Magnetization transfer histograms in clinically isolated syndromes suggestive of multiple sclerosis

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In established multiple sclerosis, magnetization transfer ratio (MTR) histograms reveal abnormalities of normal-appearing white matter (NAWM) and grey matter (NAGM). The aim of this study was to investigate for such abnormalities in a large cohort of patients presenting with clinically isolated syndromes suggestive of multiple sclerosis. Magnetization transfer imaging was performed on 100 patients (67 women, 33 men, median age 32 years) a mean of 19 weeks (SD 3.8, range 12–33 weeks) after symptom onset with a clinically isolated syndrome and in 50 healthy controls (34 women, 16 men, median age 32.5 years). SPM99 software was used to generate segmented NAWM and NAGM MTR maps. The volumes of T2 lesions, white matter and grey matter were calculated. Eighty-one patients were followed up clinically and with conventional MRI after 3 years (n = 61) or until they developed multiple sclerosis if this occurred sooner (n = 20). Multiple regression analysis was used to investigate differences between patients and controls with age, gender and volume measures as covariates to control for potential confounding effects. The MTR histograms for both NAWM and NAGM showed a reduction in the mean (NAWM, 38.14 versus 38.33, \(P = 0.001\); NAGM 32.29 versus 32.50, \(P = 0.009\); units in pu) and peak location, with a left shift in the histogram. Mean NAWM and NAGM MTR were also reduced in the patients who developed clinically definite multiple sclerosis and multiple sclerosis according to the McDonald criteria but not in the 24 patients with normal T2-weighted brain magnetic resonance imaging (MRI). MTR abnormalities occur in the NAWM and NAGM at the earliest clinical stages of multiple sclerosis.

Keywords: MTR histogram; NAWM; NAGM; multiple sclerosis; clinically isolated syndromes

Abbreviations: BPF = brain parenchymal fraction; CIS = clinically isolated syndromes; EDSS = expanded disability status scale; GMF = grey matter fraction; MTI = magnetization transfer imaging; MTR = magnetization transfer ratio; NABT = normal-appearing brain tissue; NAGM = normal-appearing grey matter; NAWM = normal-appearing white matter; NEX = number of excitations; SD = standard deviation; TE = echo time; TI = total intra-cranial volume; TR = repetition time; WMF = white matter fraction

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Introduction

Multiple sclerosis is one of the most common causes of chronic neurological disability in young adults. MRI has improved understanding of this disease by enabling sensitive and non-invasive detection of pathology occurring in the central nervous system (CNS). Using MRI evidence for dissemination in space and time, it is possible to diagnose multiple sclerosis after a single clinical event (McDonald et al., 2001).

Magnetic resonance techniques have also been used to monitor disease progression in multiple sclerosis. Much of the focus so far has been on conventional T2-weighted MRI. However, pathological changes associated with multiple sclerosis can occur in areas that look normal on conventional T2-weighted MRI (Allen and McKeown, 1979; Allen et al., 1981; Bitsch et al., 1999) in the so called normal-appearing white matter (NAWM) and grey matter (NAGM). In addition, T1-weighted MRI does not differentiate between the distinct pathological processes that occur in multiple sclerosis such as inflammation, demyelination, remyelination, axonal loss and gliosis. These factors help explain why the correlations observed between the total T2 lesion load and clinical
function in multiple sclerosis patients are generally modest (Weinshenker et al., 1989; Brex et al., 2002; Confavreux et al., 2003).

Magnetization transfer imaging (MTI) is a technique which can detect pathological changes occurring in the brain that are invisible on conventional MRI (Lexa et al., 1993, 1994; Hiele et al., 1994; Filippi et al., 1995; Loewner et al., 1995). It enables the indirect visualization of protons bound to macromolecular structures—such as myelin and cell membranes—which are not detectable on conventional MRI due to their short relaxation times. By applying an additional radiofrequency pulse at a suitable off-resonance frequency it is possible to selectively saturate this bound pool of protons. The subsequent exchange of magnetization between the bound and mobile pool causes a reduction in the magnetic resonance visible signal arising from the mobile pool of protons. The magnitude of this effect is determined by the size of the bound pool and can be quantified using the magnetization transfer ratio (MTR). Thus, MTR provides a quantitative measure of the integrity of tissue macromolecular structure (Dousset et al., 1992; van Buchem et al., 1996, 1997).

Large reductions in MTR have been reported in areas corresponding to demyelination (Dousset et al., 1992; van Waesberghe et al., 1999; Barkhof et al., 2003; Schmierer et al., 2004) and/or axonal loss (van Waesbergh et al., 1999; Evangelou et al., 2000), whereas less marked reductions in MTR may be caused by pathological processes such as oedema and inflammation (Dousset et al., 1992; Lai et al., 1996; Brochet, 1999; Gareau et al., 2000).

Brain MTR studies in patients with established multiple sclerosis have revealed significant abnormalities using region of interest (ROI) or global histogram analysis methods (van Buchem et al., 1996, 1997) applied in both the NAWM (Filippi et al., 1995, 1999, 2000a; Leary et al., 1999; Tortorella et al., 2000; Ge et al., 2002; Davies et al., 2004) and NAGM (Ge et al., 2001, 2002; Cerignani et al., 2001; Dehmeshki et al., 2003; Davies et al., 2004). Typical findings are a reduction in mean MTR, histogram peak height and peak location, with a left shift in the histogram indicating the presence of more pixels with lower MTR values (van Buchem et al., 1996; Filippi et al., 1999). These MTR abnormalities have been shown to be correlated with measures of clinical dysfunction such as the expanded disability status scale (EDSS) (Iannucci et al., 1999; Ge et al., 2001; Dehmeshki et al., 2003) and multiple sclerosis Functional Composite Scale; and cognitive scores (Filippi et al., 2000b; Rovaris et al., 2000a, b).

