PAPER

Spinal cord atrophy and disability in multiple sclerosis over four years: application of a reproducible automated technique in monitoring disease progression in a cohort of the interferon β-1a (Rebif) treatment trial

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Background: Pathology in the cervical spinal cord is considered an important cause of disability in multiple sclerosis. However, the majority of serial studies have failed to find a correlation between spinal cord atrophy and disability.

Objectives: To use a highly reproducible and accurate method to quantify spinal cord area change on three dimensional magnetic resonance imaging and relate this to disability change in patients with multiple sclerosis.

Methods: 38 patients with multiple sclerosis (20 with the relapsing–remitting (RRMS) form and 18 with the secondary progressive (SPMS) form) were imaged at baseline and at months 6, 12, 18, and 48 during two treatment trials of the high dose subcutaneous thrice weekly interferon β-1a (IFNβ, Rebif). Thirty one healthy subjects were also imaged at baseline. Upper cervical cord area (UCCA) was measured using Sobel edge detection.

Results: The intraobserver coefficient of variation of the method was 0.42%. A significant reduction in UCCA was detected at month 6 in the placebo group ($p = 0.04$) and at month 12 for INFβ ($p = 0.03$). The mean reduction of UCCA at month 48 was 5.7% for patients initially on placebo who received treatment at 24 months (RRMS) or at 36 months (SPMS), and 4.5% for those on IFNβ throughout the study ($p = 0.35$). The change in UCCA was significantly correlated with change in the expanded disability status scale at month 12 ($r = 0.4$, $p = 0.016$), month 18 ($r = 0.32$, $p = 0.05$), and month 48 ($r = 0.4$, $p = 0.016$) in the total cohort.

Conclusions: Despite the small number of patients studied and the possible confounding effects of interferon treatment, this study showed that edge detection is reproducible and sensitive to changes in spinal cord area, and that this change is related to changes in clinical disability. This suggests a role for measurement of spinal cord atrophy in monitoring disease progression and possible treatment effects in clinical trails.

Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system (CNS) and is a major cause of severe neurological disability in young adults. Recent studies have shown that axonal dysfunction and loss are common in multiple sclerosis and are likely to be the most important factors leading to irreversible disability.[1–3] Magnetic resonance imaging (MRI) provides a method to estimate tissue loss (atrophy) in the CNS and has shown that atrophy correlates with axonal pathology[4 5] and disability[6–8] in multiple sclerosis. This suggests that atrophy of the CNS reflects clinically relevant pathological processes and has the potential to provide an index to monitor the evolution of the disease and its response to treatment.

As multiple sclerosis pathology is common in the spinal cord and is a significant contributor to locomotor disability, there is much interest in evaluating atrophy of this structure using MRI. A strong correlation has been found between the area/volume of the spinal cervical cord and disability in cross sectional studies.[8–10] Longitudinally, several studies have shown a change in spinal cord area over time using a reproducible method.[11 12] However, apart from one study,[13] no correlation between the change in cord area and disability has been reported, which probably reflects the short duration of the follow up[11] or limitations of the methods used.[14–16]

In this study we aimed to measure change in spinal cord area over four years—using a highly reproducible technique[17]—in 20 relapsing–remitting (RRMS) and 18 secondary progressive (SPMS) patients with multiple sclerosis, and to relate this to change in disability.

METHODS

Subjects

Thirty eight patients classified as having clinically definite multiple sclerosis (20 RRMS, 18 SPMS)[18] were recruited for two phase III studies of subcutaneous recombinant human interferon β-1a (IFNβ, Rebif).[19 20] Both trials initially consisted of three arms: placebo, 22 μg of IFNβ given three times a week, and 44 μg of IFNβ given three times a week. The placebo controlled phase lasted two years in the PRISMS (RRMS) study and three years in the SPECTRIMS (SPMS) study, after which patients on placebo were randomised to active treatment with IFNβ at one of the two doses. Thus over the four year study period there were four treatment regimens in both RRMS and SPMS patient groups. Only one patient had a course of intravenous methyl prednisolone within 28 days of a scan. The mean numbers of courses of intravenous methyl prednisolone

Abbreviations: EDSS, expanded disability status scale; MICA, mean maximum intracranial area; PPMS, primary progressive multiple sclerosis; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; TD, time of delay; TE, time of echo; TI, time of inversion; TR, time of repetition; UCCA, upper cervical cord area

