Magnetization transfer indices and their histological correlates in post mortem multiple sclerosis brain

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Objective: To investigate histological correlates of magnetisation transfer (MT) ratio (MTR), f (macromolecular proton fraction) and T_{2B} (T_2 relaxation time of motionally restricted macromolecular protons) in post mortem (PM) multiple sclerosis (MS) brain.

Background: MT indices are being used to monitor MS. MTR changes have been related to demyelination, axonal loss and (less so) inflammation[1-3]. Using quantitative MT[4], f and T_{2B} can be obtained. The pathological correlates of f and T_{2B} in MS are unknown.

Methods (Figure 1)**:** Fresh coronal PM MS brain slices of 33 patients were studied. T_1-weighted spin echo (SE), dual SE T_2-weighted (T2W), MT and non-MT weighted SE (for MTR maps) and 2D proton density and T1-weighted gradient echo (GE) images (for T1 relaxation time maps) were acquired on a 1.5T scanner. In 19/29 brain slices ten uniquely MT-weighted datasets were also acquired using a 2D spoiled GE sequence. From these f and T_{2B} were calculated as previously described[4,5]. A stereotactic system was used to co-register regions of interest (ROIs), i.e. lesions and areas of normal appearing white matter (NAWM) on T2W MRI with the respective areas in specimens, thus guiding the dissection process[3,6]. Tissue blocks were processed for embedding in paraffin and sections stained (H&E, Luxol-Fast blue [LFB], Bielschowsky's silver impregnation). Immunohistochemistry included glial fibrillary acidic protein (GFAP) and CD68. MS lesions were classified as active (early active or chronic active, CA) or chronic inactive (CI) and as demyelinated and remyelinated. Transmittance (Tr) was obtained from LFB- and GFAP-stained slides to quantify myelin and gliosis[3], point-counting was used to quantify axons[7]. T-tests of patient means and linear regression taking into account within-patient tissue sample dependencies were used for analysis.

Results: In 82 lesions (72 demy, 10 remy; 2/82 early active, 18/82 CA, 62/82 CI) and 33 NAWM ROIs MTR and histology were studied. In 46/82 lesions (19 patients) f and T_{2B} values were available. Except for gliosis and T_{2B}, all investigated indices differed between lesions and NAWM ($p<0.001$). For correlations between the variables see table 1. Compared to demyelinated lesions, remyelinated lesions had a higher MTR ($p=0.008$), f ($p=0.021$), T_1 ($p=0.032$) and axonal count ($p=0.023$). T_1 was higher and f lower in active (CA & CI taken together) lesions, whereas MTR and T_{2B} did not differ between the two lesion types (Table 2). Multiple regression revealed that (i) MTR and f both *independently* predicted Tr_{myelin} whereas T_1 did not, and (ii) the association of T_1, f, and MTR with axonal count was secondary to the strong correlation between Tr_{myelin} and axonal count.

Conclusions: Both MTR and f are strong and independent predictors of myelin content in post mortem MS brain. The new parameter f appears to be affected by inflammation and hence may allow a distinction between active and inactive lesions.

References: 1. van Waesberghe, et al. Ann Neurol 1999;46:747-54. 2. Barkhof, et al. Arch Neurol 2003;60:1073-81. 3. Schmierer, et al. Ann Neurol 2004;56:407-15. 4. Tozer, et al. Magn Reson Med 2003;50:83-91. 5. Davies, et al. Mult Scler 2004;10:607-13. 6. Schmierer, et al. Neuropathol Appl Neurobiol 2003;29:596-601.

Table 1	Pearson correlation coefficient, p-value (n regions/n patients)				
	T_1	MTR	f	Tr_{myelin}	axonal count
MTR	-0.73, <0.001 (133/33)				
f	-0.80, <0.001 (81/19)	0.82, <0.001 (81/19)			
Tr_{myelin}	0.69, <0.001 (109/29)	-0.83, <0.001 (109/29)	-0.79, <0.001 (63/15)		
axonal count	-0.60, <0.001 (107/29)	0.70, <0.001 (107/29)	0.78, <0.001 (63/15)	-0.80, <0.001 (160/29)	
$Tr_{gliosis}$	-0.12, 0.29 (105/28)	0.12, 0.28 (105/28)	0.31, 0.028 (61/14)	-0.18, 0.04 (158/28)	0.21, 0.019 (158/28)

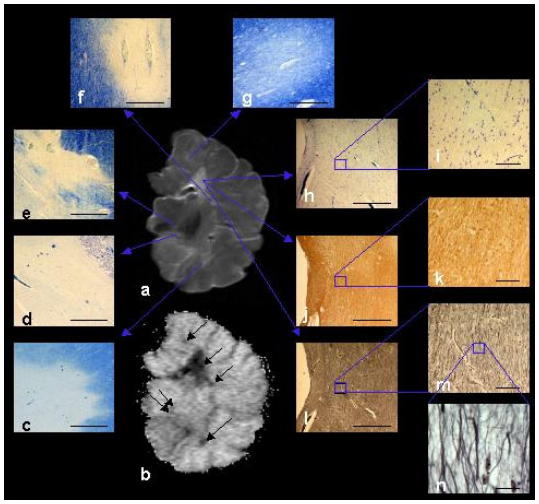

Table 2	mean (SD), n=6 patients		
	active lesions n=13	inactive lesions n=16	p-value
T_1 [ms]	1322 (436)	1594 (409)	0.032
MTR [pu]	22 (6)	20 (6)	0.35
f	3.14 (2.49)	1.77 (1.03)	0.044
T2b	11.39 (4.83)	10.62 (3.34)	0.17
Tr_{myelin}	0.76 (0.20)	0.88 (0.07)	0.26
axonal count	11.39 (7.56)	8.808 (4.19)	0.349
$Tr_{gliosis}$	0.72 (0.04)	0.73 (0.06)	0.518

Figure 1 Correlation of MRI and histopathology in *post-mortem* multiple sclerosis brain. Six regions of high signal (RHS) on T_2-weighted MRI **(a)** are pathologically confirmed MS lesions (**c-g**) (blue arrows). The regions corresponding to the RHS on T_2-weighted MRI are indicated (black arrows) on the respective MTR map **(b)**.
On sections stained for Luxol fast blue (LFB) five of the six lesions appear demyelinated (**c-f, h**), one remyelinated (**g**) (bar=1mm). For one demyelinated lesion sections stained for LFB (**h,i**), glial fibrillary acidic protein (GFAP) (**j,k**) and Bielschowsky's silver impregnation (BIEL) (**l-n**) are shown. The myelin content and the extent of gliosis were assessed by measuring transmittance (see text) on sections stained for LFB (**i**) and GFAP (**k**), respectively, at a final magnification of x125 (bar=100µm). Axonal counts were determined on sections stained for BIEL at a final magnification of x1250 (**n**) (bar=10µm).