

Abnormalities in normal appearing tissues in early primary progressive multiple sclerosis and their relation to disability: a tissue specific magnetisation transfer study

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Background: Patients with primary progressive multiple sclerosis (PPMS) often develop severe disability despite low levels of abnormality on conventional magnetic resonance imaging (MRI). This may relate to diffuse pathological processes occurring in normal appearing brain tissue (NABT) involving both white matter (NAWM) and grey matter (NAGM). Magnetisation transfer imaging (MTI) is capable of identifying these processes and may be particularly informative when applied to patients with early PPMS.

Aim: To assess the relationship between abnormalities in NABT identified by MTI and disability and other radiological data in patients with early PPMS.

Methods: We studied 43 patients within 5 years of disease onset and 43 controls. The Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC) were scored. Magnetisation transfer ratios (MTR) of NABT, NAWM, and NAGM were calculated and the following MTR parameters were measured: mean, peak height, peak location, and MTR value at the 25th, 50th, and 75th percentiles. Proton density, T2, T1, and gadolinium enhancing lesion loads were also calculated.

Results: Differences were found between patients and controls in mean, peak height, and peak location of NAWM and NAGM ($p \leq 0.001$). Weak to moderate correlations were found between MTR parameters and disability in both NAWM and NAGM. Strong correlations between MTR parameters and lesion loads were found, particularly in NAWM.

Conclusion: MTR abnormalities are seen in NAWM and NAGM in early PPMS and both are associated with disability. NAWM MTR abnormalities are more closely related to conventional MRI measures than those seen in NAGM.

The mechanisms that underlie the development of disability in multiple sclerosis (MS) are not fully understood and there is evidence from magnetic resonance imaging (MRI) studies that there may be important pathological differences between primary progressive multiple sclerosis (PPMS) and the relapsing-remitting form of the disease (RRMS).¹ In PPMS, as in other forms of MS, it is likely that processes occurring within tissues that appear normal using conventional MRI techniques, contribute to the development of disability. Pathological changes in normal appearing white matter (NAWM) have previously been described in MS, including gliosis, inflammatory cell infiltration,² and axonal loss.³ There is less information about pathological changes in normal appearing grey matter (NAGM), but cortical lesions have been demonstrated histologically⁴ and spectroscopic studies have reported reduced grey matter (GM) *N*-acetyl-aspartate.⁵ In PPMS, abnormalities within normal appearing brain tissues (NABT) have already been shown with newer MRI techniques,⁶ and have been found to be related to disability. However, it is not known how early these abnormalities arise in the disease course, as studies have tended to examine patients with well established disease. In addition, the relative contribution of abnormalities in white matter (WM) and GM has received little attention.

Conventional MRI mainly reflects the quantity of free water protons in tissue and is unable to detect protons bound to macromolecular structures due to their short T2 relaxation times. Magnetisation transfer imaging (MTI) makes use of the continuous magnetisation interchange between free protons and protons bound to macromolecules and provides an indirect measurement of tissue integrity. Magnetisation

transfer ratio (MTR) is a quantitative measurement of the amount of magnetisation transfer occurring between these two pools of protons. A low MTR indicates a reduced capacity of the macromolecules in brain tissue to exchange magnetisation with the surrounding water molecules, and indicates structural tissue damage.^{7,8} The main pathological changes leading to reductions in MTR in MS are thought to be demyelination and axonal loss,^{9–13} with other pathological changes such as oedema, ischaemia, and gliosis having a lesser role.¹⁴ Post mortem studies have also found a correlation between low MTR and the percentage of remaining axons in MS lesions.¹⁵ Thus, this technique seems to have a greater pathological specificity than conventional MRI and may provide additional information on the widespread pathological processes which may be fundamental to the development of disability. MTI studies in MS have focussed largely on RRMS, secondary progressive MS, and clinically isolated syndromes. These studies showed reduced MTR in lesions,^{12,16} whole brain (WB),¹⁷ NAWM,^{12,18–20} and NAGM.^{19,21}

