Diffusion tensor indices and their histological correlates in post mortem multiple sclerosis brain

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Objective: To explore histological correlates of diffusion tensor imaging (DTI) measures in post mortem (PM) brain of patients with multiple sclerosis (MS).

Background: DTI is being used to investigate MS. DTI is a magnetic resonance (MR) technique that probes changes in the random translational motion of water in tissue. No data on the pathological correlates of DTI in MS brain are available.

Methods: Fresh coronal PM MS brain slices (1cm thick) of 13 patients (mean age 54.3 years [SD 14.3]; disease duration 22.5 years [10.7]) were studied. Using a 1.5T scanner spin echo (SE) T1- and T2-weighted (T2W) images were acquired. For DTI a multi-shot diffusion-weighted SE-EPI sequence was employed (b-factor=1940s/mm², slice thickness=5mm; resolution=1.9x1.9mm²; TR=3s; TE=86 ms; no. shots=8; NEX=4) was used[1]. A stereotactic system was used to co-register MS lesions and areas of normal appearing white matter (NAWM) regions of interest (ROIs) on T2W MRI with the respective areas in the specimens, thus guiding the dissection[2]. ROIs on T2W MRI were visually matched with DTI b_0 images and transferred to mean diffusivity (MD) and fractional anisotropy (FA) maps (figure 1). 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 (figure 1). Lesions were classified as active, chronic active or chronic inactive and as demyelinated and remyelinated. Transmittance (Tr) was obtained from LFB- and GFAP-stained slides to quantify myelin (Tr_{myelin}) and gliosis ($Tr_{gliosis}$), point-counting was used to quantify axons[3]. T-tests of patient means and linear regression (taking into account within-patient tissue sample dependencies) were used for analysis.

Results: Thirty-four lesions visible on T2W MRI were studied. For 22/34 lesions and nine NAWM ROI of nine patients histopathological data were available. There were 19/22 demyelinated lesions and three/22 remyelinated lesions. Seven/22 lesions were chronic active, 15/22 chronic inactive. MD and FA were lower in post mortem MS tissue than in patients with MS in vivo by approximately 70% and 30%, respectively[4]. MD and FA differed significantly between lesions and NAWM (table 1). Both indices were independent predictors of myelin content, axonal count and gliosis (table 2). Tr_{myelin} and axonal count correlated strongly with each other and weakly with $Tr_{gliosis}$ (table 2). No leading correlation could be detected in the search for primary and secondary associations including Tr_{myelin}, axonal count, MD and FA in the multiple regression model. $Tr_{gliosis}$ was not independently associated with either MD or FA.

Conclusion: Both MD and FA in post mortem MS brain are primarily influenced by demyelination and axonal loss and – more moderately and secondarily – gliosis. Since the extent of gliosis is only weakly correlated to myelin content and axonal count, gliosis might independently influence MD and FA. The acquisition of a larger dataset is underway to clarify this.

References: 1. Wheeler-Kingshott, et al. Proc. Intl. Soc. Mag. Reson. Med. 2003;11:2137 & 2004;12:1239. 2. Schmierer, et al. Neuropathol Appl Neurobiol 2003;29:596-601. 3. Schmierer, et al., Ann Neurol 2004;56:407-15. 4. Ciccarelli, et al. Neurology 2001;56:926-33.

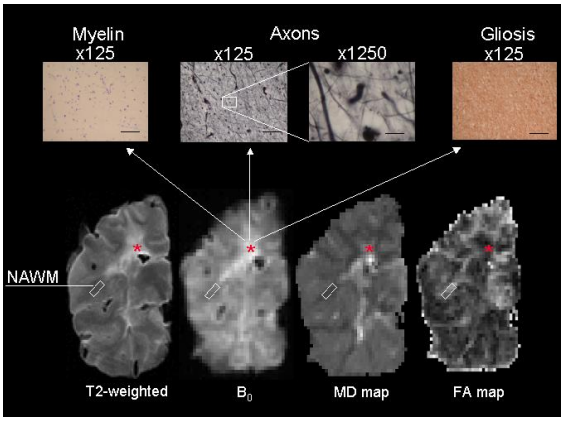

Figure 1 DTI and quantitative histopathology of *post mortem* multiple sclerosis brain. Lower panel: T2-weighted and b_0 images and mean diffusivity (MD) and fractional anisotropy (FA) maps of a coronal brain slice of a half hemisphere. Upper panel: histological sections that correspond to a chronic MS lesion (* on the MRI scans/maps). The sections are stained for Luxol fast blue (LFB, for myelin), Bielschowsky's silver impregnation (for axons) and glial fibrillary acidic protein (for gliosis). Bar=10µm for magnifications x125 and 100µm for magnification x1250.
Note the relatively preserved axons (39% axonal loss compared to normal appearing white matter [NAWM, rectangular box on the MRI scans/maps] in this case) despite the lesion being void of myelin (far left image on upper panel does not show the typical intense blue LFB staining for myelin).

Table 1	lesions	NAWM	
	mean (SD)	mean (SD)	p
MD ($x10^{-6}m^2s^-$)	357.11 (89.41)	222.48 (47.23)	<0.001
fractional anisotropy	0.24 (0.06)	0.40 (0.14)	0.0009

N=13 patients. Mean of individual patient means (averaged across tissue sample). MD=mean diffusivity.

Table 2	Pearson correlation coefficient, p-value (n regions/n patients)			
	MD	FA	Tr_{myelin}	axonal count
FA	-0.64, <0.001 (47/13)			
Tr_{myelin}	0.66, 0.001 (31/9)	-0.75, <0.001 (31/9)		
axonal count	-0.74, <0.001 (31/9)	0.75, <0.001 (31/9)	-0.80, <0.001 (160/29)	
$Tr_{gliosis}$	-0.53, 0.009 (31/9)	0.51, 0.014 (31/9)	-0.18, 0.043 (158/28)	0.21, 0.019 (158/28)