Pathological Basis of Dirty Appearing White Matter in Multiple Sclerosis: Insights from MRI and Histology

C. Laule[1], I. M. Vavasour[1], E. Leung[2], D. K. Li[1], P. Kozlowski[1], A. L. Traboulsee[3], J. Oger[3], A. L. MacKay[1,4], and G. W. Moore[2]

[1]Radiology, University of British Columbia, Vancouver, BC, Canada, [2]Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, [3]Medicine, University of British Columbia, Vancouver, BC, Canada, [4]Physics & Astronomy, University of British Columbia, Vancouver, BC, Canada

INTRODUCTION

The characteristically focal proton density (PD) and T_2 hyperintense lesions and normal appearing white matter (NAWM) in subjects with multiple sclerosis (MS) have been studied extensively using magnetic resonance (MR) imaging. It is now generally accepted that NAWM can be abnormal when examined *in-vivo* by non-conventional MR methods[1] and post-mortem with histology[2]. As disability, cognitive impairment and atrophy are better correlated with NAWM abnormalities than T_2 lesion load, NAWM abnormalities are clearly important and clinically relevant[1]. However, little is known about another diffuse form of MS white matter abnormality which has been termed "dirty-appearing white matter" (DAWM). DAWM is only mildly hyperintense, appearing isointense with grey matter on a PD weighted image (Fig. 1). It is commonly periventricular in location[3] and diffuse in nature, making it distinct from the typical focal high signal intensity MS plaque. Because the boundary of DAWM is poorly defined, it is not usually included when quantitative volume measurements of disease burden are made, even though it is evident in 25% of relapsing remitting (RR) MS patients[4] and may contribute to clinical symptoms and disease progression. Previous work has found DAWM to have reduced magnetization transfer ratio (MTR)[5] and increased T_{1free} on MT imaging[6]. We have reported preliminary observations on histopathological abnormalities of DAWM in a small (n=4) series of post-mortem cases[7]. The purpose of this study was to further elucidate the MRI and histopathologic features of DAWM in a large *in-vivo* and *ex-vivo* study.

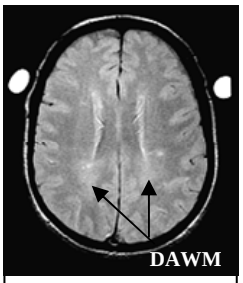

Fig.1 Example of DAWM

METHODS

DAWM was investigated in two ways: (1) *in-vivo* and (2) *ex-vivo* (formalin-fixed tissue).

1. *In-vivo*: 53 MS subjects with clinically definite MS (38RR, 13 secondary progressive, 1 primary progressive, 1 benign) were examined (33F/20M; mean age = 40yrs (23-56yrs); median Expanded Disability Status Scale = 3.0 (1.0-8.0), mean disease duration (DD) = 9.5yrs (1-35yrs)). Images were acquired using a 1.5T GE EchoSpeed scanner and included: PD/T_2 (TR=2500ms, TE=30/90ms, 256x192) and T_2 relaxation 32 echo CPMG (TR/TE=3000/10ms, one 5mm slice, 256x128, 4 averages). A subset of subjects (n=27) also underwent: T_1 Inversion Recovery (2D gradient echo/IR prepped, minimum TE, one 5mm slice, 256x128, 14 inversion times) and MT (3D gradient echo with and without a 2000Hz sinc MT pulse, TR=106ms, TE=5ms, flip angle 12^O, 256x128, 28 5mm slices). Regions of interest (ROIs) were outlined in DAWM and NAWM on the PD image and mapped to registered MT, T_1 and T_2 images using in-house software[8]. T_1 and T_2 data were analyzed using non-negative least square (NNLS)[9] to determine mean T_1, geometric mean T_2 (GMT_2), water content (WC, T_1 corrected PD relative to water standard PD) and myelin water fraction (MWF, 10ms<T_2< 40ms). MTR was defined as $(M_O - M_S)/M_O$ x 100% where M_O and M_S are the signals without and with the MT saturation pulse, respectively. MR measures from DAWM were compared to NAWM.

2. *Ex-vivo*: A total of 23 brain slices, fixed in 10% formalin, from 10 MS subjects (7 female, 3 male; mean age = 63yrs (range 35-77yrs); mean DD = 20yrs (range 11-38yrs)) with DAWM were examined with a 32 echo CPMG T_2 relaxation experiment. 15 samples were imaged on a GE 1.5T scanner using a transmit/receive head coil (TR/TE=3000/10ms, 8 averages, 256x256, 3mm thick, in plane resolution = 586µm x 586µm)), while the remaining 8 samples were imaged on a 7T, 30 cm bore, Bruker Avance MR scanner using a 7 cm i.d. quadrature volume coil (TR/TE=1500/6.673ms, 6 averages, 256x256, FOV=6 cm, 1mm thick, in plane resolution = 234µm x 234µm). ROIs were outlined in DAWM and NAWM on the first echo of the CPMG experiment. T_2 relaxation distributions were calculated using NNLS[9] and MWF was defined as the area of the short T_2 component (10-30ms at 1.5T, 10-25ms at 7T)[10] divided by total T_2 distribution. Tissue samples were embedded in paraffin, sectioned into 10µm thick sections and stained for myelin using luxol fast blue (LFB), for axons using Bielschowsky, immunohistochemically for the myelin proteins myelin basic protein (MBP) and 2',3'-cyclic nucleotide 3' phosphohydrolase (CNP) and glial fibrillary acidic protein (GFAP) for astrocytes. ROIs were mapped onto the histopathology images registered to the first echo MR image and mean optical density (OD) was determined using Image Pro Plus (Media Cybernetics, Silver Spring, MD). DAWM OD was compared to NAWM, and the OD's of the various histological stains were compared to each other.

