Title: An abnormal periventricular magnetisation transfer ratio gradient occurs early in multiple sclerosis

Running title: Periventricular MTR gradients in early MS

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Word counts:
Abstract: 326
Word count: 4859
Figures: 4 (all colour)
Tables: 3

Abstract

In established multiple sclerosis (MS), tissue abnormality - as assessed using magnetisation transfer ratio (MTR) – increases close to the lateral ventricles. We aimed to determine whether or not i) these changes are present from the earliest clinical stages of MS; ii) they occur independent of white matter lesions; and iii) they are associated with subsequent conversion to clinically definite MS and disability.

Seventy-one people had MRI scanning a median of 4.6 months after a clinically isolated optic neuritis (ON, 49 females, mean age 33.5 years) and were followed up clinically two and five years later. Thirty-seven healthy controls (25 females, mean age 34.4 years) were also scanned. In normal-appearing white matter (NAWM), MTR gradients were measured 1-5mm and 6-10mm from the lateral ventricles.

In controls, MTR was highest adjacent to the ventricles and decreased with distance from them; in ON, NAWM MTR was lowest adjacent to the ventricles, increased over the first 5mm, and then paralleled control values. The MTR gradient over 1-5mm differed significantly between the ON and control groups (+0.059 percentage units/mm (pu/mm) vs -0.033 pu/mm, p=0.010), and was significantly steeper in those developing clinically definite MS within two years compared to those who did not (0.132 pu/mm vs 0.016 pu/mm, p=0.020). In multivariate binary logistic regression the MTR gradient was independently associated with the development of clinically definite MS within two years (MTR gradient odds ratio (OR) 61.708, p=0.023; presence of T2 lesions OR 8.500, p=0.071). At five years, lesional measures overtook MTR gradients as significant predictors of conversion to MS. The MTR gradient was not significantly affected by the presence of brain lesions (T2 lesions (p=0.918), periventricular T2 lesions (p=0.580) or gadolinium-enhancing T1 lesions (p=0.724)). The MTR gradient also correlated with Expanded Disability Status Scale (EDSS) score five years later (Spearman r=0.313, p=0.027).

An abnormal periventricular MTR gradient occurs early in MS, is clinically relevant, and may arise from one or more mechanisms that are at least partly independent of lesion formation.

Keywords

Multiple sclerosis; magnetisation transfer ratio; normal-appearing white matter

Abbreviations

ANOVA = analysis of variance; BPF = brain parenchymal fraction; CDMS = clinically definite multiple sclerosis; CIS = clinically isolated syndrome; GM = grey matter; MTR = magnetisation transfer ratio; NAWM = normal-appearing white matter; ON = optic neuritis; PD = proton density; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis

Introduction

In people with established MS, it has recently been shown that magnetisation transfer ratio (MTR) is increasingly abnormal in NAWM and WM lesions close to the lateral ventricles (Liu *et al.*, 2015). Several mechanisms may be responsible for this periventricular MTR gradient, and it is unclear whether the underlying process differs from those leading to WM lesion formation. It is also not known if such a periventricular predilection for non-lesional WM pathology occurs early in MS or is associated with subsequent disease activity. An early, clinically-relevant change that is independent of lesions might represent an early treatment target.

About 80% of people with MS initially present with a clinically isolated syndrome (CIS) such as optic neuritis (ON). About two-thirds of people with a CIS have asymptomatic brain white matter (WM) lesions visible on MRI, and these are associated with a substantially greater risk of subsequently developing clinically definite (CD) MS (80% compared with 20% for those who do not have such lesions (Fisniku *et al.*, 2008)). However, WM lesions are not the only abnormality found early in people with MS: GM lesions are also seen (Calabrese *et al.*, 2007; Nielsen *et al.*, 2013), and normal-appearing (NA) WM and GM are both abnormal when assessed using quantitative MRI measures (for example (Fernando *et al.*, 2005; Gallo *et al.*, 2005; Rocca *et al.*, 2008; Henry *et al.*, 2009).

By looking for an abnormal periventricular MTR gradient in people who have recently had a CIS, we would be able to establish if it is seen from the earliest clinical stages of MS and, given that people usually have very few WM lesions immediately after a CIS, determine if it is closely associated with WM lesions. Using previously acquired MTR data from a longitudinal study of people with a recent CIS, we aimed to (1) look for gradients in NAWM abnormalities around the lateral ventricles and, if present, to determine (2) whether or not they were associated with WM lesions, or (3) were associated with the subsequent risk of developing MS and disability.

Materials and methods

Participants

From a prospectively recruited CIS cohort (Fernando *et al.*, 2005) we included data from 81 people who had presented with a clinically isolated ON, who had no previous history of neurological symptoms, were aged between 16 and 50 years at symptom onset, had 3D fast spoiled gradient echo (FSPGR) as part of their baseline MRI assessments and MTR three months later. Participants were recruited from Moorfields Eye Hospital (London) by a single experienced neuro-ophthalmologist. They were evaluated clinically and with MRI a median of 1.4 (range 0.1-3.5) months after ON onset ('baseline') and underwent a further scan (including magnetization transfer imaging) on the same scanner three months later (median 4.6; mean 4.8; range 2.0-7.5 months after ON onset); patients were followed up clinically two and five years later (Brownlee *et al.*, 2015) and had EDSS and paced serial addition test (PASAT, 3 second intervals (Cutter *et al.*, 1999)) scores assessed at five years. This work therefore describes cross-sectional MTR data (collected 4.6 months after symptom onset) with clinical data at 4.6 months plus two and five years after symptom onset. We also studied a group of 39 healthy controls with no known neurological disorder or clinical follow up. Demographic and clinical data are reported in Table 1.

