Histogram analysis of quantitative $T_1$ and MT maps from ultrahigh field MRI in clinically isolated syndrome and relapsing–remitting multiple sclerosis

Ali Al-Radaideh, Olivier E. Mougin, Su-Yin Lim, I-Jun Chou, Cris S. Constantinescu and Penny Gowland

This study used quantitative MRI to study normal appearing white matter (NAWM) in patients with clinically isolated syndromes suggestive of multiple sclerosis and relapsing–remitting multiple sclerosis (RRMS). This was done at ultrahigh field (7 T) for greater spatial resolution and sensitivity. 17 CIS patients, 11 RRMS patients, and 20 age-matched healthy controls were recruited. They were scanned using a 3D inversion recovery turbo field echo sequence to measure the longitudinal relaxation time ($T_1$). A 3D magnetization transfer prepared turbo field echo (MT-TFE) sequence was also acquired, first without a presaturation pulse and then with the MT presaturation pulse applied at $-1.05$ kHz and $+1.05$ kHz off resonance from water to produce two magnetization transfer ratio maps (MTR(--)) and MTR(+-)). Histogram analysis was performed on the signal from the voxels in the NAWM mask. The upper quartile cut-off of the $T_1$ histogram was significantly higher in RRMS patients than in controls ($p < 0.05$), but there was no difference in CIS. In contrast, MTR was significantly different between CIS or RRMS patients and controls ($p < 0.05$) for most histogram measures considered. The difference between MTR(+) and MTR(--) signals showed that NOE contributions dominated the changes found. There was a weak negative correlation ($r = -0.46$, $p < 0.05$) between the mode of $T_1$, distributions and healthy controls’ age; this was not significant for MTR(+) ($r = -0.34$, $p > 0.05$) or MTR(--) ($r = 0.13$, $p > 0.05$). There was no significant correlation between the median of $T_1$, MTR(--), or MTR(+) and the age of healthy controls. Furthermore, no significant correlation was observed between EDSS or disease duration and $T_1$, MTR(--), or MTR(+) for either CIS or RRMS patients. In conclusion, MTR was found to be more sensitive to early changes in MS disease than $T_1$. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: ultrahigh field MRI; clinically isolated syndromes; relapsing–remitting multiple sclerosis; normal appearing white matter; nuclear Overhauser effect; relaxometry; quantitative magnetization transfer

INTRODUCTION

Changes in normal appearing white matter (NAWM) may be important in understanding the discrepancy observed between lesion load detected on MRI (1) and clinical status of multiple sclerosis (MS) patients. It is thought that this discrepancy is due to the diffuse, occult disease beyond the ‘visible’ white matter (WM) lesions, as well as grey matter (GM) disease (1,2).

Post mortem studies in MS have shown that NAWM is abnormal (3), and although the origin of these changes is unclear they are thought to be due to extensive glial pathology, microglial activation, axonal loss, and inflammatory cell infiltration. It has been argued that these changes either are due to primary events such as microglial activation-induced inflammation and axonal loss, or reflect degenerative events in NAWM due to axonal transection in lesions (secondary Wallerian degeneration) (4). Studies have shown that NAWM abnormalities increase with disease duration (5) and disability (6). Small focal abnormalities independent of the macroscopic lesions have also been seen in NAWM of MS patients (3).

Diffuse, occult disease cannot be detected using conventional MRI, as it is unable to detect microscopic changes, but diffuse pathology can be studied with quantitative MR (1) measures.
including relaxometry, magnetization transfer ratio (MTR) and susceptibility mapping, diffusion weighted imaging (DWI), or MRS. It has been shown that most pathological changes occur in early relapsing–remitting MS (RRMS), and these changes can then play an important role in the degree of severity of MS disease. Techniques that could detect such early changes or even earlier changes in clinically isolated syndromes (CISs) (often indicative of MS) could assist in treating the disease prior to permanent disability.

The aim of this work is to study the changes in NAWM in patients at presentation with CISs suggestive of MS and RRMS patients compared with healthy controls, comparing $T_1$ and MTR MR measures. Data were acquired at high field (7 T) to give increased sensitivity and increased spatial resolution, which is needed to study heterogeneity in the WM. Two different MTR measures were acquired, one sensitive to standard MT effects from macromolecules (MTR(+)) and the second also sensitive to nuclear Overhauser effects (NOEs), probably arising from aliphatic protons in lipids associated with myelin (MTR(−)) (7,8).