The stage at which these abnormalities first occur in NAWM and NAGM has been less well explored. Such information is relevant since very early abnormalities may point towards pathological processes with potential to influence disease course and prognosis. One way of looking at the earliest clinical stages of the disease is to study patients who present with a first episode of suspected demyelination, known as a clinically isolated syndrome (CIS, e.g. optic neuritis or isolated brain stem or spinal cord syndromes), since this is the initial clinical manifestation in ~85% of multiple sclerosis patients. Previous MTR studies of CIS patients have reported inconsistent findings: some have shown a reduction in MTR parameters in normal-appearing brain tissue (NABT; Iannucci et al., 2000) with abnormalities evident both in the NAWM and NAGM (Traboulsee et al., 2002), while others have not detected abnormalities (Filippi et al., 1999; Kaiser et al., 2000; Brex et al., 2001); all the studies to date have investigated relatively small cohorts.

The aim of the present report are 2-fold:

(i) to more definitively investigate the NAWM and NAGM MTR by examining a large cohort of prospectively and consecutively recruited CIS patients, over 90% of whom were studied within 6 months of symptom onset. By also investigating the subgroup of patients who satisfied the McDonald (McDonald et al., 2001) and Poser (Poser et al., 1983) diagnostic criteria for multiple sclerosis at the time of the MTR examination, it was possible to study MTR histograms in the NAWM and NAGM at the earliest clinical stage of multiple sclerosis,

(ii) to explore whether MTR in the NAWM and NAGM soon after the initial clinical presentation is related to the subsequent clinical and MRI evolution of the disease for up to 3 years.

Methods

Patients

Baseline cohort

Patients who underwent MTI were participating in a prospective, multiparameter, magnetic resonance and clinical follow up study of CIS patients first seen and investigated within 3 months of symptom onset. Full details of the study design are provided elsewhere (Brex et al., 1999; Dalton et al., 2002). Appropriate investigations were undertaken to exclude alternative diagnoses. Disability was assessed using Kurtzke’s EDSS (Kurtzke, 1983). The study had approval from the Joint Medical Ethics Committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery, and the Moorfields Eye Hospitals Ethics Committee. Informed consent was obtained from all subjects before entry into the study. The magnetization transfer study, performed in 100 patients, took place at the first scheduled follow up visit 3 months after the baseline assessment; in 93% of cases this was within 6 months of onset of the CIS.

Follow up cohort

The baseline cohort patients who had MTR are being followed up clinically and radiologically after 1 and 3 years, and are being evaluated for the following: (i) development of clinically definite multiple sclerosis; (ii) development of multiple sclerosis by the McDonald criteria; (iii) number of relapses, and (iv) disability as determined by measuring the EDSS. CDMS is defined according to the Poser criteria as two separate attacks disseminated in time (at least 1 month apart) and space (with clinical evidence of two separate lesions) (Poser et al., 1983). Sixty-one patients have been followed up clinically for 3 years, and of these, 60 have had a 3-year MRI scan. Twenty additional patients are also included who have not yet been followed up for the full 3 year period, but who have already been diagnosed with...
MTR histograms in CIS


multiple sclerosis according to the McDonald criteria at their 1 year follow up; 13/20 of these subjects have also developed CDMS. The analysis of MTR abnormalities with future EDSS was confined to the 61 patients in whom a 3-year follow up measure of EDSS was available.

Controls
Using identical methods, MTI was also obtained from 50 healthy adult controls matched for age and gender.

MRI protocol
All magnetic resonance studies were performed on a 1.5 Tesla GE Signa Echospeed scanner (General Electric Medical Systems, Milwaukee, WI). The patients had baseline T2-weighted, dual echo fast spin echo (FSE) sequences and T1-weighted pre- and post-gadolinium (0.1 mmol/kg body weight) spin echo sequences of the brain and spinal cord within 12 weeks of their initial presentation (mean 5.9 weeks, SD 3.1, range 1–12 weeks). The acquisition parameters were as follows: (i) brain—46 × 3 mm contiguous, axial oblique slices (parallel to the anterior/posterior commissural line; AC/PC line) covering the whole brain [matrix 256 × 256, field of view (FOV) 24 × 18 cm, 1 NEX (number of excitations)] with repetition time (TR) 3200 ms, echo time (TE) 15/90 ms for the FSE T2-weighted sequence and TR 600 ms, TE 17 ms for the T1-weighted sequence; (ii) spinal cord—13 × 3 mm contiguous, sagittal slices (matrix 256 × 256, FOV 48 × 24 cm) with TR 2500 ms, TE 56/98 ms, 2 NEX for the FSE T2-weighted sequences and TR 500 ms, TE 19 ms, 3 NEX for the T1-weighted sequence. Patients were followed up clinically and with conventional MRI at presentation after 3 months, 1 year and 3 years. Baseline and follow-up scans were analysed for the presence and number of T2 and gadolinium enhancing lesions by an experienced neuroradiologist blinded to the clinical data. The images were reported as normal if only the symptomatic lesion was visible. After 3 months (when MTR was also performed) and at subsequent 1 or 3 year follow up, the clinical and conventional MRI findings were reviewed to determine whether patients fulfilled the McDonald diagnostic criteria for multiple sclerosis (McDonald et al., 2001).

MTI sequence
MTI was obtained at the same time as the 3 month follow up MRI scan, a mean of 19 weeks, (SD 3.8, range 12–33 weeks) following CIS onset in 100 patients. In 93/100 patients, the MTI study was performed within 6 months of the CIS onset. A dual-echo, spin-echo sequence (28 × 5 mm contiguous, axial oblique slices (parallel to the AC/PC line) covering the whole brain; TR 1720 ms, TE 30/80 ms, 0.75 NEX, matrix 256 × 128, FOV 24 × 24 cm, total acquisition time 20 min) was performed using an interleaved sequence described by Barker et al. (1996).