All participants gave written informed consent and the study was approved by our local institutional ethics committees.

Clinical assessments

MS was diagnosed on the basis of further relapses (clinically definite multiple sclerosis (CDMS, (Poser *et al.*, 1983) and using the 2010 McDonald criteria (Polman *et al.*, 2011).

Magnetic resonance imaging

All magnetic resonance studies were performed on a 1.5 Tesla (T) GE Signa Echospeed scanner (General Electric Medical Systems, Milwaukee, WI). The following sequences were acquired: (i) an FSPGR scan of the whole brain (124x1.5mm slices; matrix, 256x160, interpolated to a final in-plane resolution

of 1.1mm, TR= 10.9 ms, TE= 4.2 ms, TI= 450 ms) for volumetric measures; (ii) dual echo proton-density (PD)/T2 scans of the whole brain (46x3 mm contiguous axial-oblique slices parallel to the anterior/posterior commissural line, matrix 256x256, FOV 24x18 cm, TR=3200 ms, TE=15/90 ms), for the evaluation of WM lesions; (iii) T1-weighted pre- and post-gadolinium (0.1 mmol/kg body weight) spin echo sequences of the brain (46x3 mm contiguous axial-oblique slices parallel to the anterior/posterior commissural line, matrix 256x256, FOV 24x18 cm, TR=600 ms, TE=17 ms) to evaluate the presence of pathologically enhancing lesions; and (iv) MTR data using a dual-echo, spin-echo sequence of the whole brain (28x5mm contiguous, axial-oblique slices parallel to the anterior/posterior commissural line with an interleaved sequence described by Barker (TR=1720 ms, TE=30/80ms, number of excitations 0.75, matrix 256x128, FOV 24x24cm, MT-weighted by the application of a pre-saturation pulse (Hamming apodized 3 lobe sinc pulse, duration 64ms, flip angle 1430°, and a peak amplitude of 14.6 μT giving a normal bandwidth of 62.5 Hz, applied 1kHz from the water resonance (Barker *et al.*, 1996))). MTR maps were calculated on a voxel-by-voxel basis using the short echo data because of its higher signal to noise compared to the longer echo data. PD and T2-weighted images are included in this sequence, intrinsically registered to the MT data, and were used to facilitate registrations to native MT space as described below. The FSPGR scan was acquired at baseline (when magnetisation transfer imaging was not performed); all other scans and lesion counts used for this analysis were acquired three months later (when the FSPGR was not repeated (Fernando *et al.*, 2005)).

Image analysis

MTR map calculation

For each participant, MTR maps were calculated using the following equation: MTR (in percentage units (pu)) = ((MTR_{off} – MTR_{on}) / MT_{off}.) x 100). The interleaved nature of the MTR sequence used meant that co-registration of MT_{on} and MT_{off} data was not required. MTR values were extracted from each NAWM and lesion ring using FSLstats (http://www.fmrib.ox.ac.uk/fsl/).

Tissue segmentation

T1-hypointensities were outlined using a semi-automated edge-finding tool (JIM v6.0, Xinapse systems, Aldwincle, United Kingdom) on the 3D FSPGR images and these masks were used to perform lesion filling on the 3D FSPGR scans (Chard *et al.*, 2010; Prados *et al.*, 2016). After lesion filling the FSPGR scans were segmented into grey matter (GM), WM and cerebrospinal fluid (CSF) probability maps using the new segmentation pipeline in Statistical Parametric Mapping 12 (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK; www.fil.ion.ucl.ac.uk/). Brain parenchymal fraction (BPF) was calculated using SPM12-derived tissue segmentations for use as a covariate in the statistical models (Chard *et al.*, 2010).

Generation of NAWM masks

WM lesions were identified on the dual echo proton-density (PD)/T2-weighted scans, and outlined using JIM v6.0 (Xinapse systems, Aldwincle, United Kingdom) by one investigator (WJB). Using NiftyReg (Modat *et al.*, 2010; Modat *et al.*, 2014) the T2-weighted image was linearly co-registered with the T2-weighted image embedded in the MT sequence, thus aligning the WM lesion masks with the MTR maps. NiftyReg implements a symmetric and inverse-consistent registration ensuring that the results are unbiased towards the directionality of the registration process because the forward and backward transformations are optimised concurrently in an inverse-consistent manner. The symmetric full affine approach (Modat *et al.*, 2014) with 12 degrees of freedom is based on the asymmetric block-matching approach (Ourselin *et al.*, 2001).

The PD and T2 images embedded in the MT sequence were used to generate a pseudo-T1 image, by subtracting the PD scans from the T2 images (Hickman *et al.*, 2002). For each participant, the 3D FSPGR scan was then linearly co-registered to the pseudo-T1 using NiftyReg (Modat *et al.*, 2014) and the transformation applied to the GM, WM and CSF tissue segmentation maps. In MTR space the GM, WM and CSF maps were binarised using a probabilistic threshold of > 90% (Samson *et al.*, 2014). In MTR space, WM lesion masks were dilated by two voxels (to account for perilesional MTR abnormalities (Vrenken *et al.*, 2006)), and the dilated lesion masks were

subtracted from the thresholded WM tissue probability maps to produce NAWM masks.

