Brain Tumor Hypercellularity Detected in Diffusion Restricted Voxels Outside Contrast Enhancement in Six Human Brains Examined Ex-vivo

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AUDIENCE This study will be of interest for clinicians and scientists in the field of brain tumor imaging.

INTRODUCTION It is speculated that the edema surrounding high-grade brain tumors evident as hyperintensity on FLAIR (fluid attenuated inversion recovery) images, likely contains invading tumor cells. Radiation treatment plans therefore usually include the entire FLAIR abnormality. It would be however beneficial to be able to identify regions more specifically in order to target them and spare healthy tissue. This study uses histogram analysis[1] of ADC voxels within FLAIR hyperintensity to determine diffusion restriction cut-offs. We then examine these regions ex-vivo to determine if hypercellularity was in fact present.

METHODS Eight patients with brain tumors, who had enrolled in an ongoing IRB-approved imaging study volunteered to donate their brains upon death. At autopsy, six patients were diagnosed with glioblastoma, WHO grade 4, while the other two patients were diagnosed with grade 2 astrocytomas. The typical imaging sequence acquired prior to death included pre- and post-contrast T1-weighted acquisition, diffusion weighted imaging, and FLAIR. Apparent diffusion coefficient (ADC) maps were created from the diffusion weighted images. For processing, all MR images were co-registered to either a high resolution T1 weighted volume (SPGR) or the FLAIR volume. A mask was drawn manually on the enhancing tumor seen on the post-contrast T1 weighted image volume. This was used to exclude contrast-enhancing tissue from further analysis. *Ex-vivo* samples were taken in regions of suspected pathology and imaged under a microscope at high resolution. Using custom software, the *ex-vivo* samples were co-registered to the imaging datasets[2]. The histological images were down sampled such that the resolution of the histology matched that of the MR imaging (See **Fig.** 1). Image processing software was written to count the number of cells in histological equivalent of each MRI voxel. The cell count in each voxel was normalized to the size of the MR voxels to obtain values in the units of cells per square millimeter. Voxels within a hyperintense FLAIR region of interest (ROI) were considered for this analysis. Voxels were considered to have restricted diffusion if their ADC value was below the intersection found between two Gaussian[3] curves fit to the ADC histogram (**Fig.** 2). Cell density was then compared between regions of restricted diffusion and FLAIR hyperintensity alone using a T-test.

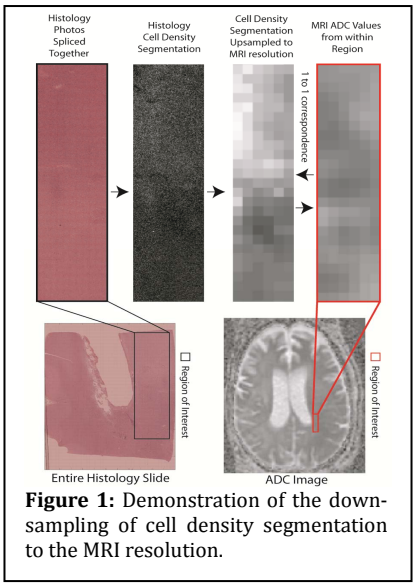

Figure 1: Demonstration of the down-sampling of cell density segmentation to the MRI resolution.

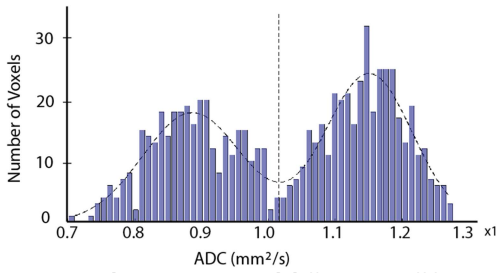

Figure 2: Selecting a restricted diffusion cutoff for an individual patient.

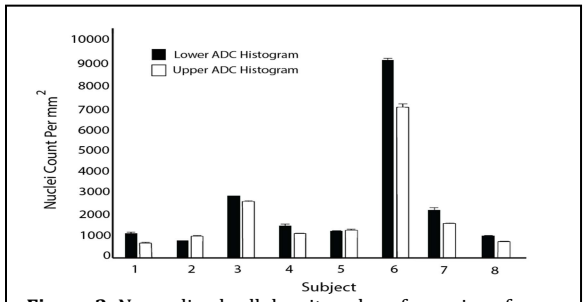

Figure 3: Normalized cell density values for regions from upper and lower Gaussian curves.

RESULTS We found that cellularity was significantly greater in regions of restricted diffusion compared to hyperintense FLAIR voxels alone in the six patients with high-grade glioblastomas ($p<0.0001$). The two patients with lower grade tumors however showed lower cellularity in regions of restricted diffusion ($p<0.001$).

CONCLUSION Regions of restricted diffusion determined from a double Gaussian distribution within abnormal FLAIR regions showed high cell density when sampled histologically in six cases of high-grade glioblastoma. This study demonstrates that regions of restricted diffusion in the presence of FLAIR hyperintensity may present as an imaging biomarker for the identification of invasive cells in high-grade tumors.

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