Magnetization transfer and non-magnetization transfer images were acquired for both TEs; the interleaved nature of the sequence removes the need for coregistration of the images. The sequence was magnetization transfer weighted by the application of a presaturation pulse. The presaturation pulse was a Hamming apodized 3 lobe sinc pulse, with a duration of 64 ms, flip angle of 1430° and a peak amplitude of 14.6 μT, giving a nominal bandwidth of 62.5 Hz, applied 2 kHz from the water resonance.

The MTR is measured in percentage units (pu) and is calculated using the following formula, MTR = \[\left(\frac{Mo - Ms}{Mo}\right) \times 100\], where Mo and Ms are mean signal intensities without and with presaturation, respectively (Fig. 1A). MTR maps were calculated on a pixel by pixel basis using the short echo data because of its higher signal to noise ratio compared with the long echo data.

MTR histogram analyses
Dispimage image display software (D. L. Plummer, University College London Hospitals, London, UK; Plummer, 1992) was used to contour lesions on the non-magnetization transfer saturated mildly T2-weighted images (Grimaud et al., 1996). These measurements were used both to calculate the volume of lesions visible on T2-weighted scans (‘T2 lesions’) for each subject, and also to create lesion masks which were used to remove the lesions from the images before subsequent analyses.

SPM99 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, Queen Square, London, UK) was used to generate probability maps representing NAWM and NAGM. The T2-weighted images (with the lesions masked out) were initially automatically segmented into grey matter (GM), white matter (WM) and CSF (Fig. 1B). A whole brain mask was generated and applied to the calculated MTR map. The MTR map was then also segmented into GM and WM, using a maximum likelihood algorithm with a probability threshold of 75% certainty, to allow separate NAWM and NAGM analyses. Partial volumes effects were minimized using a 10 pu lower threshold (Fig. 1C) and outer voxel erosions (2 erosions for WM and 1 erosion for GM (Fig. 1D)).

MTR histograms, normalized for brain volume, were generated with a bin width of 0.1 pu and a smoothing window of 0.3 pu. Histogram spikes relating to the division of integers were removed by the addition of random noise with mean zero and range ±0.5 to the data prior to MTR calculation (Tozer and Tofts, 2003). The following histogram parameters were measured for NAWM and NAGM: peak height (PH), peak location (PL), average MTR, and the MTR value at the 25th, 50th and 75th percentiles.

Measures of brain atrophy
In house software was used to calculate the volumes of WM, GM and CSF from the segmented images. The lower brainstem and spinal cord were excluded by selecting the most inferior slice immediately caudal to the cerebellum. The following measures of brain atrophy were generated:

\[\text{Total intra-cranial volume (TI)} = \text{volume (WM + GM + CSF + T2 lesions)}\]
\[\text{Brain parenchymal fraction (BPF)} = \frac{\text{volume (WM + GM + T2 lesions)}}{\text{TI}}\]
\[\text{GM fraction (GMF)} = \frac{\text{volume (GM/TI)}}{\text{WM fraction (WMF)} = \frac{\text{volume (WM + T2 lesions)}}{\text{TI}}}\]

Statistical analysis
Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc, Chicago, IL). Differences between patients and controls were assessed using multiple linear regression models, with MTR histogram parameters as the response variable; and disease status (CIS or healthy control), gender, age and atrophy measures (WMF for NAWM BPF for NAGM due to the correlations observed, see Tables 5 and 6) as covariates. Partial correlation coefficients (adjusted for age and gender) were calculated for each of the
MTR histogram parameters versus T2 lesion volumes and atrophy measures Spearman correlation coefficients were calculated for each of the MTR histogram parameters versus: (i) EDSS at baseline for all patients; (ii) EDSS at 3 years for the 61 patients who were followed up for the full 3 year period, and (iii) the total number of relapses over 3 years for the 61 patients who were followed up for the full 3 year period. A $P$-value of $<0.05$ was considered to be significant.

Fig. 1 (A) The axial gradient echo images of the brain with (right) and without (left) a magnetization transfer pulse are used to obtain the MTR map (middle). (B) T2 weighted images are segmented into GM (right), WM (left) and CSF using SPM99 software after masking lesions. (C) A whole brain mask was generated and used to segment the MTR map into NAGM (right) and NAWM (left), using a maximum likelihood algorithm with a probability threshold of 75% certainty. Partial volumes effects were minimized using a 10 pu lower threshold (D) and outer voxel erosions.
Results

Clinical and conventional MRI findings

Baseline cohort

Of the 100 CIS patients studied, 89 presented with optic neuritis, 6 with brainstem syndromes and 5 with spinal cord syndromes. Sixty seven of the CIS patients were female and 33 were male. Their median age was 32 years (range 18–50 years) and the median EDSS was 1 (range 0–6). The median age for the controls was 32.5 years (range 20–50 years), with 16 males and 34 females.

Twenty four patients had normal T2-weighted brain MRI scans at baseline, whereas 76 (76%) had one or more T2 lesions consistent with demyelination. Twenty-three of the patients with normal T2-weighted scans at baseline also had normal scans at 3 months. The remaining patient had a single new T2 lesion at 3 months. Thirty eight (38%) patients fulfilled the McDonald criteria for dissemination in space at baseline. Twenty six (26%) patients fulfilled the McDonald criteria for multiple sclerosis and 13 (13%) had CDMS at 3 months i.e. at the time that the MTR scan was acquired.

