Diffusion MRI detects early axon loss despite confounding inflammation in optic neuritis

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Purpose: Multiple sclerosis (MS) is an inflammatory demyelinating disease ultimately producing irreversible axon loss, leading to permanent neurological impairments. Optic neuritis (ON) is an early symptom of MS, characterized by inflammatory demyelination of optic nerve. The neuronal and axonal injury in ON has been suggested to reflect the progression of global CNS injury in MS. Optic nerve atrophy has been used as an image marker of axon loss. However, MRI volume measurement is confounded by the co-existing inflammation and partial volume effects. We employed the experimental autoimmune encephalomyelitis (EAE) mouse model of ON with diffusion basis spectrum imaging (DBSI) to examine whether DBSI is capable of detecting axon loss in the presence of confounding inflammation at the onset of ON in EAE mice.

Methods: *Animals:* All experiments were approved by the Washington University Animal Studies Committee and were performed on 8-week old female C57Bl/6 mice. EAE mice were actively immunized with myelin oligodendrocyte peptide (MOG_{35-55}). Visual acuity (VA), as a measure of visual function, was measured pre-immunization and then daily after immunization. Visual impairment was defined as VA ≤ 0.25 based on our previous work (1). We defined Time 1 as the day the first eye had a VA ≤ 0.25 and Time 2 as the day the second eye had a VA ≤ 0.25. *MRI Measurements:* MRI experiments were performed on a 4.7 T Agilent DirectDrive™ small-animal MRI system. Diffusion data was acquired with an icosahedral 25-direction diffusion-encoding scheme combined with one b=0 was employed. Data was analyzed with DBSI multi-tensor and conventional DTI single-tensor model analysis packages developed in-house with Matlab (2). *Optic Nerve Volume:* Optic nerve cross-sectional volume was computed as the sum of the voxels in the optic nerve multiplied by the volume per voxel. *Axon volume:* DBSI fiber fraction is the percent of signal associated with axons. Axon volume, a marker proportional to axon count and therefore axon loss, is derived by the mean fiber fraction times the nerve volume.

Results: Figure 1 shows the DTI and DBSI derived parameters for Eye 1 and Eye 2 over time. Based on VA, Time 1 and Time 2 corresponded to onset of ON for Eye 1 and Eye 2, respectively. Because there is potential for pathological dependence, it is not appropriate to compare the left-right eyes from the same mouse. DTI and DBSI-derived $\lambda_{\parallel}$ significantly decreased at Time 2 for both eyes. While DTI-derived $\lambda_{\perp}$ significantly increased at Time 2 for both eyes, DBSI-derived $\lambda_{\perp}$ only significantly increased for Eye 2 at Time 2. Cell infiltration (restricted isotropic diffusion tensor fraction) and edema (non-restricted isotropic diffusion tensor fraction) significantly increased in both eyes at Time 2, suggesting increasing inflammation. For cell infiltration and edema, the eye that was not significant at Time 1 showed a trend towards increase. Atrophy assessed by structural MRI is a common image marker of axon/neuron loss in CNS diseases. However, atrophy can be underestimated by confounding increase in tissue volume resulting from elevated cellularity and edema resulting from inflammation. We observed optic nerve atrophy at ON onset in Eye 1. The axon volume is a combination of the percent of signal associated with fibers and optic nerve volume, removing non-fiber partial volume effect, and this was significant in Eye 1 at Time 1 and both eyes at Time 2. We expect that this axon volume will be a better measurement to detect axon loss. We were encouraged that "axon volume" detected axon loss in the presence of confounding inflammation in ON.

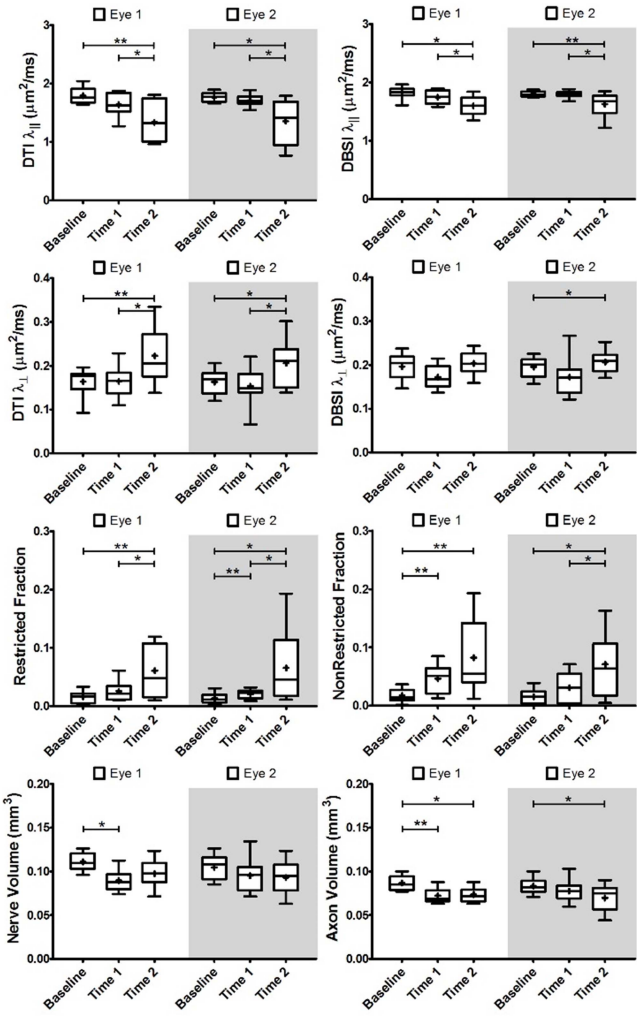

Figure 1. Changes in DTI and DBSI Metrics over time. Data was analyzed for each eye independently. Whiskers show the minimum and maximum, the line in the middle of the box is the median, and the plus sign is the mean. P<0.05 for *. and P<0.01 for **.

Discussion and Conclusion: ON pathology includes axonal injury, demyelination, and inflammation leading to visual function impairment. DBSI was able to show involvement of all these pathologies in ON. However, it is difficult to determine which of these pathologies is most strongly linked to visual function. Correlation of metrics to VA and histological markers is ongoing. Additional studies will include longer follow-up as well as therapy to ascertain the potential of these different metrics as biomarkers in inflammatory demyelinating diseases.

References: 1. Chiang, C.-W. et al. *NeuroImage* **101,** 310–319 (2014). 2. Wang, Y. et al. *Brain* **134,** 3587–3598 (2011).