Segmentation of NAWM into concentric periventricular rings

Given the voxel size of MT images (1x1x5 mm), to reduce the potential for partial volume effects between CSF and WM all analyses were limited to two axial slices orthogonal to the wall of the lateral ventricles, i.e. a slice immediately above the insula, and the slice immediately below this. To consistently identify these slices in all participants, a position marker was placed in the lateral ventricles just superior to the insula on a MNI152 brain template (Grabner *et al.*, 2006). The MNI152 brain template was registered to each participant's native MTR space with a non-linear transformation as previously described (Muhlert *et al.*, 2013) using NiftyReg (Modat *et al.*, 2010). The position of the marker was checked and corrected as needed by one investigator (JWLB) and then the two axial slices were selected relative to this template marker.

To identify the lateral ventricles we used a previously described approach (Liu *et al.*, 2015). Briefly, a mask of the lateral ventricles was created in MNI152 space using the Wake Forest University School of Medicine PickAtlas toolbox (Maldjian *et al.*, 2003) and then registered to each participant's MTR native space using the previously computed transformation. The lateral ventricle mask was then intersected with each participant's CSF mask on the two previously selected axial slices. These masks were checked and manually edited by one investigator (JWLB) to ensure anatomical accuracy, and then sequentially dilated in the axial plane by 1 voxel (1mm) using DilM (part of FSL software package (http://www.fmrib.ox.ac.uk/fsl/) producing concentric rings around the lateral ventricles (Fig. 1). The first two rings were discarded to limit partial volume effects from CSF as each of these contained CSF in over half of participants (first ring 117/120 participants; second ring 63/120). As per previous work (Liu *et al.*, 2015), WM lesional MTR and the percentage of lesioned WM were computed for the next ten rings. Residual CSF was identified in the first of these ten rings in ten people with ON and two controls

and so these participants were excluded, leaving 71 people with ON and 37 healthy controls.

We also recalculated the mean NAWM and GM MTR (as reported in (Fernando *et al.*, 2005) for use as covariates in the statistical models.

Statistical analyses

Statistical analyses were performed using SPSS (IBM SPSS version 22 for Windows (SPSS, Inc., Chicago, IL, USA)). Demographic data are presented as mean (standard deviation) and MTR data as mean (standard error), while EDSS values are presented as median (range). In each participant, mean MTR values were calculated in the 1 mm rings in both axial slices, and then averaged across both slices, weighted by the number of voxels in each ring. After discarding the first two rings (see above), we calculated periventricular MTR gradients over the next 5mm closest to the ventricles (i.e. NAWM MTR in ring five – NAWM MTR in ring one) and deep MTR gradients over the subsequent 5mm (i.e. NAWM MTR in ring ten – NAWM MTR in ring six), dividing both by the number of intervals to give the MTR change in percentage units (pu) per mm. This division was consistent with previous work (Liu *et al.*, 2015), and coincided with the point at which the MTR gradients in the ON and control groups converged (Fig. 2A).

In the ON cohort, 58/71 patients were followed-up two and five years after their baseline MRI scan. These patients were subdivided into those who developed CDMS or McDonald MS within two years of their ON. We also examined those developing CDMS and McDonald MS within five years of their ON. To assess associations with gadolinium-enhancing lesions, the ON cohort was subdivided into those with or without enhancing lesions. To explore associations with WM lesions, the ON cohort was also subdivided into those who did or did not have asymptomatic T2 WM lesions and, in those with lesions, those who did or did not have periventricular lesions. Independent sample t-tests and analysis of variance (ANOVA) tests were used to compare clinical and MRI measures between groups. For the MTR gradient measures, the same comparisons were also performed using general linear models firstly

correcting for BPF (to control for the possible effects of atrophy on periventricular MTR gradients), then correcting for mean NAWM and GM MTR (to ensure diffuse MTR changes were not driving MTR gradients). Spearman correlation was used to explore the relationship between MTR gradients and both EDSS and PASAT scores. Factors predicting conversion to CDMS status were examined with multivariate binary logistic regression. Results were considered statistically significant at the $p<0.05$ level.

Results

There were no significant demographic differences between the clinically isolated ON group and controls (Table 1). At the three-month scan, 55/71 (77.5%) of the optic neuritis group had WM lesions, 46/71 (64.8%) had periventricular lesions and 22/71 (31.0%) had gadolinium-enhancing lesions. Thirteen patients were lost to follow-up. After two years, 18/58 (31%) had experienced a further clinical relapse and so developed CDMS while 40/58 (69%) had McDonald MS. Five years after ON, these figures had risen to 31/58 (53.5%) and 45/58 (77.6%) respectively.

Periventricular MTR gradients in ON compared with controls

Table 2 and Fig. 2A show the mean and standard error (SE) NAWM MTR per ring in the ON and control groups. The mean MTR of each NAWM ring was lower in the ON group than in healthy controls. In controls, mean MTR was highest in the rings nearest the ventricles and declined with distance from them. In contrast, in the ON group, mean MTR was lowest in the ring nearest the ventricles, increased over the first 5mm and then paralleled control values.

Over the first 5mm from the ventricles, we found significantly different MTR gradients between the ON and control groups (ON: mean +0.059 pu/mm ± 0.028; controls mean -0.033 pu/mm ± 0.028, t=-2.62, p=0.010). This difference remained significant after adjustment for BPF (p=0.009). Conversely, the MTR gradient over the next five rings showed no significant difference between the groups (ON: +0.003 pu/mm ± 0.008, controls -0.009 pu/mm ± 0.012, p=0.541).