Follow-up cohort

The clinical and baseline MRI demographics of this subgroup of 81 patients (identified as described in Patients section) was similar to that of the whole cohort: there were 29 men and 52 women; the median age was 32 years (range 20–50); 70 presented with optic neuritis, 6 with brain stem syndromes and 5 with spinal cord syndromes; 20 (25%) had normal T2-weighted MRI of the brain at baseline. At last follow up, 55 had multiple sclerosis using the McDonald criteria. Twenty four patients had normal T2-weighted brain MRI and 52 after 3 years). Twenty patients had a normal baseline T2 scan and were followed up for 3 years: of these, 1 (0–6). Twenty four patients had normal T2-weighted brain MRI at last follow up, whereas 76 had one or more T2 lesions consistent with demyelination. Twenty-three of the patients with normal T2-weighted scans at baseline also had normal scans at 3 months. The remaining patient had a single new T2 lesion at 3 months. Thirty eight (38%) patients fulfilled the McDonald criteria for dissemination in space at baseline. Twenty six (26%) patients fulfilled the McDonald criteria for multiple sclerosis and 13 (13%) had CDMS at 3 months i.e. at the time that the MTR scan was acquired.

NAWM histograms (Tables 1 and 2, Fig. 2)

Baseline cohort (Tables 1, Fig. 2)

Compared with controls, the following MTR histogram parameters were significantly reduced in CIS NAWM (units in pu): mean MTR (NAWM, 38.14 versus 38.33, \( P = 0.001 \)), peak location (NAWM, 38.37 versus 38.52, \( P = 0.011 \)) and MTR at the 25th (36.76 versus 36.94, \( P = 0.003 \)), 50th (NAWM, 38.14 versus 38.31, \( P = 0.003 \)) and 75th percentile (NAWM, 39.44 versus 39.63, \( P = 0.001 \)). Similar reductions were also seen in: (i) the 76 CIS patients with abnormal baseline T2-weighted brain MRI; (ii) the 38 patients who satisfied the McDonald criteria for dissemination in space at baseline; (iii) the group of 26 who satisfied McDonald criteria for multiple sclerosis at 3 months; (iv) the 50 with abnormal T2-weighted scans who did not satisfy the McDonald criteria for multiple sclerosis at 3 months, and (v) the 13 patients who had already developed CDMS at 3 months. In contrast, CIS patients with normal T2-weighted brain MRI did not show significant reductions in any of the MTR histogram parameters compared with controls.

There were significant differences between CIS patients who satisfied the McDonald criteria for dissemination in space at baseline versus those who did not \(( P = 0.018 \) for mean MTR; \( P = 0.046 \) for peak location; \( P = 0.019 \) for the 25th percentile; \( P = 0.018 \) for the 50th percentile and \( P = 0.030 \) for the 75th percentile). No significant differences were observed between any of the other patient subgroups i.e. patients with normal versus abnormal baseline MRI and those fulfilling the McDonald criteria for multiple sclerosis and CDMS versus those who did not at 3 months.

Of the 100 CIS patients 14 had mean NAWM MTR values which fell below 2 standard deviations (SDs) for the control group. Of these 14, 13 had abnormal T2-weighted MRI at baseline, 10 satisfied the McDonald criteria for dissemination in space at baseline, 6 were positive for multiple sclerosis by the McDonald criteria at 3 months and 5 had CDMS at 3 months.

Follow up cohort (Table 2)

Of the 81 patients in the total follow up cohort, 80 were included in the analysis for the development of multiple sclerosis by the McDonald criteria (20 who fulfilled these criteria after 1 year and 60 who had both clinical and MRI evaluations after 3 years) and 74 were included in the analysis for the development of CDMS (13 who had developed CDMS after 1 year and all 61 who were followed up clinically after 3 years) (Table 2). Significant reductions in MTR were observed for those who satisfied the McDonald criteria for multiple sclerosis at last follow up \((n = 55)\) and in those who had CDMS at last follow up \((n = 41)\).

Patients who did not satisfy the McDonald criteria for multiple sclerosis at 3 years \((n = 25, Table 2)\) did not show significant reductions in any of the NAWM MTR histogram parameters compared with controls but the subgroup not developing CDMS after 3 years \((n = 33, Table 2)\) did show significant decreases.

No significant differences were observed in NAWM MTR histogram measures between any of the patient subgroups according to their follow up status, i.e. those patients fulfilling the McDonald criteria for multiple sclerosis or CDMS versus those who did not.

Of the 12 patients whose mean NAWM MTR was more than 2 SDs below the control mean and who were followed up for 3 years, 8 had developed CDMS and 10 had developed multiple sclerosis using the McDonald criteria.

NAGM histograms (Tables 3 and 4, Fig. 3)

Baseline cohort (Table 3, Fig. 3)

