Are outer cortical MTR changes caused predominantly by MR-visible cortical lesions or abnormalities in the normal-appearing grey matter?

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Target audience: Clinicians and scientists interested in measures of cortical abnormalities in multiple sclerosis (MS) and advanced image analysis methods.

Purpose: To investigate whether or not changes in inner and outer cortical grey matter (GM) magnetization transfer ratio (MTR) values seen in MS are associated with MR-visible lesions or changes beyond MR-visible lesions in different clinical MS subtypes.

Introduction: Cortical grey matter (CGM) pathology including demyelination, neuronal loss, reductions in synapse and neurite/axon density, microglial activation, and damage to the glia limitans (1-3) is commonly observed in MS. A high frequency of demyelinating lesions has been reported in the outer, subpial, cortex, especially in secondary progressive (SP) MS (4,5), but subpial lesions are difficult to detect using MR images acquired with current clinical scanners (operating at up to 3T). Recently (6), the relationship between inner and outer cortical MTR (cMTR) and clinical course was investigated in a large cohort of MS patients of different clinical subtypes and healthy controls (HC). A higher inner than outer cMTR was observed in HC, which was attributed to the higher myelin content in inner cortical layers. Both inner and outer cMTR were reduced in people with MS, which is compatible with demyelination and axonal damage, and greater outer cMTR reductions (consistent with subpial demyelination) were found particularly in SPMS. We aimed to investigate the relationship of MR-visible lesions to inner and outer cMTR changes in people with different clinical subtypes of MS, by examining the co-localisation of CGM lesions (CL) marked on phase sensitive inversion recovery (PSIR) (7) images acquired in the same scanning session as the MTR data, and examining inner-outer and between-subtype differences in CGM lesion volume, CL and normal-appearing GM (NAGM) MTR.

Methods: ***Subjects:*** Thirty-seven people with relapsing-remitting (RR) (mean age 41.8 years, median Expanded Disability Status Scale (EDSS) 1.5), 21 SPMS (54.1 years, EDSS 6.5) and 17 primary progressive (PP) (52.4 years, EDSS 6.0) MS were included in this study.

MR acquisition: We used a 3T Philips Achieva system (Philips Healthcare, Best, The Netherlands) with a 32-channel head coil and multi-transmit technology to acquire the following sequences sagittally, with field-of-view (FOV) 256x256x180mm^3; voxel size1x1x1mm^3: i) T_1-weighted (T_1w) volumes using a 3D inversion-prepared (TI=824ms) gradient echo (FFE) sequence (TR/TE=6.9/3.1ms); flip angle (α)=8°; ii) MTR data with a 3D slab selective spoiled gradient echo (FFE) sequence with 2 echoes (TR=6.4ms, TE1/TE2=2.7/4.3ms, α=9°), with and without Sinc-Gaussian shaped MT saturating pulses of nominal α=360°, offset frequency 1kHz, duration 16ms. PSIR data were acquired axially, with FOV 240x180x150mm^3; voxel size 0.5x0.5x2mm^3, TR/TE/TI=7306/13/400ms.

Image Analysis: Segmentation of lesion filled (8) T_1w volumes (with a conservative 90% threshold applied to CGM probability maps to limit partial volume effects) and division of CGM into inner and outer cortical bands was performed using methods described previously (6, 9-11). MTR data were affine registered to each subject's T_1w volume using NiftyReg (12,13), and MTR maps were calculated. CLs were marked on each subject's PSIR images by a trained observer (VS), and classified as intracortical (IC; only involving CGM) or leukocortical (LC; mixed GM-WM lesions). PSIR images were affine registered to each subject's T_1w volume, and the transformations were applied to CL masks. These were used to generate inner and outer LC (including the GM portion only), IC, total CL (TCL, combining the IC and GM part of LC lesions) and NAGM masks. Statistical analysis was performed using SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA).

Table 1: Mean inner and outer cortical lesion and NAGM MTR values and volumes (vol) for each MS clinical subtype (± SD)

