Evaluating neuroinflammation imaging as a biomarker of tissue cellularity in the post-mortem human brain

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Background

Neuroinflammation, which has been shown to correlate with the seeding and spreading of amyloid-beta (Aβ) pathology[1], may account for changes in tissue cellularity and cellular morphology in Alzheimer's disease (AD).[2,3] A diffusion MRI model, diffusion basis spectrum imaging (DBSI), has been validated to quantify neuroinflammation-associated cellularity in white matter in multiple sclerosis.[4,5] The current study evaluates next-generation DBSI, neuroinflammation imaging (NII), to quantify cellularity in both gray and white matter in AD.

Methods

Participants

Formalin-fixed left hemispheres of two post-mortem human brains were acquired from the Knight Alzheimer Disease Research Center (Table 1).

Participant	Post-mortem interval (hours)	Fixation time (months)	Sex	Age	Cause of death	Expiration CDR
Case 1	21.5	4	F	91	Cancer	0
Case 2	13.1	2	M	69	Inanition	2

Table 1. Participant details. Post-mortem interval is the time between death and start of fixation. Fixation time is the time between start of fixation and MRI acquisition. CDR is the Clinical Dementia Rating.

MRI acquisition

Diffusion-weighted datasets were acquired using the 3T Siemens Prisma scanner. Scans were performed using a EPI spin-echo sequence 1.5mm isotropic resolution, TE=77ms, TR=14200ms. Diffusion weighting was applied along 55 directions uniformly distributed over a sphere with 51 unique b-values at a maximum value of 3500s/mm^2. Total imaging time was 8 hours for each brain. The neuroinflammation imaging diffusion MRI multi-compartment biophysical model was fitted to the data to obtain estimates of cell fraction and other parameters for each voxel.

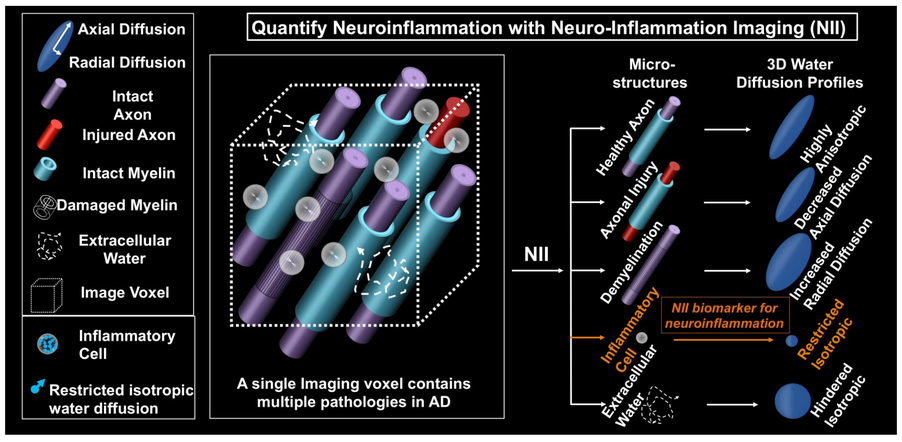

Figure 1. Neuro-inflammation imaging diffusion MRI multi-compartment biophysical model details.

Histology quantification

Histology sections were sampled by a neuropathologist from 15 standard regions in each brain for paraffin embedding and slide preparation.[6,7] Of the 15 standard regions, nine cortical and subcortical regions were available: middle frontal gyrus, superior temporal gyrus, parietal lobe, occipital lobe, hippocampus, caudate and putamen, thalamus, anterior cingulate gyrus, and amygdala. In the nine cortical and subcortical regions, six-micron TDP-43 sections counterstained for hematoxylin were digitized by slide scanner (Hamamatsu Nanozoomer) at 20x magnification (0.46 um). The digital slides were quantified to determine cell fraction using NDPItools, Python, and OpenCV.

MRI and histology comparison

Because MRI acquisition is performed before histology quantification, the diffusion-weighted datasets must be reoriented by a rotation transform such that the angle of the coronal slices matches the angle of the coronal sections taken by the neuropathologist during histology sampling. This is performed in Connectome Workbench and AFNI. Regions of Interest approximating the locations of the nine cortical and subcortical regions taken during histology sampling were drawn using FSLeyes. A linear model was fitted in R, where average cell density in each ROI is set as a predictor of NII cellularity.

References

[1] Venegas C et al. *Nature*. 2017. [2] Hopperton KE et al. *Molecular Psychiatry*. 2017. [3] Heneka MT et al. *Nature Reviews Immunology*. 2014. [4] Wang Y et al. *Brain*. 2011. [5] Wang Y et al. *Brain*. 2015. [6] Cairns N et al. *Neuropathology*. 2015. [7] Franklin E et al. *Alzheimer's and Dementia*. 2015.

Acknowledgements

This work was funded by NIH NIA 1R01AG054567-01A1 (TB and YW), NMSS RG5265 A1 (YW), and BrightFocus A2017330S (YW). We also acknowledge support from the Hope Center Alafi Neuroimaging Lab and a P30 Neuroscience Blueprint Interdisciplinary Center Core award to Washington University (P30 NS057105) as well as the Knight Alzheimer's Disease Research Center (ADRC) grants, P50AG005681, P01AG026276, and P01AG003991 (JCM).

