Quantitatively Characterize Pathological Compositions for Different Types of Multiple Sclerosis Lesion

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Target Audience: This work is for those who are interested in quantitatively characterizing white matter lesions in CNS diseases using MRI.

Purpose: MRI has significantly improved lesion detection in CNS diseases such as multiple sclerosis (MS). Conventional MPRAGE, and FLAIR images have been used to identify and classify MS lesions into different subtypes, including persistent black hole (PBH), chronic T2W hyperintensities that were non-black holes (NBH), and gadolinium-enhanced (Gd+) lesions, etc. However, these conventional images are not quantitative and are incapable to distinguish different pathological components within MS lesions. Quantitative MRI such as diffusion tensor imaging (DTI) has been used to detect and distinguish axon and myelin injury based on decreased axial diffusivity (AD) and increased radial diffusivity (RD) repectively.[1-3] Unfortunately, the most common DTI findings in different types of MS lesions have been increased AD and RD, and decreased FA,[4,5] insufficient to profile pathological compositions underlying different lesion types. Against this background, diffusion basis spectrum imaging (DBSI) was recently developed to differentiate and quantify individual pathological components in MS lesions.[6-8] The purpose of this study is to further validate DBSI using autopsy human MS spinal cord specimens and to investigate whether DBSI can quantitatively characterize the distinct pathological compositions underlying different MS lesion types, a task yet to be demonstrated by other neuroimaging approaches.

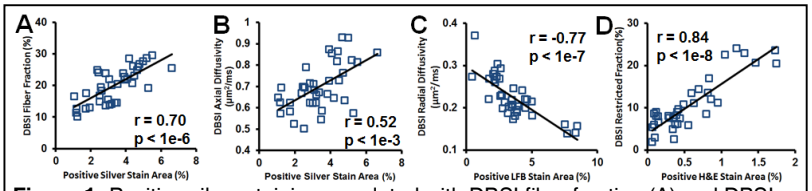

Figure 1: Positive silver staining correlated with DBSI fiber fraction (A) and DBSI AD (B). Positive LFB-PAS staining correlated with DBSI RD (C). Positive hematoxylin staining correlated strongly with DBSI restricted diffusion fraction (D).

Method: Autopsied MS spinal cord specimens: Cervical spinal cord specimens were obtained following autopsy from three MS patients. The tissues were fixed in 10% formalin in phosphate buffered saline (PBS) after autopsy. Ex vivo MRI: MS spinal cord specimens were examined using an Agilent 4.7 T magnet. TR/TE = 2000/39ms, diffusion time=17 ms. Diffusion gradients were applied in 99 directions with max b-value = 3200 s/mm^2. Voxel size=250x250 μm^2. Histology: The spinal cord specimens were embedded in paraffin and sectioned at 5-μm thick. Sections were stained with Bielschowsky's silver, Hematoxylin and Eosin (H&E), and Luxol Fast Blue-Periodic Acid Schiff (LFB-PAS) stains. Images were acquired with a NanoZoomer 2.0-HT System. The raw histology image was down-sampled and co-registered to MR images. 40 regions of interest (ROIs) were randomly selected. Positive stains were manually counted. The fraction of positive stain area was computed. Statistical Analysis: Spearman's rank correlation coefficients were used to measure the strength of monotone association between diffusion MRI metrics and positive stain quantifications. Human Subjects: Procedures involving human subjects were approved by the Institutional Review Board of Washington University. Five healthy control subjects, three MS patients (1 RRMS and 2 SPMS) were imaged. Human MRI: A 3-Tesla TIM Trio scanner (Siemens, Erlangen, Germany) was employed. Diffusion MRI data were collected at 2x2x2 mm^3 resolution with TR/TE = 10000/120ms. The max b-value was 2000 s/mm^2. The same 99-direction diffusion scheme was used for 15 minutes acquisition time. DBSI/DTI Analysis: A voxel-based DBSI analysis was performed based on the recent publication.[6] Conventional DTI was also computed. Sixteen MS lesions and anatomically matched control regions were outlined using MPRAGE and FLAIR, and assessed by Beeswarm and Boxplot.