Abbreviations: COVs, coefficients of variation; DSS, Disability Status Scale; EDSS, Expanded Disability Status Scale; FOV, field of view; FSE, fast spin echo; Gd, gadolinium; GM, grey matter; M, mean MTR; MRI, magnetic resonance imaging; MS, multiple sclerosis; MSFC, Multiple Sclerosis Functional Composite; MTI, magnetisation transfer imaging; MTR, magnetisation transfer ratio; NABT, normal appearing brain tissue; NAGM, normal appearing grey matter; NAWM, normal appearing white matter; NEX, number of excitations; P25, MTR value of the 25th percentile; P50, MTR value of the 50th percentile; P75, MTR value of the 75th percentile; PD, proton density; PH, peak height; PL, peak location; PPMS, primary progressive multiple sclerosis; pu, percent unit; RRMS, relapsing-remitting multiple sclerosis; SE, spin echo; TE, echo time; TR, repetition time; WB, whole brain; WWM, white matter

A small number of studies have used MTI to examine patients with well established PPMS and have reported reductions of MTR in WB,²² NAWM,²³ and NAGM.²¹ In these studies, the associations found with disability have been weak. This might be due to the inclusion of patients with very long disease duration.

The principal aim of this study is to determine whether MTR abnormalities in NAWM and NAGM are present in early PPMS and whether these relate to disability at this stage of the disease. A secondary aim is to study the relationship of MTR to conventional MR measurements in early PPMS.

METHODS

Subjects

Forty three patients with PPMS fulfilling the diagnostic criteria for definite or probable PPMS according to Thompson *et al.*,²⁴ and who were within 5 years of clinical onset, were recruited from clinics at the National Hospital for Neurology and Neurosurgery and other hospitals in south east England. Patients were invited to take part in a 3 year longitudinal multimodal MRI study. Patients were subdivided into cord and non-cord onset groups according to their clinical presentation. Forty three healthy controls were also studied.

All patients underwent conventional neurological assessment and were scored on Kurtzke's Expanded Disability Status Scale (EDSS)²⁵ and the Multiple Sclerosis Functional Composite (MSFC).²⁶ Using our own sample as a reference, z scores for the MSFC subtests (Paced Serial Auditory Test (z-PASAT), Nine Hole Peg Test (z-9HPT), and Timed Walk Test (z-TWT)) were calculated according to published methods. The study was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, London, UK. Written informed consent was obtained from all subjects before they were included in the study.

MRI acquisition

MRI of all subjects was carried out using a 1.5 T GE Signa scanner (General Electric, Milwaukee, IL, USA). In all subjects, WB MTI was acquired using a 2D interleaved spin echo (SE) sequence²⁷ (28 contiguous 5 mm axial slices, repetition time (TR) 1720 ms, echo time (TE) 30/80 ms, number of excitations (NEX) 0.75, acquired matrix 256×128, reconstructed matrix 256×256, field of view (FOV) 240×240 mm) with and without a saturating Hamming-apodised three lobe sinc MT pulse (duration of 16 ms, peak amplitude of 23.2 μT, bandwidth of 250 Hz applied 1 kHz off water resonance). Saturated and unsaturated images were interleaved for each TR period providing two data sets precisely co-registered. The total acquisition time was 20 min. MTR was calculated for each pixel according to the formula $[(Mo - Ms)/Mo] \times 100$ percent units (pu), where Mo and Ms represent signal intensities pre and post saturation pulse, respectively.

The following sets of images were also obtained in patients: dual fast spin echo (FSE) sequence (28 contiguous 5 mm axial slices, TR 2000 ms, TE 95 ms, NEX 1, matrix 256×256, FOV 240×180); and T1 weighted SE sequence (28 contiguous 5 mm axial slices, TR 540 ms, TE 20 ms, NEX 1, flip angle 90°, matrix 256×256, FOV 240×240) acquired before and 5 min after intravenous administration of triple dose gadolinium (Gd; 0.6 ml/kg of body weight; Magnevist, Schering, Berlin, Germany). MTI and FSE sequences were acquired on the same day. T1 weighted and T1 weighted Gd enhanced scans were acquired in a separate session from MTI (mean separation between sessions 20 days, median 13 days).

