Diffusion MRI derived immunohistochemistry equivalent "stains" of white matter pathology

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Introduction

In the classical diffusion tensor imaging (DTI) paper, Basser and Pierpaoli proposed the use DTI derived parameters to generate physiological and pathological "stains".[1] Early studies applying DTI on various animal models indeed suggested that DTI derived directional diffusivity correctly reflect white matter pathologies in vivo.[2] However, there has not been an established MRI derived immunohistochemistry (IHC) equivalent "stains" since the publication of the paper. Recently, a novel diffusion basis spectrum imaging (DBSI) was introduced to resolve the confounding effect of inflammation on diffusion MRI parameters as well as to separate and quantify the extent (volume ratio of underlying pathologies and fiber density) of coexisting white matter pathologies in a rodent model of multiple sclerosis.[3] In the current study, in vivo DBSI was performed on the spine of the experimental autoimmune encephalomyelitis (EAE) mice at various stages of the disease. By taking the advantage of DBSI derived metrics, we created diffusion MRI equivalents of IHC staining for intact axons, myelin, and cellularity in EAE mouse spines. In vivo DBSI measurements were validated by post-fixation ex vivo DBSI and IHC. Our data indicate that the DBSI derived IHC equivalent "stains", including DBSI-SMI-31 (for intact axons), DBSI-MBP (for intact myelin), and DBSI-DAPI (for cell counts), correlated well with the actual IHC staining.

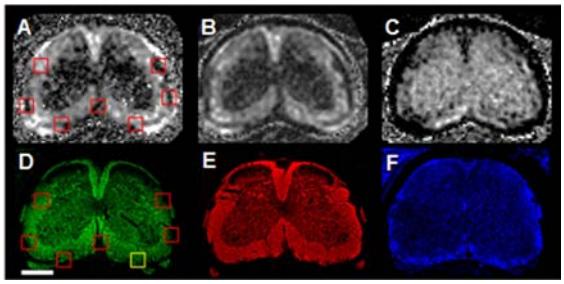

Figure 1 Representative DBSI histology equivalent maps (A-C) and the corresponding IHC images (D-F) were taken from a mouse spinal cord at the peak of EAE. The in vivo DBSI derived equivalent of SMI31 (A), MBP (B), and DAPI (C) maps show remarkable resemblance of the postmortem IHC counterparts. Scale bar = 500 μm.

Materials and Method

EAE: Rodent Model of MS

EAE was induced in twenty 8-weeks-old C57BL/6 female mice by active immunization of MOG_{35-55} peptide. Cross-sectional DBSI was performed on the spine at the baseline, onset, peak and chronic phases of EAE (n=5 each group).

DBSI and Immunohistochemistry (IHC)

High resolution ex vivo MRI was acquired after in vivo DBSI acquisition using the multiple spin-echo diffusion weighted sequence with 99 diffusion directions. The acquisition parameters were: TR 1.5s, TE 27ms, *b* 9 distinct values from 0~3200s/mm^2, △ 15ms, δ 5ms, NEX 4, slice thickness 2mm in plane resolution 62μm×62μm. The acquisition time was approximately 16 hrs. Following MRI scans, tissue were sectioned for histological validations. SMI31, MBP and DAPI staining was used to quantify the extent of axon injury, myelin damage and cellularity.

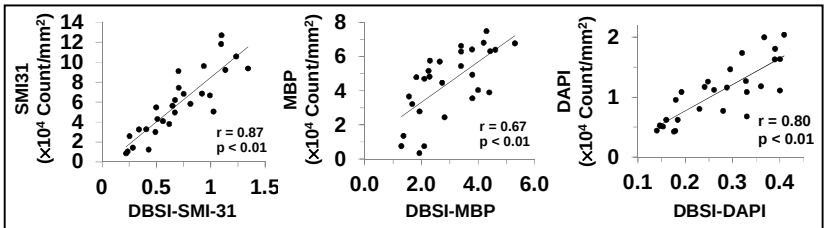

Figure 2 High positive correlations are seen between the DBSI-derived IHC equivalent "stains" and the corresponding IHC counts acquired from the ROI marked in Figure 1.