Compared with controls, the following MTR histogram parameters were significantly reduced in CIS NAGM
<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 50)</th>
<th>All CIS (n = 100)</th>
<th>Brain MRI at baseline</th>
<th>Dissemination space at baseline</th>
<th>McDonald criteria at 3 months</th>
<th>CDMS at 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 24)</td>
<td>Abnormal (n = 76)</td>
<td>Negative (n = 62)</td>
<td>Positive (n = 38)</td>
<td>Negative (n = 74)</td>
<td>Positive (n = 13)</td>
</tr>
<tr>
<td>Mean MTR</td>
<td>38.3317 (0.3078)</td>
<td>38.2269 (0.3199)</td>
<td>38.2216 (0.3789)</td>
<td>38.067 (0.4752)</td>
<td>38.1668 (0.3876)</td>
<td>38.1645 (0.3844)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Peak height</td>
<td>0.0207 (0.0018)</td>
<td>0.0200 (0.0019)</td>
<td>0.0207 (0.0016)</td>
<td>0.0206 (0.0013)</td>
<td>0.0207 (0.0016)</td>
<td>0.0207 (0.0015)</td>
</tr>
<tr>
<td>Location</td>
<td>38.5200 (0.3405)</td>
<td>38.4271 (0.3529)</td>
<td>38.4434 (0.3960)</td>
<td>38.2582 (0.5171)</td>
<td>38.3949 (0.3970)</td>
<td>38.4011 (0.4020)</td>
</tr>
<tr>
<td>25th percentile</td>
<td>36.9380 (0.3181)</td>
<td>36.8154 (0.3481)</td>
<td>36.8508 (0.3862)</td>
<td>36.6271 (0.4764)</td>
<td>36.7950 (0.3897)</td>
<td>36.7917 (0.3897)</td>
</tr>
<tr>
<td>50th percentile</td>
<td>38.3060 (0.3113)</td>
<td>38.2208 (0.3379)</td>
<td>38.2207 (0.3839)</td>
<td>38.0011 (0.4632)</td>
<td>38.1647 (0.3914)</td>
<td>38.1631 (0.3884)</td>
</tr>
<tr>
<td>75th percentile</td>
<td>39.6320 (0.3644)</td>
<td>39.5746 (0.3539)</td>
<td>39.5177 (0.4175)</td>
<td>39.3074 (0.5142)</td>
<td>39.4643 (0.4244)</td>
<td>39.4623 (0.4154)</td>
</tr>
</tbody>
</table>

Values are mean (±SD).  
*P-values are comparisons between control and patient groups.  
**P < 0.05, ***P < 0.01, ****P < 0.001 compared with controls.  
Significant P values are highlighted in bold type.
Similar reductions were also seen in: (i) the 76 CIS patients and 75th percentile (NAGM 34.63 versus 34.83, and MTR at the 50th (NAGM 32.57 versus 32.75, development of CDMS (13 who had developed CDMS after 3 years) and 74 were included in the analysis for the development of CDMS (13 who had developed CDMS after 1 year and all 61 who were followed up clinically after 3 years) (Table 4). Significant reductions in MTR were observed for those who satisfied the McDonald criteria for multiple sclerosis at last follow up (n = 55) and in those who had CDMS at last follow up (n = 41).

Of the 100 CIS patients, 12 had mean NAGM MTR values which fell below 2 SDs for the control group. Of these 12, 10 had abnormal T2-weighted brain MRI; (ii) the 38 patients who satisfied the McDonald criteria for dissemination in space at baseline; (iii) the group of 26 who satisfied McDonald criteria for multiple sclerosis at 3 months; (iv) the 50 with abnormal T2-weighted scans who did not satisfy the McDonald criteria for multiple sclerosis at 3 months, and (v) the 13 patients who had already developed CDMS at 3 months. In contrast, CIS patients with normal T2-weighted brain MRI did not show significant reductions in any of the NAGM MTR histogram parameters compared with controls.

Of the 100 CIS patients, 12 had mean NAGM MTR values which fell below 2 SDs for the control group. Of these 12, 10 had abnormal T2-weighted MRI at baseline, 9 satisfied the McDonald criteria for dissemination in space at baseline; 5 were positive for multiple sclerosis using the McDonald criteria at 3 months and 3 had CDMS at 3 months.

**Follow up cohort (Table 4)**

Of the 81 patients in the total follow up cohort, 80 were included in the analysis for the development of multiple sclerosis by the McDonald criteria (20 who fulfilled these criteria after 1 year and 60 who had both clinical and MRI evaluations after 3 years) and 74 were included in the analysis for the development of CDMS (13 who had developed CDMS after 1 year and all 61 who were followed up clinically after 3 years) (Table 4). Significant reductions in MTR were observed for those who satisfied the McDonald criteria for multiple sclerosis at last follow up (n = 55) and in those who had CDMS at last follow up (n = 41).

Patients who did not satisfy the McDonald criteria for multiple sclerosis after 3 years (n = 25, Table 4) showed a significant reduction only in NAGM MTR 75th percentile compared with controls, whilst the subgroup not developing CDMS after 3 years (n = 33, Table 4) showed significant decreases in two MTR measures (Table 4).

No significant differences were observed in NAGM MTR histogram measures between any of the patient subgroups according to their follow up status, i.e. those patients fulfilling the McDonald criteria for multiple sclerosis or CDMS versus those who did not.

Of the 10 patients whose mean NAGM MTR was more than 2 SDs below the control mean and who were followed up for 3 years, 6 had developed CDMS and 9 had developed multiple sclerosis using the McDonald criteria.

**Correlation coefficients (Tables 5 and 6)**

**T2 lesion volume**

The volume of T2 lesions in the brain correlated weakly with the following NAGM MTR histogram parameters: mean MTR (r = −0.23, P = 0.023), peak height (r = −0.32, P = 0.001), and MTR at the 25th percentile (r = −0.28, P = 0.005). There were no significant correlations between T2 lesion volumes and any of the NAWM MTR histogram parameters.
WMF

The WMF correlated weakly with the following NAWM MTR histogram parameters: mean MTR ($r = -0.25, P = 0.012$), peak location ($r = -0.26, P = 0.01$), and MTR at the 25th ($r = -0.23, P = 0.021$), 50th ($r = -0.26, P = 0.009$) and 75th percentiles ($r = -0.27, P = 0.008$). In addition, WMF also correlated with the following NAGM MTR histogram parameters: mean MTR ($r = 0.30, P = 0.003$), peak height ($r = 0.35, P < 0.001$), and MTR at the 25th ($r = 0.32, P = 0.001$) and 50th percentiles ($r = 0.26, P = 0.01$).
# Table 3

NAGM MTR histograms in controls and patients (units in pu) for the baseline cohort