Associations of periventricular MTR gradients with lesions

In the ON group, over the first 5mm periventricular MTR gradients did not differ significantly between those with or without asymptomatic WM lesions seen on a T2-weighted scan from the same scanning session (+0.058 pu/mm ± 0.024 vs +0.063 pu/mm ± 0.043 respectively, *p*=0.918). The periventricular MTR gradient in the ON group who did not have brain lesions showed a non-significant trend to be higher than healthy controls (+0.063 pu/mm ± 0.043 vs -0.033 pu/mm ± 0.028 respectively, *p*=0.064), Fig. 2B. The presence of periventricular lesions seen on a T2-weighted scan had no significant effect on the periventricular gradient (0.068 pu/mm ± 0.028 (with periventricular lesions) vs 0.043 pu/mm ± 0.030 (without periventricular lesions), *p*=0.580, Fig. 2C). The mean gradient in those with and without gadolinium-enhancing lesions similarly did not differ significantly (0.067 pu/mm ± 0.040 vs 0.055 pu/mm ± 0.025 respectively, *p*=0.794).

Associations of periventricular MTR gradients with conversion to MS and disability

Table 3 and Fig. 2D show the gradients over the first and second 5mm of NAWM extending from the ventricles in the ON groups who did or did not develop CDMS within two years.

The periventricular MTR gradient in the group who developed CDMS within two years differed from healthy controls and the ON group who did not convert to CDMS within two years (Table 3; overall effect p=0.006; converters vs. healthy controls p=0.001, converters vs. non-converters p=0.020, non-converters vs. healthy controls p=0.221). The periventricular MTR gradient also differed between those who converted to CDMS within five years and healthy controls, while the difference between converters and non-converters did not reach significance; overall effect p=0.026; converters vs. healthy controls p=0.007, converters vs. non-converters p=0.123, non-converters vs. healthy controls p=0.304). The differences between those who developed MS and HC remained significant when McDonald MRI criteria were used instead of CDMS (Table 3). The group effect on the periventricular MTR gradient did

not materially change after including BPF in the statistical models. The group effect on the periventricular MTR gradient also remained significant after adjustment for mean NAWM MTR (p=0.014) and mean GM MTR (p=0.022). When both NAWM MTR and GM MTR were added, the model remained significant (p=0.021) as did differences in MTR gradients between converters and non-converters (p=0.037) and between converters and healthy controls (p=0.006).

We also examined whether or not periventricular MTR gradients over 1-5mm had an effect on conversion to CDMS independent of the presence of lesions. A multivariate binary logistic regression showed that the MTR gradient over 1-5mm was independently associated with conversion to CDMS at two years (MTR gradient: odds ratio (OR) 61.708 p=0.023; presence of T2 lesions OR 8.500 p=0.071). A similar pattern was seen when the MTR gradient was compared to periventricular T2 lesions (MTR gradient: OR 44.100 p=0.029; presence of periventricular T2 lesions OR 5.84 p=0.040). However, the MTR gradient did not predict conversion to CDMS over five years.

There was no significant difference in 6-10 mm MTR gradients between ON groups who did or did not develop CDMS at two or five years (p=0.124 and *p*=0.231 respectively).

Unlike the periventricular MTR gradient, the mean MTR of band 1 or 5 in the ON group converting to CDMS within 2 years was not significantly higher than in those not converting to CDMS (see Supplementary Data).

Associations of MTR gradient, mean NAWM MTR and mean GM MTR with disability.

A correlation was found between the MTR gradient over the first 5mm and EDSS status five years later (Spearman r=0.313, p=0.027; EDSS measured in 50/58 patients at this time point). No significant correlation was found between inner MTR gradient and PASAT score at five years (*p*=0.815), although this was only undertaken in 31/58 patients at this time point.

No significant correlations were found between EDSS five years later and mean MTR in band 1 (p=0.165) or band 5 (p=0.540), NAWM MTR (p=0.506) or GM MTR (p=0.109).

Lesion measures

The mean MTR of lesional voxels, like the NAWM MTR, was lowest at the ventricular margin and increased with distance from it (see Fig. 3). Lesion volumes are shown in Fig. 4.

Discussion

In a previous study in people with established RRMS and SPMS we found that MS effects on NAWM MTR increased close to the lateral ventricles (Liu *et al.*, 2015). However, we could not determine how early in the clinical course of MS this abnormal periventricular MTR gradient occurred, whether or not it was due to a mechanism independent of WM lesion formation, or if it was associated with subsequent disease activity. In the present study we found that an abnormal periventricular MTR gradient is present within five months of a clinically isolated ON, is not dependent on the presence of WM lesions and is associated with the subsequent risk of developing MS and disability. Both the periventricular NAWM MTR gradient and WM lesions independently predicted conversion to CDMS over two years. This raises the possibility that lesions and NAWM periventricular abnormalities in early MS may arise from different but nevertheless clinically relevant pathophysiological processes.

In the ON group we looked for significant differences in the periventricular MTR gradients between those with and without asymptomatic WM lesions, those with and without periventricular WM lesions, and those with and without gadolinium-enhancing lesions, and found none. Consistent with previous work (Liu *et al.*, 2015) MTR in lesions also showed periventricular gradients similar to those in NAWM. Collectively, this strongly suggests that abnormal gradients in NAWM MTR are not dependent on the presence of WM lesions. In keeping with previous results (Barkhof *et al.*, 1997) periventricular lesions predicted conversion to CDMS over two years, and in a multivariate logistic regression the MTR gradient over 1-5mm also independently predicted conversion to CDMS over two years. The identification of an early abnormal periventricular MTR gradient that is not directly related to lesion formation, but linked with clinical outcomes suggests that there is a pathological process distinct to that underlying lesion formation.

