Axial and Radial Diffusivity as MR Biomarkers of Axonal and Myelin Injury in ex-vivo Cervical Spinal Cord from Multiple Sclerosis Patients

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Introduction: Multiple sclerosis (MS) is a devastating progressive demyelinating disease of the central nervous system. Unfortunately, conventional MR imaging of MS does not give specific information about the underlying pathology and does not necessarily correlate with clinical outcomes.[1-4] Cervical spinal cord lesions are present in over 94% of MS patients at autopsy[5, 6] and may provide better correlation with patient disability than brain pathology.[7] Using the diffusion tensor imaging (DTI) derived axial ($\lambda_{||}$) and radial ($\lambda_{\perp}$) diffusivity we have probed the microstructural environment of cervical spinal cord samples obtained at autopsy. This project aims to (1) establish normative values for $\lambda_{||}$ and $\lambda_{\perp}$ in normal *ex-vivo* human cervical spinal cord, and (2) validate $\lambda_{||}$ and $\lambda_{\perp}$ as MR biomarkers of axonal and myelin injury in human cervical spinal cord obtained from MS patients at autopsy with histology.

Methods: Human cervical spinal cords were obtained at autopsy from patients with a history of MS (*n*=7) and from age-matched control subjects (*n=10*). DTI was performed at 4.7 Tesla on an Oxford magnet with a custom built 8 mm solenoid coil for transmission and reception. Axial images were be obtained with diffusion sensitizing gradients applied in six orientations with b-values of 0 and 1.813 ms/μm^2, a resolution of 250 x 250 x 250 μm^2. Regions of interest (ROIs) were selected according to the major anatomic sections of the cervical spinal cord including dorsal (DW), ventral (VW), and lateral white (LW) matter and central gray matter (G). DTI parameter maps were calculated for $\lambda_{||}$, $\lambda_{\perp}$, trace, and relative anisotropy (RA) on a voxel-by-voxel basis. In addition, unique ROIs, corresponding to MS lesions, were identified based upon assessment of each MR image and independently based upon histology. Histological staining included Luxol fast blue (LFB) for myelin, antibody staining for phosphorylated neurofilaments (pNP), and immunostaining of β-APP, SMI-31, and myelin basic protein (MBP).

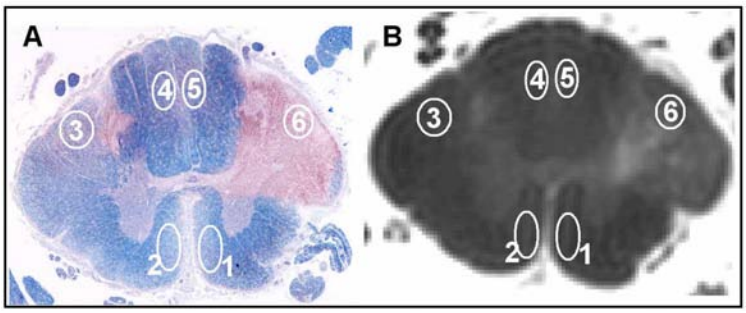

Figure 1: Histopathology **(A)** and corresponding $\lambda_{\perp}$ parameter map **(B)** for cervical spinal cord from an MS patient. **A:** Magnification views and additional stains (see methods) confirmed this sample to have normal myelin (LFP, blue) and axonal (pNF, brown) staining in the portions of lateral white (LW) matter under ROI 3 and white (DW) matter under ROI 4. The ventral white (VW) matter under ROIs 1 and 2 has relative normal myelin staining but decreased axonal density. The DW matter under ROI 5 has subtly decreased myelin (blue) staining with preserved axonal staining. The LW matter under ROI 6 has severe demyelination and relatively decreased axonal density. In **B**, these ROIs are overlaid on the corresponding $\lambda_{\perp}$ parameter map for this sample. Quantitative analysis of $\lambda_{||}$ and $\lambda_{\perp}$ for this slice is summarized below in **Table 1.**

Table 1. DTI parameters of the selected ROIs from the autopsy MS shown in Figure 1 above and the control cords.

ROI	Axial	Radial	Axonal histology	Myelin histology
MS-1	.38	.14	Decreased density	Normal
MS-2	.38	.17	Decreased density	Normal
MS-3	.48	.14	Normal	Normal
MS-4	.47	.15	Normal	Normal
MS-5	.60	.16	Normal	Decreased
MS-6	.71	.31	Intact axons at lower than normal density	Severe demyelination
Control VW (MS 1,2)	.43	.17	-	-
Control LW (MS 3, 6)	.46	.16	-	-
Control DW (MS 4, 5)	.52	.17	-	-

Results and Discussion: Normative values for $\lambda_{||}$ in *ex-vivo* normal human cervical spinal cord (+/- SD) are as follows: DW 0.518 (0.097), VW 0.434 (0.079), LW 0.463 (0.104), G 0.391 (0.034). Normative values for $\lambda_{\perp}$ in *ex-vivo* normal human cervical spinal cord (+/- SD) are as follows: DW 0.173 (0.023), VW 0.177 (0.027), LW 0.163 (0.011), G 0.223 (0.037). ROI analysis of cervical cord samples from MS patients demonstrates characteristic changes in $\lambda_{||}$ and $\lambda_{\perp}$, corresponding to discrete histopathological patterns of varying degrees of axonal and myelin loss, as summarized in Table 1 above. Quantitative DTI analysis of $\lambda_{||}$ and $\lambda_{\perp}$ is more sensitive to subtle alterations in axon density and demyelination than qualitative evaluation of the T2 and DW images and provides an unprecedented degree of specific histological correspondence. These experiments carry significant clinical implications: for patients with MS, lesions with axonal loss are currently beyond therapeutic intervention and are likely to have permanent neurological sequelae. However, lesions with demyelination only have potential for recovery. Translation of these results into *in vivo* imaging of the cervical cord will be the next stage of this project and is currently underway in our group.

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