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 50)</th>
<th>All CIS (n = 100)</th>
<th>Brain MRI at baseline</th>
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<td>Abnormal (n = 76)</td>
<td>Negative (n = 62)</td>
<td>Positive (n = 38)</td>
</tr>
<tr>
<td>Mean MTR</td>
<td>32.5028 (0.4087)</td>
<td>32.8880</td>
<td>32.3612 (0.3457)</td>
<td>32.2649 (0.4600)</td>
<td>32.3928 (0.3696)</td>
<td>32.1171 (0.4840)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.009**</td>
<td>32.2880 (0.4356)</td>
</tr>
<tr>
<td>Peak height</td>
<td>0.0127 (0.0010)</td>
<td>0.0129</td>
<td>0.0128 (0.0010)</td>
<td>0.0129 (0.0013)</td>
<td>0.0131 (0.0010)</td>
<td>0.0125 (0.0015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.006**</td>
<td>0.0125 (0.0015)</td>
</tr>
<tr>
<td>Peak location</td>
<td>33.3540 (0.3284)</td>
<td>33.1835</td>
<td>33.2396 (0.4734)</td>
<td>33.1658 (0.3661)</td>
<td>33.2452 (0.3954)</td>
<td>33.0829 (0.4037)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.015**</td>
<td>33.3540 (0.3284)</td>
</tr>
<tr>
<td>25th percentile</td>
<td>30.4120 (0.5298)</td>
<td>30.1211</td>
<td>30.2767 (0.4209)</td>
<td>30.1917 (0.6377)</td>
<td>30.3610 (0.4505)</td>
<td>29.9692 (0.7111)</td>
</tr>
<tr>
<td>50th percentile</td>
<td>32.7500 (0.3530)</td>
<td>32.5684</td>
<td>32.6283 (0.3296)</td>
<td>32.5495 (0.4005)</td>
<td>32.6516 (0.3412)</td>
<td>32.4326 (0.4164)</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.013**</td>
<td>32.7500 (0.3530)</td>
</tr>
<tr>
<td>75th percentile</td>
<td>34.8320 (0.3292)</td>
<td>34.6265</td>
<td>34.6912 (0.3358)</td>
<td>34.6061 (0.3142)</td>
<td>34.6674 (0.3188)</td>
<td>34.5597 (0.3146)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.001**</td>
<td>34.8320 (0.3292)</td>
</tr>
</tbody>
</table>

Values are mean (±SD).
P-values are comparisons between control and patient groups.
*P < 0.05, **P < 0.01 compared with controls.
Significant P values are highlighted in bold type.
### Table 4 NAGM MTR histograms in controls and patients (units in pu) for the follow up cohort

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 50)</th>
<th>McDonald criteria within 3 years (n = 80)</th>
<th>CDMS within 3 years (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (n = 25)</td>
<td>Positive (n = 55)</td>
<td>Negative (n = 33)</td>
</tr>
<tr>
<td>Mean MTR</td>
<td>32.5028 (0.04087)</td>
<td>32.3511 (0.3649)</td>
<td>32.2400 (0.4432)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.197</td>
<td>P = 0.006***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak height</td>
<td>0.0127 (0.0010)</td>
<td>0.0130 (0.0010)</td>
<td>0.0127 (0.0013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak location</td>
<td>33.3540 (0.3284)</td>
<td>33.2128 (0.3696)</td>
<td>33.1796 (0.4298)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.209</td>
<td>P = 0.018*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25th percentile</td>
<td>30.4120 (0.5298)</td>
<td>30.2888 (0.4357)</td>
<td>30.1464 (0.6135)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.462</td>
<td>P = 0.056</td>
</tr>
<tr>
<td>50th percentile</td>
<td>32.7500 (0.3530)</td>
<td>32.6036 (0.3220)</td>
<td>32.5304 (0.3959)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.154</td>
<td>P = 0.008***</td>
</tr>
<tr>
<td>75th percentile</td>
<td>34.8320 (0.3292)</td>
<td>34.6492 (0.2958)</td>
<td>34.6096 (0.3337)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.033*</td>
<td>P = 0.001**</td>
</tr>
</tbody>
</table>

Values are mean (±SD).
P-values are comparisons between control and patient groups.
*P < 0.05, **P < 0.01 compared with controls.
Significant P values are highlighted in bold type.

### Discussion

This study of a large consecutively and prospectively recruited cohort establishes that MTR histogram abnormalities are present in the NAWM and NAGM of patients within 6 months of presenting with a CIS. Previous reports of a lack of demonstrable MTR abnormalities in CIS NABT may have been due to the fact that the patient numbers were too small (Filippi et al., 1999; Kaiser et al., 2000) or because a ROI method was used (Brex et al., 2001). The study also demonstrates that MTR abnormalities are particularly evident in those CIS patients who are known from previous studies to have the highest risk of converting to CDMS (Poser et al., 1983), i.e. those with T2 lesions in the brain at presentation (Brex et al., 2002; Beck et al., 2003) and in those who satisfy the McDonald and Poser diagnostic criteria for multiple sclerosis (Poser et al., 1983; McDonald et al., 2001). The results therefore indicate that MTR NAWM and NAGM abnormalities are present at the earliest clinical stage of relapse onset multiple sclerosis.