RESULTS

***In-vivo*:** A total of 32 DAWM and 32 NAWM regions were examined on the single slice T_1 and T_2 experiments and 42 regions of DAWM and NAWM were examined on the MT experiment in 14 subjects. Table 1 summarizes *in-vivo* findings. DAWM had significantly reduced MWF and MTR and significantly increased GMT_2 and WC relative to NAWM ($p<0.05$).

Table 1 *In-vivo* mean MWF, GMT_2, WC, T_1 and MTR for DAWM & NAWM (std. dev.) (*$p<0.05$)

	MWF	GMT_2 (ms)	WC (g/ml)	T_1 (ms)	MTR (%)
NAWM	0.075 (0.004)	81 (1)	0.751 (0.007)	805 (14)	29.7 (0.3)
DAWM	0.058 (0.005)	93 (2)	0.780 (0.005)	833 (19)	28.1 (0.3)
% change of DAWM rel. to NAWM	-22.7%*	+15.1%	+3.9%*	+3.4%	-4.2%*

***Ex-vivo*:** Examining formalin fixed tissue, DAWM showed a mean reduction in MWF of 31(2)% ($p<0.0001$) compared to NAWM. The greatest OD reduction in DAWM was observed for LFB (28(2)%, $p<0.0001$), followed by Bielschowsky (16(3)%, $p<0.001$). DAWM was poorly visualized by protein stains with reductions of 12.8% ($p<0.01$), 4.3% and 1.6% for MBP, CNP and GFAP respectively. The correlation between LFB and MWF in all tissue ranged from R^2=0.53 to 0.95. LFB also correlated with Bielschowsky from R^2 =0.62 to 0.85. LFB did not correlate well with MBP, CNP and GFAP. Fig. 2 shows an example of DAWM.

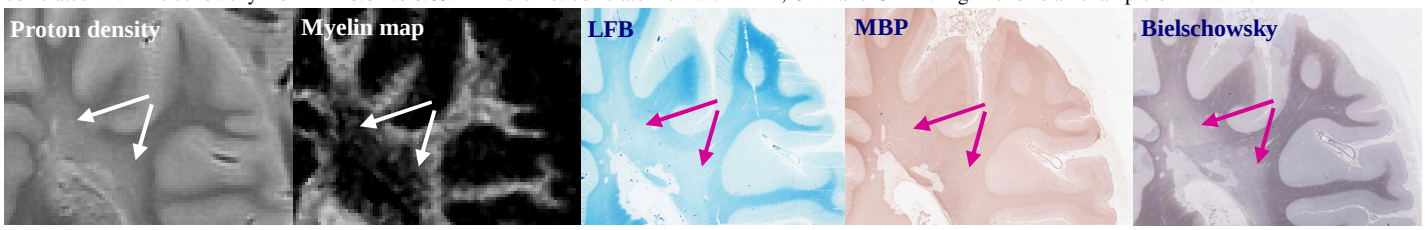

Fig. 2 – Example of DAWM (arrows) on MRI (1.5T) and histology.

DISCUSSION/CONCLUSION

In-vivo measurements of dirty-appearing white matter in MS subjects showed that it is significantly different from NAWM, in that it has a lower myelin water fraction and a lower MTR, and a higher geometric mean T_2 and water content. Histological observations correlate with the reduced MWF observed *in-vivo*. The most prominent histological abnormality was significantly reduced staining with LFB, a marker for myelin phospholipids. The reduced MWF (both in-vivo and ex-vivo) and the large LFB decrease in DAWM is consistent with a perturbation in myelin phospholipids that results in a reduction of the water trapped between the myelin lipid bilayer (ie myelin water) with relatively lesser involvement of the myelin proteins. The mild MTR change reflects non-specific loss of tissue structure likely due to a combination of the mild axonal loss and the myelin pathology. There is an associated increase in total water content (as well as T2 relaxation time) in DAWM, likely due to an influx of water into spaces formerly occupied by phospholipid, myelin proteins and axons.

(1) Miller DH, J Neurol. 2003;250:1407-1419. (2) Bruck W, In: Filippi M, Comi G, eds. Normal-appearing white and grey matter damage in multiple sclerosis, 2004: 3-8. (3) Zhao GJ, Neurol 2000;54 (suppl 3):A121. (4) Zhao G, MS 2003;9:S61. (5) Ge Y, AJNR 2003;24:1935-1940. (6) Ropele S, AJNR 2000;21:1885-1891. (7) Moore GRW J Neurol (in press). (8) Woods RP, JCAT 1992;16:620-633. (9)Whittall KP, JMRI 1989;84:134-152. (10) Laule C MS. 2006;12:747-753