Data Analysis

The DBSI diffusion parameters were derived according to the equation[2]:

$$S_k = \sum_{i=1}^{N_{Aniso}} f_i e^{-|\vec{b}_k|\cdot\lambda_{\perp\_i}} e^{-|\vec{b}_k|(\lambda_{\parallel\_i}-\lambda_{\perp\_i})\cos^2\psi_{ik}} + \int_a^b f(D) e^{-|\vec{b}_k|D} dD \quad (k=1,2,\ldots,K)$$

,where S_k signal; $|b_k|$ *b*-value of the k^{th} diffusion gradient; N_{Aniso} number of anisotropic tensors; ψ_{ik} angle between the k^{th} diffusion gradient and the principal direction of the i^{th} anisotropic tensor, $\lambda_{\parallel\_i}$ and $\lambda_{\perp\_i}$: axial and radial diffusivities of the i^{th} anisotropic tensor; f_i signal intensity fraction for the i^{th} anisotropic tensor; *a* and *b:* the low and high diffusivity limits for the isotropic diffusion spectrum *f(D)*. For comparing with the IHC images, the DBSI histology equivalent maps were generated to represent the integrity of axon (DBSI-SMI31, $f_i \times \lambda_{\parallel\_i}$), myelin (DBSI-MBP, $f_i / \lambda_{\perp\_i}$) and cellularity (DBSI-DAPI, *f(D)*, *D*<0.3). The pixel-by-pixel covariance maps were generated between the co-registered DBSI and IHC images by calculating the product of the difference from the mean of pixel intensities.[4]

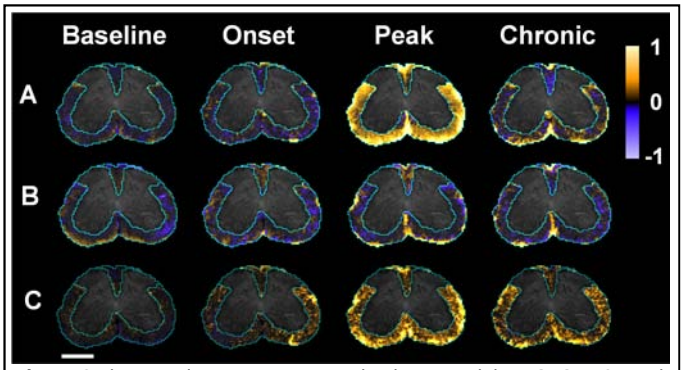

Figure 3 The covariance maps comparing between (A) DBSI-SMI-31 and actual IHC SMI-31 images, (B) DBSI-MBP and IHC MBP image, and DBSI-DAPI and IHC DAPI image at selected time points of EAE course demonstrate good correlation between the two methods. When an EAE lesion is detected in both DBSI and IHC images, both image intensities change synchronously leading to a positive correlation (color coded in orange). For example, decreased MBP (increased SMI-31 and DAPI) staining intensity in IHC and decreased DBSI-MPB (DBSI-SMI-31 and DBSI-DAPI) intensity suggests myelin damage (axonal injury and increased cellularity) detected by both methods. Scale bar: 500 μm.

Results and Discussions

In vivo DBSI equivalents of IHC resemble their postmortem IHC counterparts (Fig. 1). By co-registering in vivo DBSI equivalent map with postmortem IHC maps, ROI based analysis demonstrated a significant linear correlation between the intensity of DBSI-SMI31, DBSI-MBP, and DBSI-DAPI with IHC maps of SMI31, MBP, and DAPI (Fig. 2). To further demonstrate the utility of DBSI-IHC equivalents, high-resolution ex vivo DBSI data were co-registered with IHC staining followed by voxel-based correlation analysis (Fig. 3).[4] The proposed DBSI derived IHC equivalent "stains" exhibit the intuitively simple characteristic of the actual IHC staining, i.e., higher image intensity corresponds to increase immunoreactivity. Thus, by directly visualizing the DBSI derive IHC equivalent "stains" one can easily assess the tissue integrity as seen under the microscope. The covariance map analysis uniquely allows the correlation between DBSI derived IHC equivalent and the actual IHC staining to be examined, readily validating in vivo DBSI derived IHC equivalent "stains".

Reference [1]Basser et al. JMRB, 1996; [2]Song et al. Neuroimage, 2002; [3]Wang et al. Brain 2011; [2]Anderson, MRM, 2005, [4]Li et al., J Neuroscience, 2000