**MATERIALS AND METHODS**

**Subjects**

17 patients with CIS (10 females, 7 males; mean age 37.9 years; age range 26–41), and 11 patients with RRMS (7 females, 4 males; mean age 42.6 years; age range 20–54 years) were recruited from Nottingham University Hospital, along with 22 age matched healthy controls (9 females, 13 males; mean age 36.6 years; age range 21–48). Of the 22 controls, only three subjects were staff from the MRI centre (two of whom were extremely familiar with the 7 T), one was a medical doctor who was naive to the 7 T, and the rest were friends of the patient group, who were probably less familiar with being scanned than the MS patients.

All participants gave informed consent according to local ethics approval. Table 1 summarizes the characteristics of the three groups. Of the CIS group, six presented with optic neuritis, three with myelitis, two with a brainstem syndrome, and six with a cerebellar syndrome. Of these, three patients had higher functionals (bowel and bladder, and higher cerebral). The FS scores reflected the degree of disability across all these areas, as found on the neurological assessment. The final EDSS score was calculated on the basis of the combined/summarized FS scores, giving a value of between 0 (normal examination) and 10 (death due to MS).

**Data acquisition**

Scanning was performed on a 7 T Philips Achieva MR system, equipped with a 16-channel receive coil and head-only, volume transmit coil. MT weighted images were acquired using a 3D MT prepared turbo field echo (MT-TFE) sequence with $0.86 \times 0.86 \times 1.5$ mm voxel size; $T_E = 5.7$ ms; $T_R = 9.8$ ms; 20 slices (9). Three acquisitions were made: one with no presaturation, one with presaturation $-1.05$ kHz off resonance from water (sensitive to MT and NOEs, MTR(−)), and one with presaturation +1.05 kHz off resonance to give sensitivity primarily to MT (MTR(+)) (total scan time = 8 min 22 s for the three acquisitions). The pulsed saturation was applied as a train of off-resonance pulses applied at $\pm 1.05$ kHz (3.5 ppm). Each pulse train consisted of 20 Gaussian windowed $13.5 \mu T$, sinc, 20 ms pulses with a bandwidth of 300 Hz and 55 ms between each pulse. $T_1$ maps were created from 3D inversion recovery TFE (IR-TFE) images (also known as MPRAge images) (10) acquired at seven different inversion times (150, 300, 500, 800, 1200, 1800, 2500 ms). The acquisition parameters were the following: $T_E = 3.2$ ms; $T_R = 6.9$ ms; flip angle of the TFE readout pulse = 80°; TFE factor per inversion = 240; shot-to-shot interval = 8 s; spatial resolution = $1.25 \times 1.25 \times 1.25$ mm$^3$; field of view = $200 \times 200 \times 72.5$ mm$^3$; scan time per $T_E = 2$ min. An adiabatic, phase modulated inversion pulse with a bandwidth of 1.6 kHz and duration of 13 ms was used (11). A $T_2$ map was acquired using the two $T_E$ method (12) to correct the MT images for the effect of $T_2$ inhomogeneity and to calculate the readout flip angles for $T_1$ mapping. No $T_2$ fluid attenuated inversion recovery ($T_2$-FLAIR) was acquired at 7 T at the time of data acquisition due to some technical problems. However, for 14 CIS and 5 RRMS patients, 3D $T_2$-FLAIR scans were acquired on a 3 T Philips Achieva MR system with the following imaging parameters: $1 \times 1 \times 3$ mm$^3$ voxel size; $T_E = 125$ ms; $T_R = 11000$ ms; turbo factor = 27; 50 slices; scan time = 5 min 52 s. These were used to verify the semi-automated segmentation of lesions adopted in this work.

**Data analysis and post-processing**

$T_1$ maps were calculated from the IR-TFE images as described previously (11). The MT images acquired at positive and negative frequency offset were co-registered to the reference scan acquired with no saturation pulse using rigid body registration with six degrees of freedom in FSL (FMRI, Oxford, UK). MTR maps were then calculated on a voxel by voxel basis using

$$MTR(+) = \frac{S_0 - S_{MT(+)}}{S_0} \quad [1]$$

$$MTR(−) = \frac{S_0 - S_{MT(−)}}{S_0} . \quad [2]$$

The IR-TFE image that was acquired near the null point of GM was used to segment the GM and WM in SPM (http://www.fil.ion.ucl.ac.uk/spm/). This algorithm automatically segmented MS lesions as GM tissues. The segmented WM was displayed as a probability map, which was converted to a mask and then eroded by two voxels to exclude any partial volume effects at the GM or lesion borders. Hereafter this mask will be referred to as a NAWM mask.