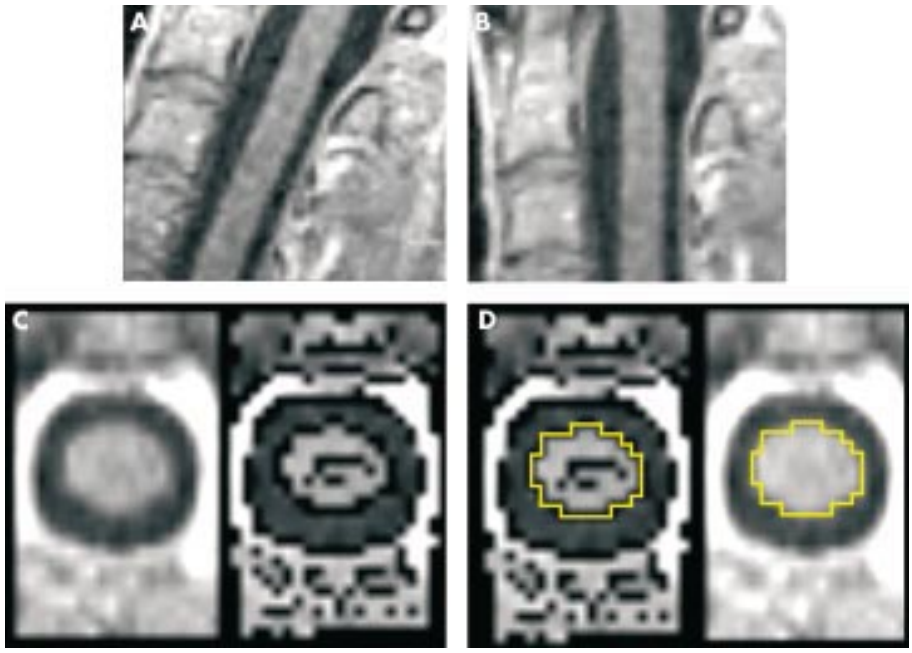

Figure 1 Sagittal spinal cord image before (A) and after rotation (B); cross sectional image of the spinal cord before and after removal of the edge voxels detected using the Sobel operator and non-maximal suppression (C), and region of interest defined automatically on the edge removed image and placed on the original image (D).

over the four years were 1.0 and 0.35 for the RRMS and SPMS groups, respectively. Disability was scored on the expanded disability status scale (EDSS)[21] within 24 hours of each MRI scan. The study was conducted with local ethics approval and with full patient consent.

MRI protocol

All patients underwent MRI on study day 1 (month 0) and at months 6, 12, 18, and 48. MRI was done with a 1.5 T system (Magnetom SP4000, Siemens, Erlangen, Germany) with a proprietary head coil. Proton density and T2 weighted dual echo sequences with 5 mm transverse contiguous images were acquired (TR 2500 ms; TE 27/81 ms; matrix 256 × 256; field of view 25 cm). T1 weighted spin echo images (TR 580 ms; TE 10 ms) and three dimensional (3D) magnetisation prepared, rapid acquisition gradient echo (MPRAGE) images (TR 10 ms; TE 4 ms; TD 100 ms; TI 300 ms; flip angle 10°) were obtained 10 minutes after intravenous gadolinium-DPTA at 0.1 mmol/kg. The MPRAGE image matrix was 256 × 256 × 180 with a 25 cm field of view, providing 128 sagittal images with a slice thickness of 1.4 mm. The same MRI protocol was used during a separate pilot study in 31 healthy subjects who were age and sex matched to the patients. These data were used for baseline comparisons with the patients.

MR images post-processing and analysis

Measurement of the cross sectional upper cervical cord area (UCCA) was done using software developed locally (by CRT) by an experienced investigator (XL). The UCCA was measured at the C2–C3 segments. Briefly, the images were first rotated such that the spinal cord was perpendicular to the axial plane (fig 1, panels A and B). Then in the mid-sagittal plane, a volume extending from the upper border of C2 rostrally to the centre of the C2/3 intervertebral disc caudally was defined. In the axial plane, the boundary between the cord and cerebrospinal fluid (CSF) was then determined using an automatic edge detection method (fig 1, panel C) based on the Sobel operator and non-maximal suppression.[22]

The Sobel operator measures an image intensity gradient in the transverse plane for each voxel. The gradient magnitude is highest for partially volumed voxels, which provides a means of detecting the boundary between cord and CSF. Thus the method does not depend directly on the voxel intensities, but requires that there be a difference in intensity to define the edge. By eliminating all voxels except those where the gradient magnitude is maximal in the direction of the gradient, the boundary may be localised precisely; this process is called non-maximal suppression.[22]

A region of interest delineating the automatically detected cord edge was defined on each axial slice (fig 1, panel D), the areas of which were then averaged to obtain the UCCA. To account for biological variation in spinal cord size,[8 23] the baseline UCCA was normalised by multiplying by the ratio of the mean maximum intracranial area (MICA) for the total population to the MICA for each individual. This is based on a previously observed result showing that the MICA was significantly correlated with UCCA in healthy controls.[17] The MICA was measured by defining a region of interest for each subject, using seed growing, at the inner table of the skull on the largest observed brain slice. In the longitudinal study of UCCA, non-normalised measures were used.

