**Histological metrics confirm microstructural characteristics of NODDI indices in multiple sclerosis spinal cord**

Francesco Grusu1, Torben Schneider1, Richard L. Yates2, Mohamed Tachrount3, Jia Newcombe1, Hui Zhang1, Daniel C. Alexander1, Gabriele C. DeLuca1, and Claudia A. M. Wheeler-Kingshott1


**TARGET AUDIENCE** Scientists investigating novel MRI biomarkers in multiple sclerosis (MS).

**PURPOSE** MS is a complex inflammatory, demyelinating, and neurodegenerative disease characterised by several pathological mechanisms. Routine diffusion-weighted (DW) MRI techniques such as diffusion tensor imaging (DTI) are sensitive to MS, but can lack in specificity. Neurite orientation dispersion and density imaging (NODDI) is a novel DW MRI method that has shown encouraging results in the MS brain, and could also be useful in the spinal cord, often extensively involved in MS. Here, we investigated the relation between NODDI metrics and histology in the MS spinal cord. We performed NODDI analysis of multi-shell DW data acquired at 9.4 T from an ex vivo specimen, and compared results to histological parameters quantifying neurite orientation dispersion and myelin density.

**METHODS MRI acquisition** Multi-shell DW data from one specimen of formalin-fixed MS lumbar spinal cord (sex: F; age at decease: 67; MS subtype: chronicle; last assessed EDSS: 7; decease to fixation time: 23 h), were acquired on a 9.4 T Agilent system at 35°C. Twenty slices were acquired sagitally with a PGSE sequence (TE/TR = 39.5/2500 ms; δ/Δ = 12/18 ms; 0.8 mm slice thickness; resolution: 0.164×0.200 mm²; field-of-view: 21×51.2 mm²). Six shells were acquired with b = [520, 2080, 4680, 8320, 13000, 18720] s/mm² and respectively [6, 15, 24, 32, 42, 51] directions, interleaved with twenty-five b = 0 images.

**NODDI model fitting** NODDI was fitted to the DW data with the NODDI Matlab toolbox, after accounting for extra diffusion-weighting due to imaging gradients. We obtained voxel-wise estimates of orientation dispersion index (ODI) and neurite density index (NDI). ODI ranges from 0 for perfectly parallel neurites to 1 for randomly oriented neurites, whereas NDI represents the estimated voxel volume fraction occupied by neurites.

**Histology** Routine histological procedures were followed to obtain four formalin-fixed-paraffin-embedded (FFPE) sections, 10 μm thick, within two consecutive MRI slices. For the histological material derived from each MRI slice, two of the four FFPE sections were stained for axons (Palmgren silver) and two for myelin (proteolipid protein (PLP) immunohistochemistry). Images of the stained sections were acquired with an Aperio slide scanner (ScanScope AT Turbo), for a final pixel dimension of 1.008x1.008 μm².

**Analysis** We identified neurite orientation dispersion-related quantitative histological features to NODDI indices. The local dominant orientation of neurite’s (θ) was estimated via structure tensor analysis of the Palmgren silver stained images, after removal of cell bodies and blood vessels. Afterwards, histologically-derived images were split into patches matching the in-plane MRI voxel size to calculate patch-wise circular variance (CV, for silver staining) and mean myelin staining intensity (MSI, for PLP images). CV quantifies the spread of θ, higher as CV grows from 0 to 1, whereas MSI is proportional to the myelin content. Several regions-of-interest (ROIs) were drawn on the b = 0 MRI image and on corresponding areas of the downsampled histological images: two normal-appearing white matter (NWM1, NWM2); two normal-appearing grey matter (NAGM1, NAGM2); three white-matter lesions (WML1, WML2, WML3); two grey matter lesions (GL1, GL2). Values of the ROIs were shown as boxplots. Lastly, we calculated Pearson’s correlation coefficients of the mean values of the metrics within the nine ROIs, for all combinations of NODDI ODI and NDI and of histologically-derived CV and MSI.

**RESULTS** Figure 1 shows NODDI maps in one MRI slice and corresponding histological indices. Lesions identified by hyperintensity of the b = 0 image corresponded to areas of reduced ODI and NDI. MSI decreased in lesions, highlighting extensive demyelination in the same regions, and CV also appeared reduced, compared to normal-appearing tissue of the same type. Correlation analysis suggested that NODDI ODI and NDI captured different pathology features in MS. NDI showed the strongest association with decrease of myelination as measured by MSI, indicating that NDI may be most sensitive to demyelination and axonal loss. On the other hand, ODI was mainly associated with CV. Both indices independently suggested an increase of orientation coherence in MS lesion tissue, which had previously been reported in vivo. In conclusion, NODDI provides imaging biomarkers that can disentangle specific pathology features in MS by histological observations. NODDI, therefore, has the potential for spinal cord imaging in MS. Future work will clarify the histopathology underlying the observed changes in NODDI indices, such as the reduction of orientation dispersion in MS lesions.

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