MRI lesion volume heterogeneity in primary progressive MS in relation with axonal damage and brain atrophy

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**Objectives:** To investigate whether axonal damage in primary progressive (PP) multiple sclerosis (MS), as measured by proton magnetic resonance spectroscopy (HMRS) imaging and brain atrophy, is a function of T2 weighted brain lesion volume.

**Methods:** 34 PP MS patients were divided into two categories: low (<3 cm³, n = 18) or high (≥3 cm³, n = 16) T2 lesion load (LL). An Index of Brain Atrophy (IBA) was calculated and HMRS metabolite ratios were derived from a central brain area centred at the corpus callosum.

**Results:** Patient groups did not differ with regard to clinical characteristics and showed lower mean IBA and mean N-acetylaspartate:creatinine (NAA:Cr) ratios compared to healthy controls.

**Conclusion:** PP patients with low and high brain T2LL have detectable brain atrophy and NAA:Cr reduction compared to healthy controls. In PP MS, T2 lesions alone are insufficient to explain the presence of brain atrophy and decrease in NAA:Cr.

Primary progressive (PP) multiple sclerosis (MS) accounts for about 10% of patients with MS. They experience a progressive worsening from onset, a lesser degree of inflammation found on histopathological reports compared to relapsing remitting (RR) and secondary progressive (SP) MS. Recent immunology studies in PP have found a positive correlation between brain T2 weighted lesion volume and lymphocyte migration, and number of intercellular adhesion molecules critical for oligodendroglial support and survival.

Recent immunology studies in PP have found a positive correlation between brain T2 weighted lesion volume and lymphocyte migration, and number of intercellular adhesion molecules critical for oligodendroglial support and survival. However, the relation to T2LL was not addressed. Several groups have proposed brain atrophy as an MRI marker for destructive tissue changes taking place in MS.

**METHODS**

**Subjects**
Baseline assessments of 34 untreated PP patients were derived from a larger ongoing multicentre PP phase II placebo controlled clinical trial using intravenous mitoxantrone. Only randomised patients at the UCSF MS Center were included. All PP patients met the following criteria: (1) abnormal cerebrospinal fluid (CSF) findings as defined by increased IgG index and/or presence of two or more oligoclonal bands not present in the serum; (2) a progressive course from onset for more than 12 months without acute exacerbation; and (3) no disease modifying or immunosuppressive therapies three months prior to the baseline scan. The Expanded Disability Status Score (EDSS) was performed the day of the MRI. Twenty five healthy age matched subjects were imaged. Informed consent was obtained for all subjects in accordance with the UCSF Ethics Committee.

**MRI acquisition and lesion load post-processing**
A 1.5T GE scanner was used to obtain proton density (PD)/T2 weighted (T2) images. Contiguous 3 mm axial PD/T2 slices were acquired using TR/TE1/TE2 = 2500/20/80 ms with matrix size of 192×256×44 (FOV 180×240×144 mm³). T1 weighted images (spooled gradient echo) were acquired (TR/TE = 27/6 ms, flip angle = 40°) as high resolution 1.5 mm contiguous axial slices with matrix size of 192×256×124 (FOV×240×186 mm³). Lesions on PD images were drawn based on a semiautomated threshold method using software that allows simultaneous access to PD/T2/T1 images. PP patients were divided into two groups based only on their volumetric quantification of T2LL: high (≥3 cm³) or low lesion volume (<3 cm³). This cut off was chosen in concordance with our previous work looking at heterogeneity of T lymphocyte functions in PP MS.

**Brain atrophy measurement**
An index of brain atrophy (IBA) was measured using in-house software based on a similar technique previously reported. Supratentorial brain and CSF masks are created to remove skin, skull, and subcutaneous lipids. Both masks are used to calculate IBA as the ratio of (supratentorial brain parenchyma + supratentorial parenchyma + CSF) × 100. Reproducibility was determined by calculating the IBA of 10 healthy controls during two or three separate MRI sessions <15 days apart. Scan-rescan coefficients of variation (COV) (100% standard deviation/mean) were calculated between repeated measurements. Mean COV was 1.0%, representing about 99% reproducibility.

**HMRS imaging**
Brain HMRS imaging was obtained immediately following T1/T2 weighted images. Twenty eight subjects (18 PP patients, 10 healthy controls) were imaged. A 1.5T GE scanner was used to obtain proton density (PD)/T2 weighted (T2) images. Contiguous 3 mm axial PD/T2 slices were acquired using TR/TE1/TE2 = 2500/20/80 ms with matrix size of 192×256×44 (FOV 180×240×144 mm³). T1 weighted images (spooled gradient echo) were acquired (TR/TE = 27/6 ms, flip angle = 40°) as high resolution 1.5 mm contiguous axial slices with matrix size of 192×256×124 (FOV×240×186 mm³). Lesions on PD images were drawn based on a semiautomated threshold method using software that allows simultaneous access to PD/T2/T1 images. PP patients were divided into two groups based only on their volumetric quantification of T2LL: high (≥3 cm³) or low lesion volume (<3 cm³). This cut off was chosen in concordance with our previous work looking at heterogeneity of T lymphocyte functions in PP MS.