The pathological basis of the abnormal periventricular MTR gradient is uncertain. MTR correlates with myelin and axonal density (Schmierer *et al.*, 2007) and may also be reduced by tissue oedema and inflammation (Dousset *et al.*, 1992; Gareau *et al.*, 2000). To the best of our knowledge, there have

been no histopathological studies looking for a gradient of pathology in extra-lesional periventricular WM. Several mechanisms could underlie the periventricular MTR changes seen, perhaps in combination, but without knowing the underlying pathological substrate we can only speculate which are responsible.

Considering first factors external to the brain, CSF-mediated and ependymal processes may both influence MS pathology, particularly in tissues close to the surface. CSF from people with a CIS or MS has been found to cause neuronal death in vitro (for example (Alcazar *et al.*, 2000; Vidaurre *et al.*, 2014)), and when sampled at the time of relapse in RRMS induces oligodendrocyte apoptosis (Menard *et al.*, 1998). In addition, leucocytes can enter the CSF space through the choroid plexus, and in people with MS the concentration of the tight junction protein claudin-3 - which helps maintain the blood-CSF barrier integrity - is lower when compared with healthy controls (Kooij *et al.*, 2014). 'Granular ependymitis' has been reported at post-mortem in some people with MS, however while it is associated with 1 in 10 periventricular lesions it is also evident distant from lesions, and is seen in non-inflammatory central nervous system conditions (Adams *et al.*, 1987).

Within the brain, there are several mechanisms through which a periventricular pathological gradient might arise. Axons and oligodendrocytes are susceptible to hypoxia, and the periventricular venous watershed renders this region intrinsically susceptible to hypoperfusion and hypoxia (Andeweg, 1996); periventricular hypoperfusion is significantly greater in MS than controls and is evident even after a CIS (Dewar *et al.*, 2003; Varga *et al.*, 2009; Beggs, 2013). WM lesions nearly always form around veins, and the high venular density around the lateral ventricles likely accounts for WM lesions' periventricular predilection (Brownell and Hughes, 1962; Adams *et al.*, 1987; Narayanan *et al.*, 1997; Evangelou *et al.*, 2000). However, we think that lesions themselves are unlikely to directly explain the WM periventricular MTR gradient, as we have excluded lesions plus a two voxel-layer NAWM perilesional cuff, and extending this to a four voxel-layer cuff in our previous work did not materially alter the results (Liu *et al.*, 2015). A tract-mediated

effect of lesions may also contribute to gradients: adjacent to the lateral ventricles run multiple WM tracts (e.g. those in the corpus callosum, and so it may be expected the remote effects of axonal transection in lesions (Trapp *et al.*, 1998) will be more apparent in regions with high compared with low densities of parallel tracts. Again, we think that it is unlikely that this explains our present results as the periventricular MTR gradient did not significantly differ between those who did and those who did not have additional asymptomatic brain lesions, and the mean lesion load was low (~1.5 ml).

Elucidating the responsible mechanism(s) may have significant implications for early MS treatment. For example, in established MS, neuropathological work has shown a cortical gradient in neuronal loss (Magliozzi *et al.*, 2010) where an MTR gradient has also been identified (Samson *et al.*, 2014). If a gradient in axonal loss were confirmed around the ventricles, one might infer that the *in vivo* periventricular MTR findings indicate a neurodegenerative process that should be targeted from the earliest clinical stages of MS.

There are a few study limitations worth noting. First, we used previously acquired MTR data with lower resolution (1x1x5mm) than that used in our recent study examining periventricular gradients in established MS (1x1x1mm, (Liu *et al.*, 2015) and given this, partial volume effects were a greater concern. To minimize these, we restricted our analysis to two axial slices perpendicular to the lateral ventricular wall, so avoiding oblique CSF-WM boundaries within periventricular voxels, and through plane smoothing of periventricular MTR gradients (recalling that these were seen over the first 5 mm around the ventricles, and the axial slices were 5 mm thick). Despite this, the first two periventricular rings still contained CSF in the majority of participants, and so these rings were also excluded from subsequent analyses. Noting that the periventricular MTR gradient seen in previous work was steepest close to the ventricles (Liu *et al.*, 2015), it is likely that excluding these rings will have significantly reduced our sensitivity to gradients. In 10/81 participants with ON and 2/39 healthy controls, there was still CSF in the third ring so these participants were also excluded from the analyses, further reducing the power of the study to detect periventricular gradients. While we

were very careful to avoid partial volume effects between CSF and WM, partial volume between periventricular rings will tend to smooth MTR gradients, and so reduce sensitivity to disease effects. Similarly, restricting our analysis to two axial slices, while limiting the risk of MTR gradients being smoothed through plane, substantially reduced the volume of each ring studied when compared to our previous study using 1x1x1mm MTR data; this too is likely to have reduced sensitivity to disease effects. To allow for brain atrophy, which could exacerbate this further, we included BPF as a covariate when looking for differences in MTR gradients between clinical subgroups, and found no material difference in the results. To provide context with established MTR metrics and confirm that the difference in MTR gradient between converters and non-converters was not simply driven by diffuse tissue differences, we added NAWM and GM MTR as covariates and the MTR gradient remained a significant factor.