There have been a considerable number of MTR studies of NAWM and NAGM in established multiple sclerosis that have used either a ROI or histogram analysis approach and that have consistently reported the presence of abnormalities in both tissues (Filippi et al., 1995, 1999, 2000a; Leary et al., 1999; Tortorella et al., 2000; Cercignani et al 2001; Ge et al., 2001, 2002; Dehmeshki et al., 2003; Davies et al., 2004). Other studies in multiple sclerosis have reported abnormalities from histogram analysis of whole brain (Filippi et al., 1999; Dehmeshki et al., 2001; Kalkers et al., 2001) or NABT (Tortorella et al., 2000; Traboulsee et al., 2003) that may increase over time (Rovaris et al., 2003; Davies et al.,...
and are more severe in secondary progressive than relapsing remitting multiple sclerosis (Traboulsee et al., 2003). Although the MTR acquisition and analysis methods used in the present CIS cohort have not been evaluated in similarly sized cohorts of patients with more established and advanced multiple sclerosis (thus it is not possible to make a direct comparison of the different stages of disease), the spectrum of abnormalities seen in the CIS cohort appears to be relatively mild as would be expected on clinical grounds.

Fig. 3 MTR histograms for NAGM (baseline cohort).
**NAWM**

The absence of a significant reduction in MTR histogram parameters in CIS patients with normal T2 scans (although there is a clear trend to reduced MTR) and the presence of significant reductions in MTR in those with T2 lesions suggests that the occurrence of lesions and abnormalities in NAWM in CIS patients are not entirely unrelated. It is also possible that many in the group with a normal T2 scan are a distinct subpopulation that is unlikely to ever develop multiple sclerosis (70% of such individuals developed no new MRI lesions or relapses over 3 years). However, the absence of a significant correlation between NAWM MTR histogram parameters and T2 lesion volumes suggests that the two processes occur at least in part independently of each other. MTR may therefore complement the information obtained from conventional T2-weighted MRI.

The presence of T2 lesions in CIS patients is associated with a relatively high risk of relapses leading to a diagnosis of CDMS (Brex et al., 2002; Beck et al., 2003; Minneboo et al., 2004; Tintore et al., 2005) but is only related to a modest extent to future disability (Brex et al., 2002; Minneboo et al., 2004; Tintore et al., 2004). A key question is whether the presence or extent of NAWM MTR abnormalities may also influence the subsequent clinical course of multiple sclerosis, especially the long term risk for disability, which cannot be predicted reliably for individual patients using existing clinical (Weinshenker et al., 1989; Confavreux et al., 2003; Eriksson et al., 2003) or conventional MRI lesion (Brex et al., 2002; Minneboo et al., 2004; Tintore et al., 2004) measures of early disease status. Follow up studies are required to investigate this further.

Histopathological studies of multiple sclerosis NAWM reveal diffuse astrocyte hyperplasia and perivascular lymphocytic infiltrates (Allen and McKeown, 1979; Allen et al., 1981). Microglial activation is also described (Allen et al., 2001). There may also be axonal loss (Evengelou et al., 2000) and patchy demyelination (Allen and McKeown, 1979; Allen et al., 1981). Histopathological studies of NAWM in CIS patients are not available but several of the changes reported in established multiple sclerosis could potentially reduce the MTR at an early stage. The previous report of an increase in myo-inositol on proton magnetic resonance spectroscopy (Fernando et al., 2004) would be consistent with glial cell proliferation occurring at this early stage; it is possible that glial cell proliferation per se leads to a decrease in MTR although fibrillary gliosis appears to be unrelated to MTR.

### Table 5

Correlation coefficients for NAWM MTR histograms in patients

<table>
<thead>
<tr>
<th>T2 lesion volumes</th>
<th>BPF</th>
<th>WMF</th>
<th>GMF</th>
<th>EDSS at baseline</th>
<th>EDSS at 3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MTR</td>
<td>-0.295</td>
<td>0.4970</td>
<td>0.2989</td>
<td>0.1210</td>
<td>-0.0270</td>
</tr>
<tr>
<td>Peak height</td>
<td>-0.3204</td>
<td>0.3628</td>
<td>0.3488</td>
<td>-0.0281</td>
<td>-0.2370</td>
</tr>
<tr>
<td>Peak location</td>
<td>-0.1330</td>
<td>0.1760</td>
<td>0.1677</td>
<td>-0.0073</td>
<td>0.1200</td>
</tr>
<tr>
<td>25th percentile</td>
<td>-0.2804</td>
<td>0.4945</td>
<td>0.3190</td>
<td>0.1003</td>
<td>-0.1050</td>
</tr>
<tr>
<td>50th percentile</td>
<td>-0.058</td>
<td>0.4725</td>
<td>0.2593</td>
<td>0.1381</td>
<td>0.0010</td>
</tr>
<tr>
<td>75th percentile</td>
<td>0.0054</td>
<td>0.4100</td>
<td>0.1943</td>
<td>0.1494</td>
<td>0.1080</td>
</tr>
</tbody>
</table>

**P < 0.05, **P < 0.01, ***P < 0.001.
Significant P values are highlighted in bold type.

### Table 6

Correlation coefficients for NAGM MTR histograms in patients

<table>
<thead>
<tr>
<th>T2 lesion volumes</th>
<th>BPF</th>
<th>WMF</th>
<th>GMF</th>
<th>EDSS at baseline</th>
<th>EDSS at 3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MTR</td>
<td>0.411</td>
<td>0.2664</td>
<td>0.2592</td>
<td>0.0630</td>
<td>-0.1080</td>
</tr>
<tr>
<td>Peak height</td>
<td>0.0222</td>
<td>0.0413</td>
<td>0.2664</td>
<td>0.0088</td>
<td>0.0100</td>
</tr>
<tr>
<td>Peak location</td>
<td>0.2761</td>
<td>0.0839</td>
<td>0.2629</td>
<td>0.2820</td>
<td>0.1060</td>
</tr>
<tr>
<td>25th percentile</td>
<td>0.056</td>
<td>0.1173</td>
<td>0.2854</td>
<td>0.2603</td>
<td>0.0100</td>
</tr>
<tr>
<td>50th percentile</td>
<td>0.327</td>
<td>0.2500</td>
<td>0.2887</td>
<td>0.0160</td>
<td>0.2480</td>
</tr>
<tr>
<td>75th percentile</td>
<td>0.283</td>
<td>0.0585</td>
<td>0.2592</td>
<td>0.0088</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