The number of T1-gadolinium enhancing lesions (T1-Gd lesions) was measured from the 3D MPRAGE images using axial slices with a slice thickness of 3 mm.[24] T2 lesion volume was determined by outlining observer identified lesions on T2 weighted images using a semiautomated technique on ANALYZE (Biomedical Imaging Resource, Mayo Foundation, Minnesota, USA).

Reproducibility and quality control

To evaluate the intrarater reproducibility, the same observer repeated the UCCA measurements twice on 16 randomly selected subjects at least one week apart. Five healthy controls (three male, two female) age matched to the RRMS patients underwent serial imaging on the same MRI scanner for periods of six to 24 months (median 23 months). These data were used to estimate the errors such as change in the scanner field over time and diurnal variation.

Neutralising antibody assay

Neutralising antibodies were measured by cytopathic assay according to the method of Kawade[25]; the assays were done by LCG-RBM (Livrea, Italy) on behalf of Serono International SA.

Table 1 Demographics of controls and patients

	Control (n=31)	RRMS (n=20)	SPMS (n=18)
Age (years)	37.5 (7.3)	32.9 (7.8)	39.9 (6.2)
M : F	14:17	6:14	7:11
EDSS	–	1.7 (1.1)	5.5 (1.1)*
Disease duration (years)	–	5.5 (4.4)	12.8 (6.6)*
Relapses¶	–	3.8 (1.4)	1.1 (1.3)*
MICA (cm^2)	197.8 (14.4)	199.3 (12.8)	192.8 (12.4)
Normalised UCCA (mm^2)	80.4 (5.7)	77.7 (10.1)	74.5 (11.6)†

Values are mean (SD).
*Significant *v* RRMS; †significant *v* control.
¶Relapse rate during two years before the study.
EDSS, expanded disability status scale; MICA, maximum intracranial area; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; UCCA, upper cervical cord area.

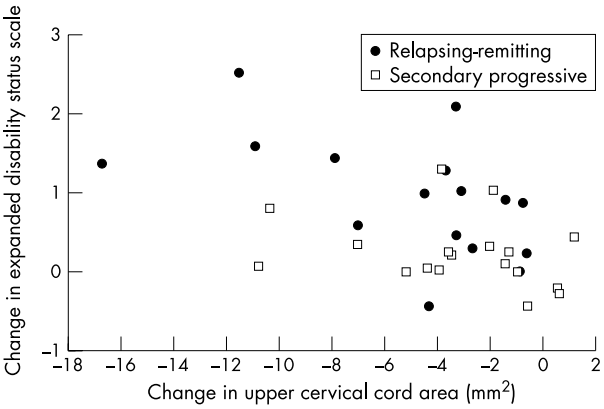

Figure 2 Correlation between change in upper cervical cord area (UCCA) and change of expanded disability status scale (EDSS) in the total patient cohort at month 48 ($r = 0.4$, $p = 0.016$).

Statistical analysis

For comparison of baseline clinical and MRI data between groups we used the unpaired *t* test. The significance of any within-group change in UCCA from baseline was assessed using a paired *t* test for both the placebo and the IFNβ treated patients. This analysis was undertaken for both absolute and percentage change in UCCA estimates. The "summary measure" statistic described in Liu *et al* was used to define EDSS change.[26 27] In brief, EDSS was calculated using the trapezoidal rule to estimate the area under the EDSS curve and above the baseline EDSS score for each patient. The EDSS curve for each patient included all EDSS scores from baseline to the 48 month visit. Correlations were explored with Spearman's rank coefficient.

RESULTS

Reproducibility

The intraobserver coefficient of variation for the UCCA measurements was 0.42% (0.34 cm^2). The reproducibility of the normalised UCCA was 0.53% (0.42 cm^2). The mean (SD) UCCA in five normal controls was 80.29 (3.8) and 80.4 (3.3) at baseline and follow up, respectively, with a mean change of 0.5%.