By five years, about ~55% of the ON group developed CDMS, while at the time of their MTR scan only ~10% had done so (Table 1). The pattern of group differences in periventricular MTR gradient were still apparent, albeit with less statistical significance, following exclusion of those converting before the MTR scan (see Supplementary Data). As such, we think it is unlikely that the apparent predictive power of the periventricular MTR gradient for conversion to CDMS within 5 years is simply due to such a gradient being present in those who had already developed CDMS by the time of their MTR scan. Thirteen of the 71 people with ON studied at baseline did not have clinical follow-up. There were no significant differences in any baseline demographic features between those who were or were not followed up (see Supplementary Table 1), so we believe this is unlikely to have biased our results in favour of detecting differences in periventricular MTR gradients between groups, but it may have reduced our sensitivity to them.

While we have assessed cross-sectional differences in MTR measures between the HC and ON groups, it would be of considerable interest to see how these differences evolve with time, and whether or not these changes relate more closely to clinical outcomes. Some longitudinal MTR data is

available for the present cohorts (HC n = 18, ON n = 44), but the resolution of the 2D MTR scans (1 x 1 x 5mm) means that it is not possible to accurately align the same axial slices over time. When we looked for changes in periventricular MTR gradients over time without registering scans, we found no significant change over 5 years.

It is well recognised that a substantial number of people with MS may have significant cognitive deficits (Langdon, 2011). However, only about half (31/58) of those followed-up at 5 years underwent cognitive testing, of whom only 12 had developed clinically definite MS. Given this, it is perhaps unsurprising that we did not find a correlation with periventricular MTR gradients in this cohort, and this would perhaps be better examined in a group of people with progressive MS who are more likely to have cognitive impairment (Langdon, 2011).

The parent cohort's principal presenting CIS was ON, and for consistency we excluded three non-ON participants who would otherwise have been eligible for our study. In some cohorts, ON appears to carry a lower risk of conversion to CDMS when compared with other presentations (Tintore *et al.*, 2015) and it would be of interest to see if periventricular gradients differ dependent on the type of CIS.

While inner periventricular MTR gradients independently contributed to the prediction of conversion to CDMS and McDonald MS, there was substantial overlap in the range of values between groups (see Supplementary Data) and no clear threshold was found beyond which conversion to MS by 5 years was inevitable. As such measurement of periventricular MTR gradients using the methods employed in this study is unlikely to be useful in clinical practice. However, this does not negate the clinical relevance of the pathological processes underlying abnormal MTR gradients, which - independent of those leading to WM lesion formation - may be a potential target for treatments.

In conclusion, our findings show that an abnormal periventricular MTR gradient occurs soon after a CIS and is associated with subsequent

conversion to MS and disability. The abnormal periventricular MTR gradient was not significantly affected by the presence of WM lesions, and therefore seems likely to arise from an at least partly independent mechanism. Histopathological studies are warranted to elucidate the nature of these MTR gradients.

Acknowledgements

The authors thank the people who took part in the study, the MS Society of Great Britain and Northern Ireland and the National Institute for Health Research University College London Hospitals Biomedical Research Centre for financial support.

Funding

The Queen Square MS centre is supported by the MS Society of Great Britain and Northern Ireland. J.W.L.B is funded through a Next Generation Fellowship funded by the Grant Charity of the Freemason's. M.P. is supported by the non-profit Karol Wojtila Association (Lavagna, Italy). F.P. is funded by the National Institute for Health Research University College London Hospitals Biomedical Research Centre (NIHR BRC UCLH/UCL High Impact Initiative-BW.mn.BRC10269). S.O. receives funding from the EPSRC (EP/H046410/1, EP/J020990/1, EP/K005278), the MRC (MR/J01107X/1), the NIHR Biomedical Research Unit (Dementia) at UCL and the NIHR BRC UCLH/UCL (BW.mn.BRC10269).

Table 1: Participant characteristics.

	Healthy Controls	**People with optic neuritis**
N	N= 37	N = 71
Mean age, years ± SD (range)	34.4 ± 4.8 (27-47)	33.5 ± 6.7 (20-48)
female : male	25 : 12	49 : 22
Median baseline EDSS (range)	-	1 (0-3)
Number with abnormal T2 scan (excluding symptomatic lesion)	-	55
Number with periventricular lesions	-	46
Number with Gd-enhancing lesions	-	22
Mean T2 lesion volume (ml) ± SD	-	1.47 ± 3.32
Mean brain parenchymal fraction ± SD	0.84 ± 0.04	0.85 ± 0.03
Number attaining CDMS at time of MTR scan	-	8
Number attaining McDonald MS at time of MTR scan	-	33
Number with clinical follow-up at two and five years	-	58
Number converting to CDMS within two years of ON (of 58 with clinical follow up)	-	18
Number converting to McDonald MS within two years of ON (of 58 with clinical follow up)	-	40

Number converting to CDMS within five years of ON (of 58 with clinical follow up)	-	31
Number converting to McDonald MS within five years of ON (of 58 with clinical follow up)	-	45
Median (range) EDSS score at five year follow-up (assessed in 50/58)	-	1 (0-8.5)
Median (range) PASAT score at five years follow-up (assessed in 31/58)	-	46.5 (17-59)

In the optic neuritis group, MTR scans were undertaken three months after first enrolment in the study, and so lesion measures from scans obtained at three months are shown. Volumetric brain scans were acquired at baseline but not three months. CDMS = clinically definite multiple sclerosis. EDSS = Expanded Disability Status Scale. ON = optic neuritis. PASAT = paced serial addition test, 3 second intervals. SD = standard deviation.