Results

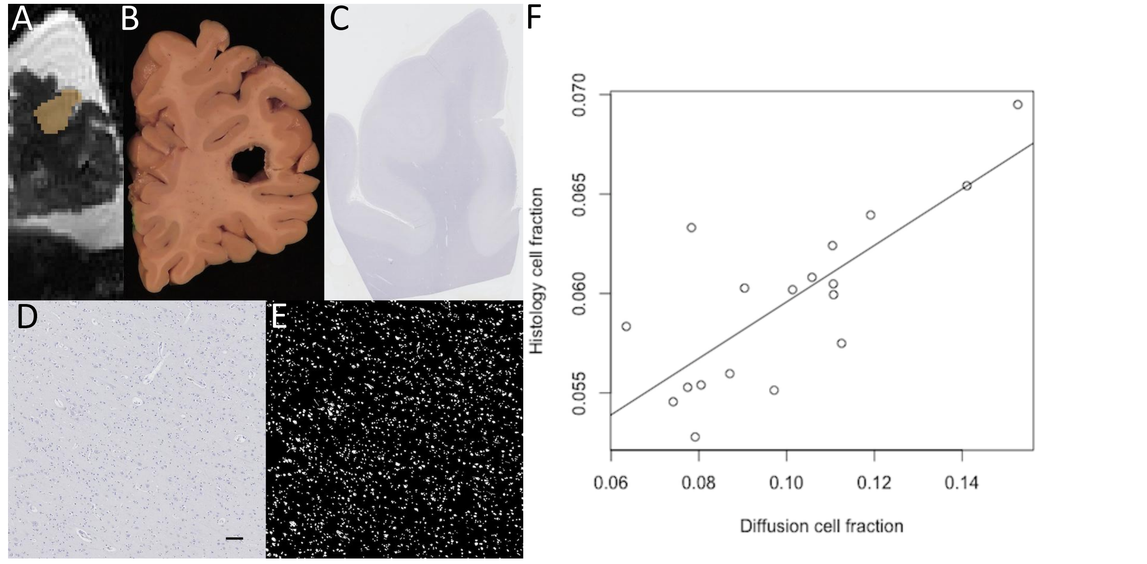

Figure 2. An example workflow to compare diffusion and histology results. (A) a ROI (middle frontal gyrus of Case 1) drawn on a mean diffusivity map in the same space as the restricted ratio map derived from the neuro-inflammation imaging model to define values taken for diffusion cell fraction; (B) photo of coronal slice (roughly equivalent to chosen MRI slice where the middle frontal gyrus was identified) taken of the fixed brain at autopsy; (C) six-micron TDP-43 control slide counterstained for hematoxylin imaged at 20x magnification (0.46 microns); (D) a 1.5 x 1.5 mm tile (scale bar 100 um) derived from the histology section (to approximate the dimensions of the MRI voxel); (E) a binary image labeling cells derived from the image tile; (F) a plot of histology cell fraction estimates (derived from the number of pixels labeled as cells over the number of pixels labeled as tissue, the latter not shown here) versus diffusion cell fraction estimates (derived from NII cellularity estimates from the restricted ratio map) where each data point represents values averaged over every image tile for the respective histology section and over every voxel within the respective ROI drawn on the mean diffusivity map. The relationship between histology cell fraction versus diffusion cell fraction appears to be linear, with an adjusted R-squared value of 0.563 and a p-value of 0.000204, and suggests a statistically significant positive linear correlation between cell density and NII cellularity across ROIs.

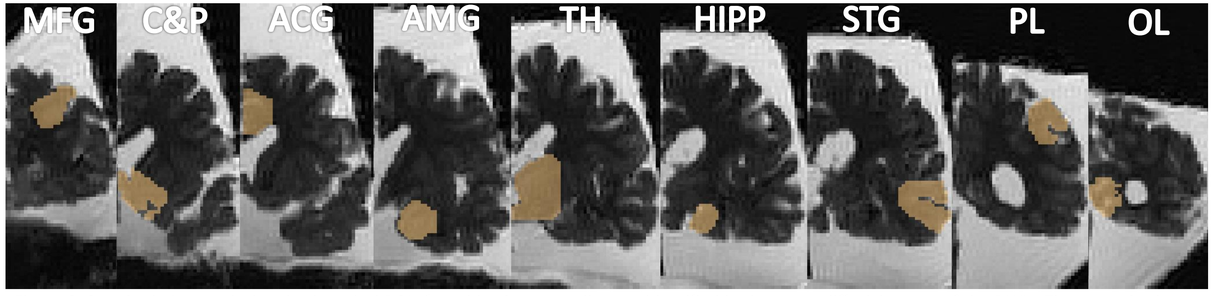

Figure 3. Standard nine cortical and subcortical ROIs from histology sampling are identified in the mean diffusivity map of the DWIs. The example shown here is Case 1 with ROIs from left to right (slices ordered from anterior to posterior): Middle frontal gyrus (MFG), caudate and putamen (C&P), anterior cingulate gyrus (ACG), amygdala (AMG), thalamus (TH), hippocampus (HIPP), superior temporal gyrus (STG), parietal lobe (PL), occipital lobe (OL).

Conclusions

Good agreement between cell density determined from gold standard histology and NII cellularity suggests NII estimates can be used to quantify tissue microstructure. Future work aims to determine whether NII can be used to track tissue cellularity and cellular morphology changes specifically of microglia and astrocytes associated with gliosis to serve as a longitudinal whole brain imaging biomarker for preclinical and symptomatic AD.