**Abbreviations:** CB, central brain; COV, coefficient of variation; Cr, creatinine; CSG, cerebrospinal fluid; EDSS, Expanded Disability Status Score; HMRS, proton magnetic resonance spectroscopy; IBA, Index of Brain Atrophy; LL, lesion load; MRI, magnetic resonance imaging; MS, multiple sclerosis; NAA, N-acetylaspartate; NAWM, normal appearing white matter; PD, proton density; PP, primary progressive; ROI, region of interest; RR, relapse remitting; SP, secondary progressive
10 healthy controls) underwent a three dimensional echo planar spectroscopic imaging (3D-EPSI) sequence recently described, operating at TE = 144 ms, TR = 2000, FOV of 24×24×16 cm covering the entire supratentorial brain. From this large area a smaller central brain (CB) region of interest (ROI) measuring 9×9×2 cm (160 cm³) centred at the middle of the corpus callosum was delimited. Fifteen subjects (six PP patients, nine healthy controls) underwent a two dimensional chemical shifting imaging (2D-CSI) GE sequence operating at TR/TE = 1000/144 ms (same TE as 3D-EPSI), 24×24×1.5 cm and a press box positioned at CB, covering a slab of approximately 160 cm³ centred also at the middle of the corpus callosum. NAA:Cr ratios were estimated from voxels within the CB ROI using a linear regression analysis between height intensity of NAA over Cr (NAA:Cr).

**Statistical analysis**

Group comparisons were assessed using the a Wilcoxon test to compare age, EDSS, disease duration, NAA:Cr, and IBA. Spearman’s correlation analysis was performed to compare T2LL, IBA, and NAA:Cr. In this study, a value of p < 0.05 is regarded as significant.

**RESULTS**

Table 1 presents clinical and MRI characteristics of all PP patients and controls. Female: male gender ratios for all PP patients (19/15) and controls (14/11) were similar: 1.267 and 1.272 respectively. Clinical characteristics for high and low LL groups did not differ with regard to mean EDSS (p = 0.6), mean disease duration (p = 0.6), and mean age (p = 0.9). The subset (n = 24) of patients for which NAA:Cr was available had a mean EDSS of 4.7 (SD 1.1; p = 0.8), mean disease duration of 10.1 years (SD 10.2; p = 0.6), and mean age of 49.6 (SD 8.4; p = 1.0) that did not differ from the entire cohort (n = 34) of PP patients.

Comparative results from both HMRS imaging methodologies were calculated. Mean (SD) NAA:Cr ratios from CB ROI for controls (1.963 (0.085) vs 1.972 (0.176), p = 0.9) and for patients (1.736 (0.164) vs 1.778 (0.127), p = 0.6) did not differ between the two HMRS sequences. No corrections were necessary and CB NAA:Cr ratios were combined for subsequent group comparisons. The volume of CB ROI used to estimate the NAA:Cr ratios encompassed ~2% of the total T2LL. The rest of the CB ROI volume contained NAWM, grey matter, and CSF.

Table 1 summarises both CB NAA:Cr ratios and IBA results from all subjects. Reductions of mean NAA:Cr were found for both high (p < 0.00001) and low (p = 0.001) LL patient groups and all PP patients combined (p < 0.00001) compared to controls. Similarly, brain atrophy was more pronounced for both high (p = 0.0004) and low (p = 0.03) LL patient groups, and all PP patients combined (p = 0.0004) compared to controls. Figure 1 shows bar graphs comparing NAA:Cr and IBA for both high and low LL PP groups. Although the high LL group did show a lower mean NAA:Cr and mean IBA compared to the low LL group, the NAA:Cr metric did not reach statistical significance (p = 0.1) but the brain atrophy did (p = 0.03).

Finally, Spearman’s correlation coefficients for all PP patients were found to be weak and statistically non-significant between T2LL and NAA:Cr (r = −0.35, p = 0.1), T2LL and IBA (r = −0.36, p = 0.1), and NAA:Cr and IBA (r = 0.36, p = 0.1).

**DISCUSSION**

We have used a highly reproducible automated global brain atrophy measurement in conjunction with NAA:Cr metabolite ratios from CB to evaluate PP patients. Patients were divided into two separate groups based on their T2LL. Both patient groups had significant reduction of NAA:Cr and brain atrophy compared to controls. We also found no significant correlations between IBA and T2LL and between NAA:Cr and T2LL. The presence of brain MR visible T2 changes seems insufficient to explain the overall pathological process leading to brain atrophy and axonal injury. We speculate that PP MS may represent a more diffuse and global axonopathy. The formation and presence of focal MS lesions encompassing
transected axons seem to have only an added effect to this axonal pathological process. This does not diminish the importance of such lesions, but their contribution to the development of more extensive brain tissue injury is perhaps marginal.

NAA:Cr ratio in CB has been proposed as an index of axonal damage and/or dysfunction for more than a decade as creatine concentration was found to be relatively constant in both lesions and NAWM of MS patients, although others have detected increased creatine. Several studies have found reduced NAA:Cr and concentration of NAA in MS lesions, lesions and NAWM of MS patients, detected increased creatine. Few investigators evaluated PP specifically.

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