Table 2: Mean ring MTR in healthy controls and people with optic neuritis.

Ring	**Mean ± SE MTR in pu**		**Mean ± SE MTR in pu**		
	Healthy controls (n=37)	*All Optic Neuritis* (n=71)	*Optic Neuritis subgroups*		
			Convert to CDMS <2y (n=18)	Convert to CDMS 2-5y (n=13)	Did not convert to CDMS (n=27)

1	38.364 ± 0.138	37.670 ± 0.102 ***p* = 0.000**	37.371 ± 0.183 ***p* = 0.000**	37.831 ± 0.180 *p* = 0.062	37.805 ± 0.203 ***p* = 0.013**
2	38.334 ± 0.113	37.836 ± 0.085 ***p* = 0.001**	37.639 ± 0.166 ***p* = 0.001**	37.773 ± 0.205 ***p* = 0.020**	37.951 ± 0.156 ***p* = 0.043**
3	38.254 ± 0.095	37.912 ± 0.080 ***p* = 0.007**	37.862 ± 0.176 *p = ns*	37.946 ± 0.209 *p = ns*	37.903 ± 0.133 *p = ns*
4	38.293 ± 0.080	37.959 ± 0.075 ***p* = 0.003**	37.944 ± 0.158 *p = ns*	37.942 ± 0.190 *p = ns*	37.918 ± 0.132 *p = ns*
5	38.232 ± 0.080	37.936 ± 0.076 ***p* = 0.009**	37.901 ± 0.163 *p = ns*	37.916 ± 0.204 *p = ns*	37.859 ± 0.126 *p = ns*
6	38.109 ± 0.087	37.827± 0.077 ***p* = 0.017**	37.822 ± 0.162 *p = ns*	37.807 ± 0.209 *p = ns*	37.765 ± 0.129 *p = ns*
7	38.006 ± 0.083	37.751 ± 0.074	37.751 ± 0.166	37.772 ± 0.194	37.706 ± 0.124

		***p* = 0.025**	*p = ns*	*p = ns*	*p = ns*
8	38.024 ± 0.084	37.774 ± 0.073 ***p* = 0.027**	37.754 ± 0.174 *p = ns*	37.805 ± 0.192 *p = ns*	37.740 ± 0.115 *p = ns*
9	38.113 ± 0.084	37.864 ± 0.075 ***p* = 0.030**	37.805 ± 0.187 *p = ns*	37.942 ± 0.192 *p = ns*	37.822 ± 0.117 *p = ns*
10	38.187 ± 0.085	37.952 ± 0.078 ***p* = 0.044**	37.850 ± 0.191 *p = ns*	38.048 ± 0.198 *p = ns*	37.945 ± 0.123 *p = ns*

Independent sample t-tests were used to compare the healthy control and whole optic neuritis groups; one-way ANOVA to compare the optic neuritis subgroups. pu = percentage units. ns = not significant.

Table 3: Periventricular (1-5mm) and deep (6-10mm) MTR gradients in normal appearing white matter (NAWM) of people with optic neuritis (ON) and healthy controls (HC).

	Clinical classification at 2 years	**N**	**MTR gradient mean ± SE (pu/mm)**	
			Periventricular (1 to 5 mm)	*Deep (6 to 10 mm)*
Healthy controls		37	-0.033 ± 0.028	-0.009 ± 0.012
Optic Neuritis	Did not convert to CDMS	40	0.016 ± 0.029	0.020 ± 0.010
	Converted to CDMS	18	0.132 ± 0.041*	-0.010 ± 0.019
	Did not convert to McDonald MS	18	0.015 ± 0.048	0.021 ± 0.015
	Converted to McDonald MS	40	0.069 ± 0.028**	0.006 ± 0.011

Optic neuritis group divided according to conversion to clinically definite multiple sclerosis (CDMS) status within two years. *Converters vs. healthy controls p=0.001, converters vs. non-converters: p=0.020, non-converters vs. healthy controls p=0.221). Optic neuritis group also divided according to conversion to McDonald MS within two years. **converters vs healthy controls p=0.014; otherwise, no significant differences in gradients detected.

Supplementary data

Supplementary Table 1. Comparison of baseline clinical and radiological demographics in those with and without follow up at two and five years.

	People with optic neuritis		**Significance**
	People with clinical follow-up	**People without clinical follow-up**	
N	N = 58	N = 13	
Mean age, years ± SD (range)	33.5 ± 6.4 (27-48)	33.6 ± 8.1 (21-46)	*p* = 0.966
female : male	40 : 18	9 : 4	*p* = 1.000
Median baseline EDSS (range)	1 (0-3)	1 (0-2)	*p* = 0.585
Number with abnormal T2 scan (excluding symptomatic lesion)	46	9	*p* = 0.471
Number with periventricular lesions	40	6	*p* = 0.197
Number with Gd-enhancing lesions	19	3	*p* = 0.741
Mean brain parenchymal fraction ± SD	0.85 ± 0.04	0.85 ± 0.03	*p* = 0.388
Mean inner MTR gradient ± SE	0.05 ± 0.02	0.09 ± 0.04	*p* = 0.378

Comparative results: capability of mean MTR in ring 1 and ring 5 for predicting conversion to CDMS and EDSS at 5 years

Conversion to CDMS – ring 1.