Image post processing

An automated segmentation procedure, using SPM99 software (Wellcome Department of Cognitive Neurology, University College London, UK), was used to extract, starting from T2 weighted images, WM, GM, cerebrospinal fluid, and non-brain tissues from the WB. A WB mask, which excludes CSF and other non-brain parenchyma, was generated in SPM99 and applied to the calculated MTR map to obtain the WB MTR map. Then, a maximum likelihood algorithm, using the WM and GM probability outputs from SPM99, was used to segment the WB MTR map into WM and GM MTR maps. Macroscopic lesions were identified, contoured, and segmented using a reproducible semiautomated local thresholding technique (DispImage; Plummer, Department of Medical Physics and Bioengineering, University College London, UK) on the unsaturated MT proton density (PD) weighted images, T2 weighted FSE images, and T1 weighted Gd enhanced images. Lesions in PD, T2, T1, and Gd enhanced scans were used to calculate lesions loads. In addition, lesions in PD weighted scans were used to create a lesion mask. Where WM lesions were encountered in controls (five subjects, either non-specific or thought likely to be due to incidental small vessel disease), the same methodology was applied in order to obtain the NABT of these subjects. The lesion mask was applied to the previously generated WB, WM, and GM MTR maps to obtain MTR maps of NABT, NAWM, and NAGM. Partial volume voxels were minimised with a 10 pu threshold and inner and outer voxel erosions of WM and GM.

SPM99 uses a histogram based segmentation procedure depending on voxel intensity that may be biased by the presence of abnormal tissue intensity.²⁸ In patients with low lesion load, the segmentation is correct, but in patients with high lesions loads, it tends to include WM tissue in GM. This misclassification has the potential to alter the NAGM MTR values observed. Therefore, we decided to exclude from NAGM analysis those patients with T2 lesion loads above 50 ml. This may have reduced the chances of observing significant differences as the excluded patients were likely to display greater abnormality.

An MTR histogram generation algorithm was implemented for each NABT, NAWM, and NAGM. Inter subject variability in specific tissue volumes was adjusted for by normalising the MTR histograms. This was achieved by dividing the number of counts in each sampling bin by the total number of voxels for each tissue type. Normalisation was used to allow comparison of histograms from subjects with different brain volumes. From each normalised histogram, mean MTR (M), peak height (PH), peak location (PL), and the MTR value of the 25th, 50th, and 75th percentiles (P25, P50, P75) were measured. Coefficients of variation (COVs) for 18 healthy control subjects in scans performed 1 year apart were 0.56% for WB, 0.43% for WM, and 1.048% for GM. COVs for 14 patients in scans performed 1 month apart were 0.61% for WB, 0.60% for NABT, 0.69% for WM, 0.62% for NAWM, 0.84% for GM, and 0.85% for NAGM.

Statistical analysis

Statistical analysis was performed using SPSS 10.0 (Chicago, IL, USA). MTR parameters of NABT, NAWM, and NAGM were compared between patients and controls using general linear modelling including age and gender in the model to account for slight differences in these variables between the two groups. The Bonferroni correction for multiple comparisons was applied with a p value of 0.003 or less regarded as significant. Pearson's correlation coefficients were used to assess the presence of associations between MTR parameters and clinical and radiological variables. Spearman's rank correlation coefficient was used for EDSS comparisons. Two

Table 1 Magnetisation transfer ratio (MTR) values for controls and patients.

	NABT			NAWM			NAGM		
	Controls (n=43)	Patients (n=43)	p value	Controls (n=43)	Patients (n=43)	p value	Controls (n=43)	Patients (n=39)	p value
M	35.174±0.358	34.026±1.279	<0.001	38.238±0.399	36.938±2.290	<0.001	32.254±0.470	31.358±0.776	<0.001
PH	0.0113±0.001	0.0105±0.001	<0.001	0.0201±0.002	0.0180±0.003	<0.001	0.0125±0.001	0.0107±0.001	<0.001
PL	37.598±0.376	36.688±1.307	<0.001	38.470±0.448	37.570±1.089	<0.001	33.181±0.348	32.731±0.616	0.001
P25	21.163±1.393	21.379±2.216	0.872	23.384±1.837	23.095±2.476	0.328	20.572±1.195	20.733±1.228	0.851
P50	32.184±2.795	32.628±4.410	0.857	32.786±2.485	32.398±2.914	0.160	30.967±2.355	31.321±2.448	0.881
P75	43.186±4.216	43.586±6.783	0.990	42.156±3.547	41.674±3.969	0.144	41.340±3.544	41.882±3.679	0.894

Comparisons have been performed using general linear modelling adjusting for age and gender.

All values are presented as mean±standard deviation. M, mean MTR; NABT, normal appearing brain tissue; NAGM, normal appearing grey matter; NAWM, normal appearing white matter; P25, MTR at the 25th percentile; P50, MTR at the 50th percentile; P75, MTR at the 75th percentile; PH, peak height; PL, peak location.

tailed *t* tests were used for comparison of radiological variables between clinical subgroups of PPMS (cord and non-cord onset).