| Table 1. Characteristics of study controls and subjects with CIS and RRMS |
|-----------------------------|-----------------------------|-----------------------------|
| Subjects | Controls | CIS patients | RRMS patients |
|-------------|-----------------------------|-----------------------------|
| N | 22 | 17 | 11 |
| Sex, F/M | 9/13 | 10/7 | 7/4 |
| Mean age ± SD, years | 36.55 ± 8.06 | 37.94 ± 9.3 | 42.6 ± 12.87 |
| Age range, years | 21–48 | 26–41 | 20–54 |
| Mean EDSS ± SD | – | 1.91 ± 1.08 | 3.26 ± 1.27 |
| EDSS range | – | 0–4 | 2–6.5 |
| Mean disease duration ± SD, years | – | 1.45 ± 1.18 | 13.16 ± 9.73 |
| Disease duration, years | 0.4–4.5 | 1.67–34 |

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The reference volume in the MT sequence was then co-registered to the IR-TFE image used to create the NAWM mask, with rigid-body image registration using the FLIRT linear registration algorithm from the FSL platform (FMRIB). Because of the difference in contrast between the IR-TFE image and reference scan, the cost function was the "mutual information" with a low number (100) of bins. The registration matrix was applied to the registered MTR(−) and MTR(+) maps to transfer them into the same space as the IR-TFE image. Finally, the NAWM mask was then used to extract the NAWM tissue values from the $T_1$, MTR(−), and MTR(+) maps.

Both MTR(−) and MTR(+) were found to depend strongly on $B_1$ amplitude, such that the peripheral WM tended to have lower MTR values than the more central parts regardless of any abnormalities. Therefore, the relative effect of $B_1$ field inhomogeneity on MTR values within the NAWM mask was corrected using a method described by Ropele et al. (13).

Maps indicating the asymmetry of the MT spectrum (asymmetry maps, which will be dominated by NOE effects) were calculated on a voxel by voxel basis for the NAWM using the following equation:

$$\text{ASYM}_{\text{NAWM}} = \frac{\text{corrected MTR}(−)_{\text{NAWM}}}{\text{corrected MTR}(+)_{\text{NAWM}}}. \quad [3]$$

The asymmetry of the $z$-spectrum can theoretically measure NOE effects, but in practice it is confounded by a number of factors and is potentially noisy due to $B_0$ inhomogeneity. Nonetheless, it provides a convenient method to investigate other contributions to the $z$-spectrum. Color coded maps were produced in MATLAB to show the spatial distribution of $T_1$, MTR(−), MTR(+), and ASYM$_{\text{NAWM}}$ values within the NAWM masks. The lower and upper limits of the $T_1$, MTR and ASYM histograms were [500, −2000 ms], [0.1, −0.7], and [0, −0.2], respectively, and the histograms were normalized by the size of the NAWM mask.

To parameterize the shape of each histogram for each subject, the median, full width at half maximum (FWHM), and peak position (mode) were calculated, and the $T_1$ or MTR value corresponding to the cut-off for the upper quartile of the $T_1$ histogram and lower quartile of the MTR histograms was calculated. The median and peak positions of the histograms were plotted against age for healthy controls, and against EDSS and disease duration for CIS and RRMS patients separately. An averaged histogram was produced for each MRI measurement, for each group of subjects.

Joint histograms (scatter plots) of MTR(−) versus $T_1$ values within the NAWM masks were plotted against each other on a voxel by voxel basis for all slices for each subject to investigate any correlation between these parameters. Similar joint histograms were created for MTR(−) and MTR(+).

All statistical analysis was performed in SPSS 17.0 (SPSS, Chicago, IL, USA). Normality was tested using the Kolmogorov–Smirnov test. One way ANOVA with Tukey post hoc test was performed on the parametric data and the Kruskal–Wallis with Mann–Whitney pairwise multiple comparisons test was performed on non-parametric data. The significance level in the multiple comparisons between groups was calculated using a false discovery rate (FDR) test. Correlation coefficients were calculated using Pearson correlation.

The reproducibility of $T_1$ maps was tested by scanning the same healthy subject on five occasions. Another healthy subject was scanned five times with the MT-TFE and $B_1$ mapping sequences. Masking and histogram analysis was applied to each repeated data set as above.

RESULTS

Figure 1 shows $T_1$, MTR(−), MTR(+), and ASYM maps of the NAWM for the same three age matched subjects (control, CIS, and RRMS). Blue color was assigned to long $T_1$ and low MTR and ASYM values, since these are thought to correspond to abnormal changes in NAWM in MS. These maps are from three typical subjects, but similar trends in $T_1$, MTR, and ASYM between patient groups were found in all subjects, as indicated by the subject averaged histograms of the NAWM values shown.