In one-way ANOVA, the mean MTR of ring 1 in the ON group who developed CDMS within two years was significantly smaller than in healthy controls, but was not significantly different when compared to the ON group who did not develop CDMS within two years (converters vs healthy controls p=0.000; converters vs non-converters p=0.077; non-converters vs healthy controls p=0.007). The results for conversion at five years were broadly similar (converters vs healthy controls p=0.000; converters vs non-converters p=0.301; non-converters vs healthy controls p=0.014).

Conversion to CDMS – ring 5.

In one-way ANOVA, the mean MTR of ring 5 in the ON group who developed CDMS within two years was not significantly smaller than in healthy controls or the ON group who did not develop CDMS within two years (converters vs healthy controls p=0.062; converters vs non-converters p=0.893; non-converters vs healthy controls p=0.012). The results at five years were broadly similar (converters vs healthy controls p=0.031; converters vs non-converters p=0.764; non-converters vs healthy controls p=0.017).

Disability

We found no correlation between the mean MTR values in rings 1 and 5 with EDSS score at five years (p=0.165 and p=0.540 respectively).

<u>Effect of conversion to CDMS before MTR scan.</u>

As a confirmatory analysis we excluded those 8 ON subjects who converted to MS in the interval between the baseline clinical MRI and the MTR acquisition. Despite the reduced power, the pattern in group differences in periventricular MTR gradients was maintained: ON vs healthy controls (0.050 pu/mm ± 0.022 vs. -0.033 pu/mm ± 0.028, p=0.022); ON converting to CDMS within two years vs HC (0.114 pu/mm ±0.051 vs. -0.033 pu/mm ± 0.028,

p=0.016); ON converting to CDMS within two years vs non-converters (0.114 pu/mm ±0.051 vs. 0.016 pu/mm ± 0.028, p=0.102).

MTR gradient threshold calculations

The range of periventricular MTR gradients found in those who developed CDMS within 2 years (-0.20 to 0.40 pu/mm) compared to those who did not (-0.41 to 0.62 pu/mm) - and in those who developed McDonald MS within two years (-0.41 to 0.40 pu/mm) compared to those who did not (-0.35 to 0.62 pu/mm) - overlapped substantially, and there was no clear cut off beyond which conversion from a CIS to MS was inevitable. The same pattern was seen when examining conversion versus no conversion at 5 years (CDMS: -0.41 to 0.40 pu/mm; -0.35 to 0.62 pu/mm) (McDonald MS: -0.41 to 0.40 pu/mm; -0.35 to 0.62 pu/mm).

When we use the mean MTR gradient of the group who did not develop CDMS within 2 years as a cut-off (n=40), 13/18 (72%) of those converting to CDMS within 2 years had a greater MTR gradient; if a mean + 1 SD threshold is used, 7/18 (39%, expected by chance 16%); and greater than the mean + 2 SD 1/18 (6%, expected by chance 2%). In comparison, the proportion of controls exceeding these thresholds were: mean 14/37 (38%); mean + 1 SD 3/37 (8%); mean + 2 SD 1/37 (3%). When we repeated this using the mean MTR gradient of controls as the threshold (n=37), 15/18 (83%) of those converting to CDMS within 2 years had a greater MTR gradient compared to 26/40 (65%) of those not converting within 2 years; if a mean + 1 SD threshold was used, respectively 9/18 (50%) and 9/40 (23%) exceeded the threshold, and greater than the mean + 2 SD 4/18 (22%) and 1/40 (3%).

Figure Legends

Figure 1. A: Normal appearing white matter (NAWM) from two 5mm axial slices were extracted at (B) and immediately below (C) the level of the insula. Rings of concentric MTR values from the normal-appearing white matter around the ventricles were then extracted (D and E). The first two rings were excluded to minimise partial volume effects.

Figure 2A: Normal-appearing white matter (NAWM) MTR of rings in healthy controls (HC - blue) and people with optic neuritis 4.6 months after symptom onset (ON - red). Mean ± 2 standard error (SE). MTR is expressed as percentage units (pu). Ring 1 is closest to the ventricular surface.

Figure 2B: Normal appearing white matter (NAWM) MTR in healthy controls (HC - blue) and people with optic neuritis 4.6 months after symptom onset (ON), divided into those with (green) and without (yellow) asymptomatic lesions on their baseline scan. Mean ± 2 standard errors (SE) is shown. MTR is expressed as percentage units (pu). Ring 1 is closest to the ventricular surface.

Figure 2C: Normal appearing white matter (NAWM) MTR in people with optic neuritis 4.6 months after symptom onset (ON) plus asymptomatic lesions, divided into those with (n=46, green circles) and without (n=9, yellow circles) periventricular lesions. Mean ± 2 standard error (SE). MTR is expressed as percentage units (pu). Ring 1 is closest to the ventricular surface.

Figure 2D: Normal appearing white matter (NAWM) MTR in healthy controls (HC - blue) and people with optic neuritis 4.6 months after symptom onset (ON), divided into those who did convert to clinically definite multiple sclerosis (CDMS) within two years (gold) and those that did not (green). Mean ± 2 standard errors (SE) is shown. MTR is expressed as percentage units (pu). Ring 1 is closest to the ventricular surface.

Figure 3: Mean MTR of white matter (WM) lesional voxels in patients with optic neuritis 4.6 months after symptom onset (ON). Mean ± 2 standard error (SE). MTR is expressed as percentage units (pu). Ring 1 is closest to the ventricular surface.

Figure 4: Percentage of lesional white matter (WM) per ring in patients with optic neuritis 4.6 months after symptom onset (ON). Mean ± 2 standard error (SE). Ring 1 is closest to the ventricular surface.

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