RESULTS

Descriptive data

We studied 43 patients with PPMS (24 males and 19 females) with a mean age of 45.12±10.84 years (range 22–65 years) and a mean disease duration of 3.33±0.87 years (range 2–5 years). The median EDSS was 4.5 (range 3.0–7.0). Forty three controls (23 males and 20 females) were also scanned (mean age of 37.21±8.99 years; range 23–59 years).

Comparison of MTR parameters between patients and controls

There were significant differences between patients and controls in M, PL, and PH of NABT, NAWM, and NAGM ($p \leq 0.001$) (table 1). Within the group of patients with PPMS, only NABT M was statistically different between cord (mean 34.228±1.120) and non-cord (mean 33.138±1.619) presentation at onset ($p = 0.028$).

Clinical correlations of MTR parameters

Weak to moderate correlations were found between MTR parameters in NABT and the two clinical measures: EDSS (table 2 and fig 1) and MSFC (table 2). Of the constituents of the MSFC, the z-9HPT correlated more strongly with NABT than either NAGM or NAWM, and the z-TWT correlated more strongly with NAWM MTR parameters than with NAGM, while the z-PASAT correlated more strongly with NAGM MTR parameters than with NAWM.

MRI correlations of MTR parameters

Strong correlations were found between MTR parameters of NABT and NAWM, and PD, T2, and T1 lesion loads and less so for NAGM parameters (table 3 and fig 2). Enhancing lesion number correlated with MTR parameters in NABT and NAWM but not in NAGM.

DISCUSSION

This study confirms the existence of MTR abnormalities in PPMS. We have now shown that these abnormalities can be detected in the early stages of the disease, affect both NAWM and NAGM, and are related to clinical status and abnormalities on conventional MRI.

MTR abnormalities of NABT are present in early disease

Previous studies in PPMS,^{21–23} using different methodologies and assessing different tissue fractions, have also found differences between patients and controls in some MTR parameters. Studies using histogram analysis found differences in some WB,²² NAWM,²¹ and NAGM²¹ MTR parameters, while studies analysing regions of interest found MTR differences in the corpus callosum, the frontal lobes, and the centrum semiovale.²³ In our study, we have found consistent differences in the most sensitive and informative MTR parameters (M, PL, and PH) in all the normal appearing brain tissues (NABT, NAWM, and NAGM), suggesting quite extensive change and underlining the sensitivity of MTR measurements in detecting subtle change. These MTR abnormalities observed between patients and controls are significantly larger than the values estimates by the reported

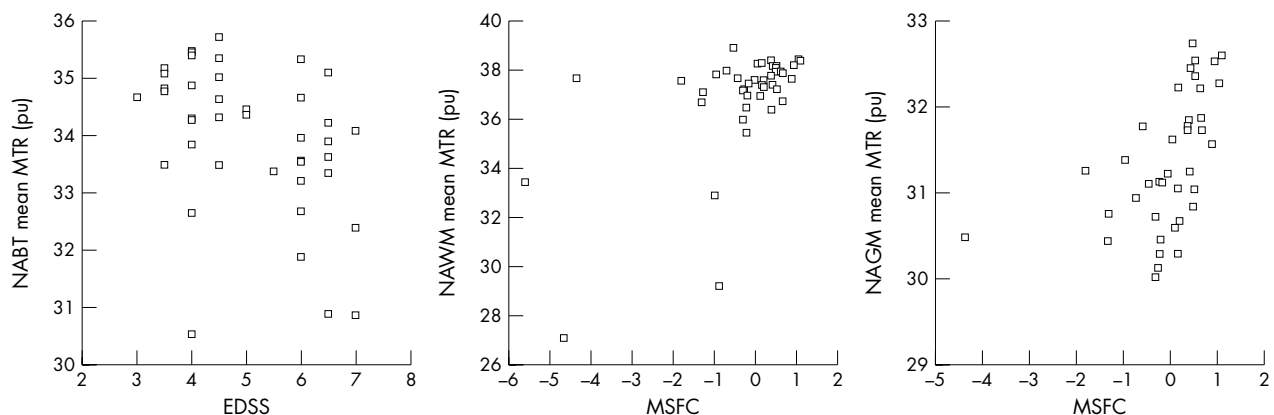


Figure 1 Normal appearing brain tissue (NABT) mean magnetisation transfer ratio (MTR) plotted against EDSS. Normal appearing white matter (NAWM) and normal appearing grey matter (NAGM) mean MTR plotted against Multiple Sclerosis Functional Composite (MSFC).

