that axonal density in the area with partial demyelination (J) is comparable to that of NAWM (G). The wedge-shaped area of high-SI change (D) corresponds with complete demyelination (K) and gliosis (I).
as patients with a PP disease course during life tended to focal high-SI lesions. In this study, we found a similar trend, of diffuse SI increases on PD-weighted SE images, without extensive abnormalities in the spinal cord, which consisted which we found that PP multiple sclerosis patients often had areas of mild SI increase, which is in accordance with our in vivo findings. However, we did not quantitate axonal density or the amount of myelinated axons. We will now discuss the possible nature of the areas of mild SI increase.

First, we found that the environment of focal multiple sclerosis plaques often showed areas of mildly increased SI. This also corresponds well with the histopathology findings in the brain in multiple sclerosis patients, since the NAWM surrounding multiple sclerosis lesions usually shows subtle abnormalities (Newcombe et al., 1991; Barbosa et al., 1994; Husted et al., 1994; Gasperini et al., 1996). Secondly, a mild SI increase may represent a different pathological process, leading to partial demyelination and extending more diffusely. This also corresponds well with the histopathology findings in PP multiple sclerosis patients compared with SP multiple sclerosis patients (Revesz et al., 1994). Of course, our findings with regard to the relation between the type of abnormality and the disease course must be interpreted with some caution as there was some overlap, as illustrated by some patients with a slow disease course who did show extensive abnormalities (both high SI and mild increases in SI). This discrepancy may be explained partly by the fact that the disease course was assessed retrospectively. Furthermore, we did not examine the brains of our patients, nor did we examine the spinal cord over its entire length. Imaging the whole CNS post-mortem should theoretically improve the relation between MRI and clinical variables.

Thirdly, an explanation for the occurrence of areas of mild SI increase could be Wallerian degeneration. In our samples, this seemed not to be very likely as Bodian staining revealed only subtle changes compared with the surrounding NAWM; apparently, axonal loss is not the main factor contributing to the signal increase on the corresponding PD-weighted MRIs. However, we did not quantitate axonal density or the amount of myelinated axons. We will now discuss the possible nature of the areas of mild SI increase.

Table 2 Signal intensity changes in different white matter columns, studied at 4.7 T in 19 cases of multiple sclerosis

<table>
<thead>
<tr>
<th></th>
<th>Anterior white matter</th>
<th>Lateral white matter</th>
<th>Posterior white matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median high-SI lesion load</td>
<td>1.5% (0–100)</td>
<td>13% (0–100)</td>
<td>16% (0–100)</td>
</tr>
<tr>
<td>Median mild SI increase</td>
<td>0% (0–100)</td>
<td>20% (0–67)</td>
<td>10% (0–100)</td>
</tr>
<tr>
<td>Median total lesion load</td>
<td>3% (0–50)</td>
<td>30% (0–51)</td>
<td>19% (0–50)</td>
</tr>
</tbody>
</table>

Lesion load was calculated as the total score per white matter column divided by the maximal score possible, and is expressed as a percentage. SI = signal intensity.

Table 3 MRI findings in 19 multiple sclerosis patients at 1.0 and 4.7 T

<table>
<thead>
<tr>
<th></th>
<th>1.0 T</th>
<th>4.7 T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of abnormal specimens (%)</td>
<td>21 (100)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Median high-SI LWM involvement (range)</td>
<td>20 (3–56)</td>
<td>13 (0–48)</td>
</tr>
<tr>
<td>Median mild SI increased LWM involvement (range)</td>
<td>0 (0–20)</td>
<td>29 (0–50)*</td>
</tr>
<tr>
<td>Median high-SI PWM involvement (range)</td>
<td>15 (0–43)</td>
<td>22 (0–48)</td>
</tr>
<tr>
<td>Median mild SI increase PWM involvement (range)</td>
<td>0 (0–7)</td>
<td>9 (0–38)+</td>
</tr>
<tr>
<td>Median high-SI AWM involvement (range)</td>
<td>0 (0–43)</td>
<td>0 (0–48)</td>
</tr>
<tr>
<td>Median grey matter involvement (range)</td>
<td>28 (0–56)</td>
<td>27 (0–48)</td>
</tr>
<tr>
<td>Number of specimens with recognizable grey matter (%)</td>
<td>10 (48)</td>
<td>17 (81)</td>
</tr>
</tbody>
</table>

*P = 0.007; †P = 0.01; ‡P = 0.028 (Wilcoxon test). SI = signal intensity; LWM = lateral white matter; AWM = anterior white matter; PWM = posterior white matter.

Table 2 Signal intensity changes in different white matter columns, studied at 4.7 T in 19 cases of multiple sclerosis

plaques and the neuropathologist did not recognize all such areas prospectively, resulting in some discrepancy with the MRI findings. However, when the histopathology and MRIs were viewed jointly, all areas of mild SI increase were found to correspond with areas of partial demyelination. This illustrates the usefulness of our MRI protocol as an adjunct to histopathological analysis in relatively subtle abnormalities. On Bodian stains, areas of mild SI change showed only subtle changes compared with the surrounding NAWM; apparently, axonal loss is not the main factor contributing to the signal increase on the corresponding PD-weighted MRIs. However, we did not quantitate axonal density or the amount of myelinated axons. We will now discuss the possible nature of the areas of mild SI increase.