Baseline UCCA

Demographics of controls and patients are presented in table 1. There was a significant difference in normalised UCCA between the SPMS group and controls ($p = 0.04$), but not between the RRMS group and controls. The normalised UCCA did not correlate with EDSS in the total cohort of patients ($r = -0.2$, $p = 0.3$).

At baseline, the patients in the placebo and INFβ groups were matched for age (35.4 (7.8) *v* 36.5 (8.1) years), disease duration (9.8 (6.8) *v* 8.5 (6.6) years), EDSS (3.3 (2.2) *v* 3.6 (2.3)), baseline UCCA (72.3 (12.3) *v* 77.6 (9.4) mm^2), T2 lesion volume (9.05 (11.1) *v* 8.3 (9.7) cm^3), and number of T1-Gd lesions (0.67 (1.4) *v* 1.16 (1.86)).

Longitudinal change in UCCA

There were no significant differences in the change of UCCA between RRMS and SPMS groups; thus the data for the two groups were analysed together. The UCCA values and their changes over the study period are given in table 2. In patients initially on placebo, a significant reduction in UCCA was detected at six months compared with baseline ($p = 0.04$). Further reductions were seen at subsequent months, and by month 48 there was a mean reduction of 5.7% compared with baseline ($p = 0.001$). In patients on INFβ throughout the study, a significant reduction in UCCA was observed at 12 months compared with baseline ($p = 0.03$). Further reductions were seen throughout the study, and by month 48 the UCCA was reduced by 4.5% compared with baseline ($p = 0.002$). At no time point was the UCCA change in the INFβ treatment-throughout group significantly different from that in the patients initially on placebo (table 2).

Table 2 Upper cervical cord area measurements and percentage changes

		Placebo (RR=7; SP=6)	INFβ (RR=13; SP=12)	p Value†
Month 0	UCCA (mm^2)	72.3 (12.3)	77.6 (9.4)	
Month 6	UCCA (mm^2)	71.1 (12.5)*	75.9 (8.7)	
	Change (%)	−1.7 (2.3)	−1.0 (3.6)	0.56
Month 12	UCCA (mm^2)	70.1 (11.6)*	75.7 (9.3)*	
	Change (%)	−2.8 (3.0)	−1.5 (3.3)	0.23
Month 18	UCCA (mm^2)	69.7 (11.8)*	75.3 (8.9)*	
	Change (%)	−2.9 (3.3)	−1.8 (3.6)	0.21
Month 48	UCCA (mm^2)	68.1 (11.6)**	73.2 (8.1)*	
	Change (%)	−5.7 (4.6)	−4.5 (5.6)	0.35

Values are mean (SD).
†Difference in UCCA change between placebo and INFβ at each time point.
*$p < 0.05$, **$p = 0.001$ *v* baseline.
Placebo: patients on placebo at 18 months and received interferon treatment at 24 months (RRMS) and 36 months (SPMS) onwards; INFβ: patients on interferon throughout the study.
MS, multiple sclerosis; RR, relapsing–remitting; SP, secondary progressive; UCCA, upper cervical cord area.

In the overall patient cohort, the change of UCCA was significantly correlated with the change of EDSS at month 12 ($r = 0.4$, $p = 0.016$), month 18 ($r = 0.32$, $p = 0.05$), and month 48 ($r = 0.4$, $p = 0.016$) (fig 2). The median change of EDSS in patients on INFβ throughout did not differ from patients initially on placebo: 0.3 (interquartile range 0.008 to 1.2) *v* 0.23 (0.02 to 0.73) over 48 months.

The neutralising antibodies were measured for the RRMS group. Five patients (one on the 44 μg dose, four on the 22 μg dose) in the IFNβ throughout group were positive. None of the patients initially on placebo developed neutralising antibodies. The percentage change of UCCA did not differ between patients with and without neutralising antibodies (−6.3 (9.8) *v* −4.12 (5.3), $p = 0.62$).

DISCUSSION

The cervical spinal cord is known to be a common site of involvement in multiple sclerosis and to be of particular importance in the development of disability.[28] In cross sectional studies several groups have shown an association between cord atrophy and disability,[8–11] suggesting that cord atrophy is a potential marker of axonal loss and irreversible neurological dysfunction. Manual outlining techniques for cross sectional area measurements have been used[15 16] but have limited reproducibility and make the serial detection of small changes difficult.[16] Using a highly reproducible semiautomated method, Stevenson *et al* detected a longitudinal change in the cervical cord area for patients with RRMS and PPMS (primary progressive multiple sclerosis),[11 12] but failed to detect a correlation between change in cord size and disability.

