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 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 centre at the corpus callosum.

RESULTS: Patient groups did not differ with regard to clinical characteristics and showed lower mean IBA and mean N-acetylaspartate:creatine (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, fewer brain magnetic resonance imaging (MRI) lesions on conventional imaging, and 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 intra-axonal lymphocytes. Whether those T2 brain lesions have a significant impact on brain atrophy and axonal injury in PP remain to be defined. Histopathological studies have reported axonal injury within and distant from MS lesions, referred to as normal appearing white matter (NAWM). New MRI metrics could improve the lack of specificity—that is, oedema, inflammation, demyelination, gliosis, and axonal loss, and poor correlation of T2 weighted lesions and disability offered by conventional imaging. Proton magnetic resonance spectroscopy (HMRS) imaging reveals the extent of axonal involvement through N-acetylaspartate (NAA), an amino acid found mainly within mature neurones and axons. Using single voxel HMRS, decreases in NAA and NAA:Cr have been reported in the NAWM of PP patients, but 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. As no reports have focused exclusively on brain atrophy and HMRS imaging in PP MS, we are investigating whether axonal injury is a function of T2LL.

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 before 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/TE = 2500/20/80 ms with matrix size of 192x256x4 (FOV 180x240x144 mm³). T1 weighted images (spoilded 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 192x256x124 (FOV 240x186 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 included. A 1.5T GE scanner was used to obtain T1, T2, and proton density (PD) images. Lesions were identified on PD images and contiguously 3 mm thick PD/T2 slices were acquired using TR/TE1/TE2 = 2500/20/80 ms with matrix 192x256x44 (FOV 180x240x144 mm³). T1 weighted images were acquired (TR/TE = 27/6 ms, flip angle = 40°). 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).

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
Table 1 Clinical and MRI characteristics of studied patients and healthy controls

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PP, primary progressive; T2LL, T2 weighted lesion load; EDSS, Expanded Disability Status Scale; IBA, Index of Brain Atrophy; N/A, not applicable.

Statistical analysis

Group comparisons were assessed using the Wilcoxon test to compare age, EDSS, disease duration, NAA:Cr, and IBA. Spearman’s correlation analysis was performed to compare age, EDSS, disease duration, NAA:Cr, and IBA. Spearman’s correlation analysis was performed to compare age, EDSS, disease duration, NAA:Cr, and IBA.

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) v 1.972 (0.176), p = 0.9) and for patients (1.736 (0.164) v 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 nonsignificant 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, whereas concentration was found to be relatively constant in both detected increased creatine.

Few investigators evaluated PP specifically.

ACKNOWLEDGEMENTS

Development of more extensive brain tissue injury is perhaps the importance of such lesions, but their contribution to the pathology of primary and secondary progressive multiple sclerosis. Brain 1994;117:759-65.

REFERENCES


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