First, we found that the environment of focal multiple sclerosis plaques often showed areas of mildly increased SI. This also corresponds well with the histopathology findings in the brain in multiple sclerosis patients, since the NAWM surrounding multiple sclerosis lesions usually shows subtle abnormalities (Newcombe et al., 1991; Barbosa et al., 1994; Husted et al., 1994; Gasperini et al., 1996). Secondly, a mild SI increase may represent a different pathological process, leading to partial demyelination and extending more diffusely. This also corresponds well with the histopathology findings in PP multiple sclerosis patients compared with SP multiple sclerosis patients (Revesz et al., 1994). Of course, our findings with regard to the relation between the type of abnormality and the disease course must be interpreted with some caution as there was some overlap, as illustrated by some patients with a slow disease course who did show extensive abnormalities (both high SI and mild increases in SI). This discrepancy may be explained partly by the fact that the disease course was assessed retrospectively. Furthermore, we did not examine the brains of our patients, nor did we examine the spinal cord over its entire length. Imaging the whole CNS post-mortem should theoretically improve the relation between MRI and clinical variables.

Thirdly, an explanation for the occurrence of areas of mild SI increase could be Wallerian degeneration. In our samples, this seemed not to be very likely as Bodian staining revealed only subtle changes, and the areas of mild SI increase were often not continuous throughout the specimen, as would be expected in Wallerian degeneration. Nevertheless, it is conceivable that Wallerian degeneration contributes at least partially to the mild SI changes seen in the spinal cord of multiple sclerosis patients. Quantitative studies of axonal density are warranted in order to resolve this issue, especially in the light of recent studies demonstrating axonal loss in humans with multiple sclerosis (Trapp et al., 1998) and in a
Correlation between total area of abnormalities (both focal high-SI changes and areas of mildly increased SI on 4.7 T MRIs), and histopathological measurement of areas of demyelination. $r = 0.86; P = 0.00$. For this analysis a subset of 81 histopathology sections from 10 patients were used, chosen to represent a wide range of abnormalities.

Box plot showing relation between disease course and percentage involvement of the spinal cord at 4.7 T (both high-SI and mild SI changes). Bars represent the median amount of abnormalities, expressed as percentages of the maximal amount possible.

Fig. 6

Fig. 7

Murine demyelination model (McGavern et al., 2000). Indeed, a recent study of the axonal density of spinal cord samples of multiple sclerosis patients clearly showed considerable axonal loss, even in NAWM (Lovas et al., 2000).

Our study was limited by the lack of other MRI sequences which may provide a more accurate distinction between mild and severe lesions, such as magnetization transfer imaging (Miki et al., 1999), T$_1$-weighted MRI (Truyen et al., 1996) and diffusion-weighted MRI (Larsson et al., 1992). Indeed, it is well known that conventional PD- or T$_2$-weighted MRI has limited power in detecting lesion heterogeneity. Another limitation in our study was the use of formalin fixation, as it limited us to the use of long-TR, short-TE imaging, as T$_1$ relaxation and MTR (magnetization transfer ratio) are affected by fixation (Carvlin et al., 1989). Furthermore, we did not use quantitative histopathological markers, e.g. the amount of axonal degeneration, which has been found to correlate with T$_1$ prolongation (van Walderveen et al., 1998). Such in-depth studies could further elucidate the nature of the pathological heterogeneity that is described in this study.

In this study, conventional imaging at 1.0 T was sensitive for multiple sclerosis abnormalities, and image contrast between abnormalities and normal spinal cord tissue was generally good. However, it was limited in detecting lesion heterogeneity and exactly localizing abnormalities within the white matter columns and grey matter. Both limitations probably reflect the relatively poor spatial resolution of conventional MRI at present. Hopefully, future MRI developments, such as higher field strength and improvements in receiver coils and gradients, will improve spatial resolution, so that lesion morphology and heterogeneity may be better appreciated in patients during life.

In conclusion, we present the results of a study in which spinal MRI in multiple sclerosis was optimized by increasing spatial resolution. The MRI findings correlated well with histopathological findings, which suggested the differentiation of multiple sclerosis abnormalities into (i) focal high-SI abnormalities representing typical demyelinated plaques, and (ii) less well-defined areas of mildly increased SI representing partial demyelination histopathologically. Since the latter
type of abnormality was associated with PP disease, we suggest that such abnormalities may be comparable with the diffuse SI changes observed in vivo. The results of imaging at conventional field strength suggest that, despite its good sensitivity, its main shortcoming is lack of spatial resolution, resulting in limited power to differentiate between several types of abnormality.

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References


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