We have developed a method of measuring the cross sectional area of the spinal cord using edge detection on MRI.[17] By combining Sobel edge detection with non-maximal suppression, the partial volume boundary between the cord and the surrounding CSF is automatically detected. The technique is independent of image intensity and so may be used even when there are intensity variations because of, for example, MRI radio frequency inhomogeneities.[29] This further implies that intensity change resulting from pathology, which may appear as hyperintense or hypointense lesions on MRI, will not affect the measurement. Another important advantage of this study is the use of a 3D sequence that has high resolution and thus is sensitive to small changes.

In this study, we have shown a significant change in UCCA from month 6 in the placebo group and month 12 in the INFβ treated group. At 18 months, the annual average rate of loss of 2% of UCCA in our mixed placebo group (seven RRMS cases and six SPMS cases) is in accordance with that reported previously in a natural history study.[11] Higher rates of cord atrophy during a one year follow up study have been detected in PPMS patients,[12] which is not surprising if we consider that the cervical cord is the predominant site of disease in this patient group.[30 31] In the current study, the progression of spinal cord atrophy was accompanied by progression of disability throughout. This is not surprising as EDSS is strongly weighted towards locomotion. This suggests a role for the UCCA measure as a potential surrogate marker in monitoring disease progression and efficacy of therapeutic intervention.

Evidence of the efficacy of IFNβ treatment on brain atrophy is scarce. The PRISMS study (Rebif) has not shown a significant effect in slowing progression of brain atrophy over two years despite strong clinical and MRI outcome benefit.[32] This observation seems to support our data showing that the lack of effect of IFNβ on spinal cord atrophy is a true result. In another study, brain volume continuously decreased in 52 RRMS patients who received either 11 μg or 33 μg of IFNβ by subcutaneous injection three times a week for two years.[33] However, no placebo group was compared in that study. It is of interest that IFNβ-1a (Avonex) slows atrophy in RRMS when given at a weekly dose of 30 μg intramuscularly, although this effect was only marginally significant in a subpopulation of patients, and only in the second year of treatment.[34] The differences in dose, route, and frequency of administration, or the pharmaceutical preparation of Rebif compared with Avonex might explain the lack of treatment effect on atrophy in our study. Another possible explanation is that all patients in the present study had received some treatment with IFNβ at some stage, and this may have masked the treatment effect. Also it has been shown that the development of neutralising antibodies, which increases with the frequency and subcutaneous route of administration,[35 36] may be associated with loss of treatment efficacy.[19 37] In the present study, the difference in UCCA reduction between neutralising antibody positive and negative patients was not significant. This may reflect the small number of patients in this study, and the small number of neutralising antibody positive patients. Further research in this direction is needed. Finally, inflammatory activity may only partly be responsible for the development of spinal cord atrophy, and axonal degeneration or transection can be caused by mechanisms unrelated to direct inflammatory demyelination.[38] Thus treatments that reduce inflammatory activity and lesion accumulation may not be translated into a similar effect on progressive tissue loss.[39 40]

To our knowledge, this is the first report of a long term serial study of spinal cord area in multiple sclerosis patients. We recognise that the study contained a small number of patients, and spinal cord tissue loss may be confounded by interferon treatment that all patients received at some point, but the observed change in UCCA during the placebo phase is comparable with previous reports on untreated patients.[11] The consistent reduction detected in UCCA throughout this study is in agreement with the observation that axonal injury/loss is a continuous process once compensatory mechanisms fail.[41] The serial data from healthy controls is further evidence that the change in UCCA detected in patients during the study period is unlikely to reflect a change in the scanner or diurnal variation.

Conclusions

Serial reduction in upper cervical cord area can be detected over a short period of study using edge detection on MRI, and is consistently correlated with change of disability during the study. This extends previous reports on the relation between spinal cord atrophy and disability in patients with multiple sclerosis. These findings suggest that edge detection is a reliable and sensitive method of detecting small changes in UCCA in multiple sclerosis, and provides a paraclinical measure of outcome for monitoring the evolution of multiple sclerosis and possible therapeutic efficacy in short term clinical trials.

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Competing interests: XL, BT, and CSC were reimbursed by Serono for attending conferences; CSC has received honoraria from Biogen for organising educational programmes, and research support from Serono and Biogen; LDB has received occasional speakers' honoraria and consultancy fees from Serono.

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