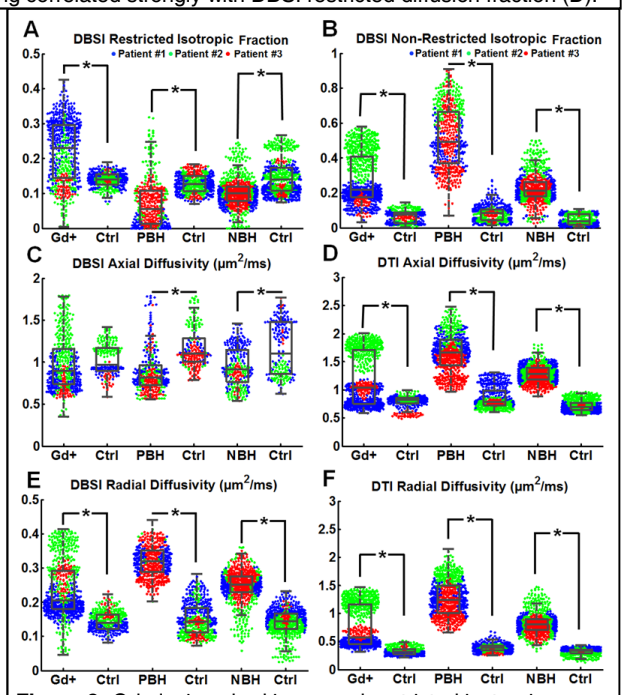

Figure 2: Gd+ lesions had increased restricted isotropic diffusion fraction compared to PBH and NBH lesions (A). PBH displayed increased non-restricted isotropic diffusion fraction (B). Decreased DBSI AD was observed in PBH and NBH, but not in Gd+ lesions (C). DTI AD (D), DBSI RD (E) and DTI RD (F) significantly increased for all MS lesion types. *p < 0.05

Results and Discussion: DBSI fiber fraction and AD correlated with the area of silver stain (Fig. 1A; r=0.70; p<1e-5; Fig. 1B r=0.52; p<1e-3). DBSI RD negatively correlated with the area of LFB stain (Fig. 1C; r=-0.77; p< 1e-7). DBSI-restricted isotropic diffusion fraction correlated with the area of nuclei detected by hematoxylin stain (Fig. 1 D; r=0.84; p<1e-8). Findings suggested that DBSI metrics and quantitative histology measure the same pathological components supporting in vivo application of DBSI to characterize MS lesions. For the six PBHs, six NBHs, and four Gd+ lesions (1551voxels total) examined by DBSI. The restricted isotropic diffusion fraction increased in Gd+ lesions (Fig. 2A). The RRMS patient (#1, blue) had the largest number of voxels with increased restricted isotropic diffusion fraction, characteristic of increased cellularity (Fig. 2A). The restricted isotropic diffusion fraction decreased in PBH and NBH, whereas the DBSI non-restricted isotropic diffusion fraction increased in all three MS lesion types (Fig. 2B). DBSI AD significantly decreased in PBH and NBH (Fig. 2C), while did not change significantly in Gd+ lesions. Consistent with literature reports, DTI AD increased in all three lesion types compared to that of the control (Fig. 2D). RD derived by both DBSI and DTI increased in all lesion types (Fig. 2E and F). Gd+ lesions displayed the greatest increased restricted isotropic diffusion fraction, significantly increased DBSI RD and minimally decreased DBSI AD, suggesting active inflammatory demyelination with slight axonal injury. Significantly increased DBSI non-restricted isotropic diffusion fraction suggested severe tissue loss with increased free water in PBHs.

Conclusion: DBSI metrics and quantitative histology measure the same pathological components. DBSI can quantitatively characterize different types of MS lesions in vivo, a task yet to be demonstrated by other neuroimaging approaches.

References: 1. Song SK, et al., 2005, *NeuroImage* 26(1): 132-140; 2. Kim JH et al., 2006, *Neurobiology of disease* 21(3):626-632; 3. Sun SW et al., 2006, *NeuroImage* 32(3):1195-1204; 4. Naismith RE et al., 2013, *Neurology* 80(24):2201-2209; 5. Oh J et al., 2013, *Neurology* 80(6):540-547; 6. Wang Y et al., 2011, *Brain* 134(Pt 12):3590-3601; 7 Chiang CC et al.,2014, *NeuroImage* 310-319; 8 Wang XJ et al., 2014, *NMR in biomedicine* 27(7) 843-852