	PPMS	SPMS	RRMS
Inner IC lesion MTR (pu)	32.9±1.30	31.8± 1.75	32.8±1.12
Outer IC lesion MTR (pu)	30.7±1.08	30.0±1.47	30.9± 1.41
Inner LC lesion MTR (pu)	31.3±2.28	30.5±2.45	30.9±5.62
Outer LC lesion MTR (pu)	29.5±2.14	28.9±2.47	29.1±5.32
Total inner CL MTR (pu)	32.1±1.91	31.2±2.09	32.3±1.31
Total outer CL MTR (pu)	29.9±1.75	29.4±2.10	30.4±1.39
Inner cNAGM MTR (pu)	33.9±0.63	33.6±0.92	34.1±0.80
Outer cNAGM MTR (pu)	31.3±0.87	30.1±1.52	31.4±1.27
Inner IC vol (ml)	0.131±0.120	0.199± 0.193	0.155±0.114
Outer IC vol (ml)	0.108±0.124	0.177±0.201	0.129±0.115
Inner LC vol (ml)	0.106±0.103	0.205±0.249	0.151±0.311
Outer LC vol (ml)	0.100±0.099	0.201±0.219	0.149±0.382
Total inner CL vol (ml)	0.238±0.165	0.404±0.386	0.306±0.392
Total outer CL vol (ml)	0.208±0.168	0.377±0.333	0.278±0.466
Inner cNAGM vol (ml)	177.9±28.5	178.3±25.4	179.1±20.7
Outer cNAGM vol (ml)	215.6±32.7	214.4±27.0	218.5±21.4

Results: Figure 1 shows a single subject's PSIR image with CLs marked, and their MTR map with inner and outer cortical bands, as well as CLs, superimposed. Mean inner and outer CL and NAGM MTR values and volumes are given in Table 1 (± standard deviations [SD]). Paired t-tests performed on the whole MS cohort showed that outer CL volumes(as a fraction of the total cortical band volume, were lower than inner CL volumes ($p<0.01$ for LC, $p<0.001$ for IC and all lesions combined). Examining different clinical subtypes separately, fractional IC lesion volumes were higher in the inner than the outer cortex (PPMS and SPMS $p<0.05$; RRMS $p<0.001$), but no significant inner-outer cortex differences in fractional LC lesion volumes were observed.

Fractional outer IC (and total) lesion volumes were lower in PPMS than SPMS ($p<0.05$), and inner LC ($p<0.05$) and fractional outer LC (and total) lesion volumes were lower in RRMS than SPMS ($p<0.01$), but there were no significant fractional CL volume differences between PPMS and RRMS (investigated using a Mann Whitney U test).

Paired t-tests of the whole MS cohort demonstrated that MTR values were lower in the outer compared to the inner cortex, in IC and LC lesions, and all lesions combined, as well as in cortical NAGM (cNAGM), as expected.

Inner cortical IC lesion MTR was reduced in SPMS compared to PPMS ($p=0.025$) and RRMS ($p=0.05$), but no significant differences were observed for LC. No differences between clinical subtypes were observed for outer CL MTR values. Inner cNAGM MTR was lower in SPMS than RRMS ($p<0.05$), and outer cNAGM MTR was decreased in SPMS compared to RRMS ($p<0.001$) and PPMS ($p<0.01$) (tested using one-way analysis of covariance (ANCOVA) (with post-hoc paired comparisons), adjusted for age and gender).

Discussion and Conclusions: The lower outer than inner CL volumes observed in this study despite the overall larger outer than inner cortical band MTR reductions (6) could be interpreted in two ways. First, it may indicate a potentially larger role for non-lesional rather than lesion CGM abnormalities in outer cMTR changes. Second, it could be due to unseen subpial lesions, which are the most extensive type of CL observed in histopathological studies, but are rarely detected using PSIR or Double Inversion Recovery (DIR) (7). The outer cNAGM MTR demonstrated significant differences between subtypes, and was most reduced in SPMS, however no significant difference between outer CL MTR was seen between subtypes, again indicating that changes outside of lesions observed on PSIR images may be responsible for the outer cMTR reductions seen in different MS clinical subtypes. In addition to difficulties with CL detection, another technical consideration is that the cortical masks used to extract MTR values were substantially smaller than segmentations used for atrophy measures (obtained using SPM8) and reflect the deliberately conservative nature of segmentation designed to exclude partial volume influences on the MTR findings (6), but having the effect that some CLs and NAGM may have been excluded from both cortical bands.

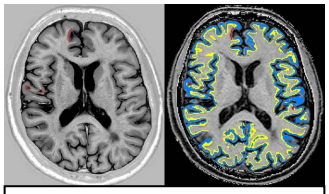

Figure 1: Single subject PSIR image (left) with cortical lesions marked in red, & MTR map (right) with inner (yellow) and outer (blue) cortical bands, as well as cortical lesions, superimposed.

Future work with PSIR data acquired at higher spatial resolution would be of interest as it may enable smaller CLs, or subpial lesions, to be detected. Combined MRI-histology studies would also help clarify if CLs unseen on MRI or non-lesional CGM changes are mostly responsible for the cNAGM abnormalities observed.

This is relevant when we consider the overall pathological burden of CLs and non-lesional abnormalities in MS subtypes, and so treatment targets.

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