![Figure 1](image-url). Representative $T_1$ (A), MTR(−) (B), MTR(+) (C), and asymmetry (D) maps of the NAWM for three age matched subjects (control, CIS, and RRMS).
in Figure 2. The $T_1$ maps from the control and CIS patient are very similar, whereas the RRMS patient shows both diffuse and perilesional increase in $T_1$ values. In contrast, the differences in MTR between groups are more obvious, although this is particularly clear in the RRMS patient around the lesions. Similar trends are seen for MTR(+) and MTR(−), although the MTR(+) values are generally lower than those for MTR(−).

Figures 1(d) and 2(c) demonstrate that the RRMS and CIS patients show less MTR asymmetry than do the control subjects.

Figure 3 plots the values of the median, peak position, and FWHM and the position of the upper/lower quartile of the $T_1$/MTR NAWM histograms for each subject in the three groups. The mean value of each parameter for each group is indicated by the red dash. Table 2 summarizes the statistical comparison of these measures between the three groups. For the $T_1$ histograms, only the upper quartile measure showed a significant difference ($p < 0.05$) between groups, being significantly larger in RRMS than in CIS patients or controls with a weak trend toward being larger in CIS patients than controls. This change in the tail of the histograms was consistent with the local changes seen around lesions in the maps in Figure 1. For the MTR(−) histograms, all measures except FWHM were significantly different between

Figure 2. Normalized MTR(−) (A), MTR(+) (B), asymmetry (C), and $T_1$ (D) averaged histograms of the NAWM for the three groups of subjects.

Figure 3. The median, peak position (A, D, and G), FWHM (B, E, and H), '75th percentile' (C), and '25th percentile' (F and I) statistics derived from each subject’s $T_1$, MTR(−), and MTR(+) histograms for each of the three groups.
groups. However, the pairwise comparisons showed that these measures were significantly different between controls and CIS or RRMS patients but not between the CIS and RRMS patients. For the MTR(+) histograms, all measures were significantly different between groups. The pairwise comparisons showed that both the median and lower quartile were significantly different between controls and CIS or RRMS patients. The peak position was significantly different between controls and CIS patients, with a trend towards a difference between the controls and RRMS patients, whilst the FWHM only showed a significant difference between controls and RRMS. Again, no significant difference was found in any measure between CIS and RRMS patients.

Figure 4 shows that there was a weak negative correlation ($r = -0.46, p < 0.05$) between the peak position of the $T_1$ histogram and the age of healthy controls, but this was not significant for MTR(+) ($r = -0.34, p > 0.05$) or MTR(−) ($r = 0.13, p > 0.05$). There was no significant correlation between the median of $T_1$, MTR(−), or MTR(+) and the age of healthy controls. Furthermore, no significant correlation was observed between EDSS or disease duration and $T_1$, MTR(−), or MTR(+) for either CIS or RRMS patients.

Figure 5 shows the joint histogram of $T_1$ and MTR(−) for the three subjects illustrated in Figure 1. Again this shows that CIS resulted in reduced MTR(−) but not increased $T_1$, and RRMS showed a larger reduction in MTR(−) combined with a smaller increase in $T_1$ but with some NAWM voxels showing significant changes in both parameters. Similar trends were found in joint histograms for the other subjects.

Figure 6 shows a similar joint histogram of MTR(+) versus MTR(−) and demonstrates that these two measures are correlated, particularly for the RRMS patients. Since MTR(−) is sensitive to both MT and NOE effects, it will tend to be larger than MTR(+) in healthy NAWM. Therefore, the slope of this plot is expected to be less than unity averaging over all subjects. The average gradients of these scatter plots for the control, CIS, and RRMS subjects were $0.849 \pm 0.021$, $0.85 \pm 0.026$, and $0.86 \pm 0.022$, respectively (assuming zero intercept).

**DISCUSSION**

This work has shown that MT MR measures are sensitive to changes in NAWM in an earlier stage of demyelinating disease than are $T_1$ measures.

The study was carried out at ultrahigh field (7 T) to give increased sensitivity to MTR changes in particular, and also to allow data to be acquired with smaller voxels, providing better information about the heterogeneity of any changes occurring in WM. However, it is likely that these results can be used to optimize 3 T acquisitions to allow these methods to be translated to 3 T.