**P < 0.05, **P < 0.01, ***P < 0.001.
Significant P values are highlighted in bold type.
In addition, the reported reduction in whole brain N-acetyl-aspartate (NAA) of CIS patients could indicate early axonal dysfunction or loss, although its location was unclear (Filippi et al., 2003). The weak negative correlation observed between NAWM MTR and WMF may reflect pathological processes such as oedema and/or inflammatory cellular infiltration that on the one hand will tend to increase WM volume (and in doing so mask the atrophic effect of loss of myelin and axons) whilst on the other hand decrease MTR.

**NAGM**

In contrast to NAWM, the histogram parameters for CIS NAGM are weakly correlated with the volume of T2 lesions. This suggests that the MTR abnormalities occurring in the NAGM may be partly secondary to processes occurring in T2 lesions.

Although GM has been relatively less well investigated in multiple sclerosis, cortical lesions have been described (Kidd et al., 1999; Peterson et al., 2001). Cortical lesions are poorly seen on conventional T2-weighted MRI. Since the lesion masks that we used were based on T2-weighted scans, it is possible that some cortical lesions may have been misclassified as NAGM due to their lack of visibility. This may account for some of the reductions seen in NAGM MTR. If GM and WM lesion loads were correlated this could contribute to the correlation seen between NAGM MTR and (WM) T2 lesion volume.

A second potential explanation for the lesion load-NAGM MTR correlation is that some cortical lesions are continuous with adjacent lesions in the subcortical WM (Kidd et al., 1999; Peterson et al., 2001). A third factor might be axonal transection in T2 visible WM lesions in multiple sclerosis (Trapp et al., 1998) with secondary retrograde degeneration of neurons contributing to the MTR abnormalities observed in CIS NAGM. Consistent with the latter mechanism would be the correlations that exist between WM atrophy (reduced WMF) and reduced NAGM MTR; and between GM atrophy (reduced GMF) and reduced NAWM MTR. These findings are consistent with other studies in established multiple sclerosis that have demonstrated correlations of WM T2 lesion volume with both NAGM MTR (Cercignani et al., 2001; Ge et al., 2001, 2002; Bozzali et al., 2002) and GM atrophy (Chard et al., 2002). Notwithstanding these associations, it is recognized that the magnitude of the correlations is low, and as for NAWM, it seems likely that the early NAGM abnormalities are at least partly independent of T2 lesions.

**Atrophy as a confounding factor**

Since MTR is known to be influenced by atrophy, several steps were taken to ensure that the observed abnormalities have not been confounded by atrophy per se. Firstly the effects of partial volume were minimized using a 10 pu lower threshold and outer voxel erosions (2 erosions for WM and 1 erosion for GM). Secondly, the MTR histograms were normalized for brain volume. Thirdly, atrophy measures were allowed for in the regression analyses such that segmented NAWM and NAGM histograms were adjusted for the relevant segmented tissue volumes. It is therefore likely that the MTR abnormalities observed do reflect intrinsic tissue pathology independent of atrophy.

**Prognostic value of MTR histograms in the NAWM and NAGM after a CIS**

With regard to predicting the development of multiple sclerosis, it is notable that there were no significant differences between patient subgroups who did or did not develop either CDMS or multiple sclerosis diagnosed using the McDonald criteria within 3 years. The use of MTR in CIS patients in determining conversion to multiple sclerosis therefore appears to have little, if any role, particularly when one considers the much better defined prognostic value of conventional MRI lesions (Miller et al., 2005).

The trend for the weak correlation that was seen between some NAWM MTR measures and EDSS at 3 years suggests that NAWM MTR may have the potential to provide some prognostic information with respect to the likelihood of future disability after a CIS. Any potential relationship between MTR and clinical function in these patients may have been masked because the EDSS scores for our patients fall into a narrow dynamic range (the EDSS score was 0–1 for 80% at baseline and 70% at 3 years). Longer term follow up studies are therefore required to investigate this relationship in more depth perhaps also using additional measures of clinical function such as the multiple sclerosis Functional Composite score or more detailed evaluation of cognitive function.

**Limitations of this study**

The majority of patients in this study presented with optic neuritis and further studies of larger cohorts with isolated brain stem and spinal cord syndromes is warranted to definitively characterize NAWM and NAGM MTR in those cohorts. The study also highlights the potential limitations of the EDSS in determining clinical function at the earliest clinical stages of the disease and the importance of using more sensitive measures to assess clinical function in this group of patients.

**Conclusions**

This study provides evidence for subtle MTR abnormalities occurring in the NAWM and NAGM soon after a CIS. These abnormalities are particularly evident in those CIS patients who are known from previous studies to have the highest risk of converting to CDM, i.e. those with T2 lesions in the brain at presentation (Brex et al., 2002; Beck et al., 2003) and in those who satisfy the McDonald and Poser diagnostic criteria for multiple sclerosis (Poser et al., 1983; McDonald et al., 2001). The results therefore indicate that MTR histogram abnormalities are present at the earliest clinical stages of multiple sclerosis. Since the NAWM MTR abnormalities were not
correlated with lesion load, they may originate at this early stage of disease from mechanisms independent of those involved in lesion formation and provide information which complements that derived from conventional T2-weighted MRI. Follow up studies are needed to determine whether the abnormalities occurring in the normal appearing tissues are related to the subsequent clinical evolution of the disease.

**Acknowledgements**

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**References**


