SHORT REPORT

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 ($<3 \text{ cm}^3$, $n = 18$) or high ($\geq 3 \text{ cm}^3$, $n = 16$) 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.¹ They experience a progressive worsening from onset,² fewer brain magnetic resonance imaging (MRI) lesions on conventional imaging,³ and a lesser degree of inflammation found on histopathological reports⁴ 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 (T2LL), lymphocyte migration, and number of interferon γ secreting cells using ex vivo peripheral blood T lymphocytes.⁵ Whether those T2 brain lesions have a significant impact on brain atrophy and axonal brain injury in PP remain to be defined. Histopathological studies have reported axonal injury within and distant from MS lesions,^{6,7} 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.^{8,9} 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.^{10,11} Using single voxel HMRS, decreases in NAA and NAA:Cr have been reported in the NAWM of PP patients,¹² 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.^{13,14} 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 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/TE/TE₂ = 2500/20/80 ms with matrix size of 192×256×44 (FOV 180×240×144 mm³). T1 weighted images (spoiled 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 ($\geq 3 \text{ cm}^3$) or low lesion volume ($< 3 \text{ cm}^3$). This cut off was chosen in concordance with our previous work looking at heterogeneity of T lymphocyte functions in PP MS.⁵

Brain atrophy measurement

An index of brain atrophy (IBA) was measured using in-house software based on a similar technique previously reported.¹⁴ 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,

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

	PP high T2LL	PP low T2LL	All PP	Controls
Age, y				
Mean	49.3	49.7	49.5	44.8
SD	9.1	6.2	7.5	9.9
Range	30.7–65.1	40.5–61.9	30.7–65.1	26.8–61.8
n	16	18	34	25
n for MRSI	10	14	24	19
Disease duration, y				
Mean	9.8	8.3	9.0	N/A
SD	9.0	6.2	7.5	N/A
EDSS				
Mean	4.9	4.7	4.8	N/A
SD	1.1	1.4	1.2	N/A
T2 lesion load				
Mean	9.49	1.17	5.09	N/A
SD	6.71	0.97	6.22	N/A
IBA				
Mean	0.685	0.729	0.708	0.761
SD	0.065	0.040	0.057	0.051
p values	0.0004	0.03	0.0004	N/A
NAA:Cr				
Mean	1.679	1.783	1.740	1.967
SD	0.124	0.165	0.156	0.131
p values	0.00001	0.001	<0.00001	N/A

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

10 healthy controls) underwent a three dimensional echo planar spectroscopic imaging (3D-EPSI) sequence recently described,¹⁵ 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 phase encoding matrix (FOV of 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 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) v 1.972 (0.176), $p = 0.9$) and for

Figure 1 Bar graph showing mean NAA:Cr ratio (A) and global brain atrophy (B) measurement (mean IBA) comparison between low and high lesion load primary progressive patient groups. p values are based on Wilcoxon test. Error bars represent one $\pm$ SD.

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 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 decade¹⁶ as creatine concentration was found to be relatively constant in both lesions and NAWM of MS patients,^{17,18} although others have detected increased creatine.¹⁹ Several studies have found reduced NAA:Cr and concentration of NAA in MS lesions, NAWM, and whole brain^{20–22} of RR and SP patients, but only a few investigators evaluated PP specifically.^{12,19,23} In our study, the mean T2LL of all PP patients was 5.09 cm³ (ranging from 0.62 to 24.77 cm³), representing <2% of the CB ROI volume used to derive NAA:Cr ratios (table 1). Therefore, the decrease in NAA:Cr is not likely to be explained by changes in T2 weighted lesions. The extent of such reduction could reflect axonal injury in NAWM¹² and perhaps in grey matter.^{24,25} Both patient groups showed marginal differences (fig 1) compared to each other. The mean NAA:Cr ratio in the high LL group did not differ from the low LL group, although it was slightly lower. IBA seems to differ statistically between the two groups, favouring patients with lower T2LL. This does not necessarily suggest that CB NAA:Cr and IBA are unlinked measures in PP, although those metrics were found to be modestly correlated ($r = 0.35$, $p = 0.1$) in this study. Possible explanations for this discrepancy could be that the difference between the two groups is very subtle and requires a larger sample size, reduction of NAA might be an early event that could precede detectable tissue loss, and/or axonal injury being not the only element contributing to brain atrophy.

The relation between MR visible lesions and brain atrophy has also been challenged in numerous MS studies.^{14,26} Changes in brain volume and reduction of lesions in progressive MS patients treated with cladribine have been reported (70% SP and 30% PP patients).²⁶ Although they observed a significant reduction in the number of gadolinium enhancing and volume of T2LL in the treated group, no effect was detected on brain atrophy. The authors concluded that MR lesions have a marginal role in the development of brain atrophy. Stevenson and colleagues²⁷ found a significant change in brain atrophy between baseline and year 1 in a group of PP patients, but no correlations between brain atrophy and T2LL were reported.

We showed that axonal injury, as measured by HMRS imaging and brain atrophy are taking place in two groups of PP patients divided by their T2LL. Focal lesions seem to make a minimal contribution to global axonal injury, and other pathophysiological processes need to be explored in this particular form of MS. Based on this information, drug therapies in PP MS should not only target lesion formation but should also offer neuronal protection.

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