Table 2 Correlations between magnetisation transfer ratio (MTR) parameters and disability measurements

		EDSS	MSFC	z-9HPT	z-PASAT	z-TWT
NABT (n=43)	M	-0.46**	0.53**	0.65**	0.38*	-0.35*
	PH	-0.51**	0.43**	0.81**	0.39**	-0.18
	PL	-0.35*	0.44**	0.50**	0.32*	-0.30
	P25	-0.30*	0.22	-0.01	0.13	-0.23
	P50	-0.29	0.21	-0.02	0.12	-0.22
	P75	-0.23	0.19	-0.06	0.16	-0.20
NAWM (n=43)	M	-0.34*	0.58**	0.42**	0.31*	-0.49**
	PH	-0.33*	0.49**	0.61**	0.33*	-0.32*
	PL	-0.31*	0.51**	0.51**	0.33*	-0.37*
	P25	-0.48**	0.48**	0.34*	0.23	-0.41**
	P50	-0.43**	0.42**	0.33*	0.28	-0.34*
	P75	-0.30*	0.32*	0.27	0.26	-0.24
NAGM (n=39)	M	-0.34*	0.51**	0.54**	0.48**	-0.22
	PH	-0.40*	0.49**	0.65**	0.39*	-0.19
	PL	-0.18	0.57**	0.34*	0.31	-0.43**
	P25	-0.34*	0.33*	0.22	0.23	-0.22
	P50	-0.33*	0.32	0.20	0.23	-0.21
	P75	-0.31	0.31	0.19	0.23	-0.21

Pearson's correlation coefficients were used except for the EDSS (Spearman's rank correlation coefficient). EDSS, Expanded Disability Status Scale; M, mean MTR; MSFC, Multiple Sclerosis Functional Composite; NABT, normal appearing brain tissue; NAGM, normal appearing grey matter; NAWM, normal appearing white matter; P25, MTR at the 25th percentile; P50, MTR at the 50th percentile; P75, MTR at the 75th percentile; PH, peak height; PL, peak location; z-9HPT, z score for the Nine-Hole Peg Test; z-PASAT, z score for the Paced Auditory Serial Addition Test; z-TWT, z score for the Timed Walk Test. Significance: *p<0.05; **p<0.01.

COVs, indicating that these differences are partly due to group effect (controls or patients) and not only to the technique used or to a time effect.

MTR abnormalities of NABT are associated with clinical status

This study suggests that abnormalities in both NAWM and NAGM are relevant to the development of disability in early PPMS. Patients with PPMS take on average 8 years to progress in disability to DSS (Disability Status Score) 6, and then a further 18 years to progress from DSS 6 to 8.²⁹ It is possible that in the early years of the disease, when the greatest changes in disability are occurring, the pathological changes are correspondingly greater. The mean disease duration of patients in the present study was 3.3 years in comparison to mean durations of 6, 7, and 8 years in previous studies.²¹⁻²³

In the present study, weak to moderate correlations were found with disability as measured by the EDSS and the MSFC. These were mainly between M, PH, and PL MTR and were observed in NABT, NAWM, and NAGM. This is in contrast to previous studies, where correlations between WB MTR histogram parameters and the EDSS were either weak²¹⁻²³ or non-existent.²² The differences in results between the present and previous studies may relate to the use of different methodologies (using regions of interest only some

parts of the brain are analysed, while using histogram analysis the entire brain is studied) or, more interestingly, might reflect a greater clinical relevance of MRI measurable pathological processes in early disease, as mentioned earlier. EDSS correlates more strongly with NABT than with NAWM and NAGM. This may indicate widespread and clinically relevant tissue damage in early PPMS. Our data also showed that MTR parameters correlate more strongly with MSFC and its components than with EDSS. We found interesting correlations between the different components of the MSFC and the different brain tissues suggesting tissue specific effects on disability (table 2): while 9HPT performance relates similarly to both NAGM and NAWM, poorer PASAT performance is more related to GM changes and slower walking times (TWT) relate strongly to changes in WM.