This is the first study in which MTR was measured in MS with saturation at two different frequencies placed symmetrically.
about the water resonance. The positive frequency offset saturation (MTR(+)) produces images that are largely sensitive to MT with the semisolid proton pool related to macromolecules. It also has some sensitivity to chemical exchange saturation transfer effects, particularly from amide proton transfer (APT) at this frequency offset and power, although the APT signal is generally associated with increased protein synthesis and therefore is not expected to be particularly large in either healthy brain tissue or MS. However saturation at the negative offset (MTR(−)) produces images that also have some sensitivity to the NOE, which is

Figure 5. Joint histogram of MTR(−) versus $T_1$ for the control (blue), CIS (magenta), and RRMS (cyan) subjects shown in Figure 1. The histograms are overlaid, but also shown separately as insets for clarity.

Figure 6. Joint histogram of MTR(−) versus MTR(+) for the control (blue), CIS (magenta), and RRMS (cyan) subjects shown in Figure 1. The histograms are overlaid, but also shown separately as insets for clarity.
thought to be due to aliphatic protons, probably associated with lipids, and since distribution of the NOE signal in the brain is similar to the distribution of WM it is assumed that NOE is related to myelination (7). It should be noted that in both cases, for the sequence used here, MT with the semisolid proton pool will dominate over APT or NOE effects. MTR(−) was generally greater than MTR(+) in all groups, implying that the combined effects of NOE and MT were larger than the effects of APT and MT, as expected in the normal brain (8), so the asymmetry image (ASYM) is largely sensitive to NOE. The proper way to investigate different contributions to the MTR signals would be to acquire a z-spectrum showing the effect of off-resonance saturation at a range of frequencies and RF pulse amplitudes, but this would be very time consuming and would not currently be feasible at such high spatial resolution.

Significant differences between the three groups of subjects were found in histograms of NAWM from all these maps, with MT changes apparent earlier in disease progression (in CIS patients rather than in RRMS patients), as shown by the color maps (Fig. 1), the subject averaged histograms (Fig. 2), the summary measures from the histograms (Table 2 and Fig. 3) and the joint histograms between $T_1$ and MTR(+) (Fig. 5). Analysis of the ASYM maps (not shown) found no statistically significant difference in ASYM between the three groups, except that the ASYM peak position was significantly lower in RRMS than in CIS subjects, and a similar trend is seen in Figure 6 (suggesting smaller NOE contribution in RRMS). This variability apparent in the asymmetry map shown in Figure 1 was characteristic of the data from other subjects and is probably due to the noisiness of this measure. This variability apparent in the asymmetry map shown in Figure 1 was characteristic of the data from other subjects and is probably due to the noisiness of this measure. This variability apparent in the asymmetry map shown in Figure 1 was characteristic of the data from other subjects and is probably due to the noisiness of this measure.

Abnormalities in $T_1$ and $T_2$ relaxation times (5,16–22), MT (6,19,23–27), diffusion weighting (28), and spectroscopy have previously been reported in NAWM of MS patients, with some studies showing that these NAWM abnormalities increase with disease duration (5) and disability (6). The increase in $T_1$ values in NAWM in MS has been related to the effects of demyelination, gliosis, axonal loss, and edema on the mobile water pool (29–33). However, macrophage activity and metal deposition will decrease $T_1$, possibly limiting the sensitivity of $T_1$ mapping for detecting changes in MS (34,35), and its specificity to demyelination (36).

Some studies have failed to detect changes in MTR, reflecting how sensitive this parameter is to the exact pulse sequence used (18,37,38). However most studies have indicated that MTR is decreased in both MS lesions and normal appearing brain tissue (NABT) (39). This has been attributed to processes such as demyelination, axonal loss, and swelling, which lead to a decrease in the concentration of macromolecules.

Fewer studies have been performed in CIS patients. Several studies have reported reductions in MTR of NAWM (40,41) or NABT (25,42) in CIS patients compared with controls. However, others (43,44) found no change in MTR in the NAWM of CIS patients even within carefully selected ROIs. Changes in myo-inositol have also been detected in early CIS (45), but this has not been found by another group, maybe due to a smaller sample size (46,47). There are again mixed results with diffusion based measures, with some studies finding changes (48–50) and others not (51,52), sometimes even for the same measures. Another study has shown no change in $T_2^*$ (possibly related to oxygenation) in CIS (53), but another has noted a reduction in NAWM perfusion (54). To the best of our knowledge, this is the first study that investigates the $T_1$ relaxation time along with MTR at both offsets in NAWM of the CIS patient for ultra-high field MRI.

This group of subjects will be followed up over three years to determine whether NAWM changes locally or globally are

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<th>Table 3. Coefficient of variance (%) of the repeated measures of $T_1$, MTR(−), and MTR(+)</th>
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predictive of disease progression, as has been previously found for MTR changes in CIS patients (25).

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