MTR abnormalities of NABT are strongly associated with lesion load

We found strong correlations between lesion load and a number of MTR histogram parameters in both NAWM and NAGM, but mainly in the former. A previous study found weak correlations between T2 and T1 lesion loads and mean NABT.³⁰ The weakness of this association was interpreted as suggesting that NABT pathology in PPMS might not merely reflect wallerian degeneration of axons traversing macroscopic lesions, but might, in addition, relate to the occurrence

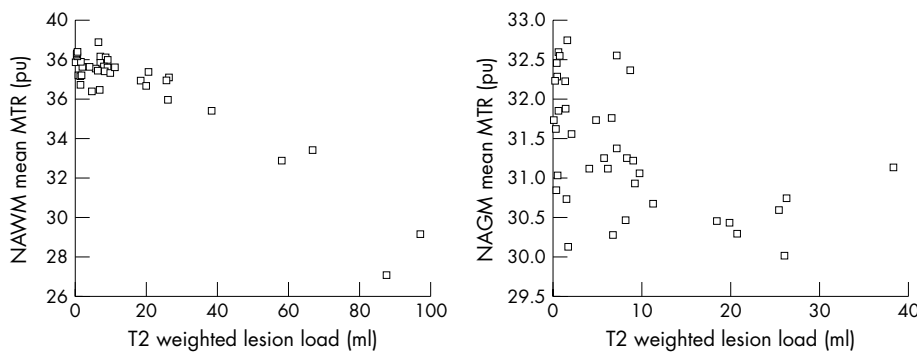


Figure 2 Normal appearing white matter (NAWM) and normal appearing grey matter (NAGM) mean magnetisation transfer ratio (MTR) plotted against T2 weighted lesion load.

Table 3 Correlations between magnetisation transfer ratio (MTR) parameters and lesion loads

		PD	T2	T1	GLN	GLV
NABT (n=43)	M	-0.86**	-0.79**	-0.69**	-0.47**	-0.20
	PH	-0.63**	-0.59**	-0.51**	-0.33*	-0.25
	PL	-0.84**	-0.83**	-0.73**	-0.44**	-0.18
	P25	-0.03	0.00	-0.08	-0.08	-0.18
	P50	-0.01	0.01	-0.07	-0.07	-0.07
	P75	0.02	0.04	-0.05	-0.05	-0.14
NAWM (n=43)	M	-0.88**	-0.94**	-0.91**	-0.58**	-0.25
	PH	-0.73**	-0.81**	-0.82**	-0.42**	-0.33*
	PL	-0.85**	-0.86**	-0.81**	-0.43**	-0.17
	P25	-0.41**	-0.45**	-0.46**	-0.34*	-0.08
	P50	-0.46**	-0.43**	-0.38*	-0.34*	-0.12
	P75	-0.41**	-0.35*	-0.27	-0.28	-0.12
NAGM (n=39)	M	-0.60**	-0.53**	-0.57**	-0.10	0.08
	PH	-0.47**	-0.32	-0.55**	-0.12	-0.08
	PL	-0.37*	-0.33*	-0.34*	-0.14	-0.01
	P25	-0.23	-0.29	-0.39*	-0.24	-0.29
	P50	-0.21	-0.26	-0.37*	-0.23	-0.28
	P75	-0.20	-0.26	-0.37*	-0.22	-0.28

Pearson's correlation coefficients were used in all comparisons. GLN, gadolinium enhanced lesion number; GLV, gadolinium enhanced lesion volume; M, mean MTR; NABT, normal appearing brain tissue; NAGM, normal appearing grey matter; NAWM, normal appearing white matter; P25, MTR at the 25th percentile; P50, MTR at the 50th percentile; P75, MTR at the 75th percentile; PD, proton density weighted lesion load; PH, peak height; PL, peak location; T1, T1 weighted lesion load; T2, T2 weighted lesion load.
Significance: * $p < 0.05$; ** $p < 0.01$.

of subtle pathology, independent of macroscopic lesions. Our results seem to suggest that a greater association between NABT MTR abnormalities and the process of lesion formation is present in early disease. A weak to moderate correlation between NAWM and gadolinium enhancing lesion number was also found. This relationship supports an association between inflammation and MTR abnormalities in NAWM. MTR correlations with lesion load were strongest in NAWM, where the lesions are found on conventional MRI, but moderate correlations were also found in NAGM. This might be explained by lesion formation in WM causing retrograde damage in the GM or by a pathological process affecting both NAWM and NAGM indiscriminately.

In summary, this study supports the existence of a diffuse disease process operating in the early stages of PPMS which affects both NAWM and NAGM, and which relates to both disability and lesion presence. Further longitudinal follow up study of this cohort will seek to extend these findings, assess the value of MTI as a marker of prognosis, and examine the relationship of MTR to other pathologically specific MRI techniques.

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