Precise ex-vivo histological validation of heightened cellularity in regions of dark ADC in three cases of high-grade glioma

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INTRODUCTION It has been generally accepted that the edema surrounding brain tumors, which is apparent as abnormally high signal on FLAIR (fluid attenuated inversion recovery) images, is likely comprised of invading tumor cells. Consequently, radiation treatment plans usually include the entire FLAIR abnormality. If however, there was a way to visualize the location of invading cells within these regions, radiation therapy could be optimized to maximally target these sites while minimizing radiation to normal areas. In an effort to address this issue we have developed a new imaging metric named AFMM (ADC FLAIR MisMatch), which is defined as an area of low ADC within FLAIR abnormality. The aim of this study was to address the hypothesis that areas of AFMM represent areas of increased cellularity by histologic analysis of spatially-correlated tissue acquired and processed ex-vivo.

METHODS Patient Population Three patients with high-grade gliomas, who had enrolled in an ongoing IRB approved imaging study, volunteered to donate their brains upon death. Patient A and B underwent biopsy followed by radiation, chemotherapy and eventually bevacizumab at recurrence. Patient C presented with imaging findings and refused treatment. At autopsy, all three patients were diagnosed with glioblastoma, WHO grade 4. Eight additional patients with meningioma were included for purposes of ADC threshold definition. Imaging Each patient underwent routine clinical imaging at our Institution using a 1.5T GE MRI scanner (GE, Waukesha, WI). The typical imaging sequence consisted of a conventional pre- and post-contrast T1-weighted acquisition. Diffusion weighted images were acquired with a TE/TR: 102.2/8000ms, slice thickness of 5mm skip 1.5mm, matrix of 256x256, FOV 240mm, including two b-values b=0 and b=1000s/mm². ADC maps were then calculated from these images. Axial FLAIR imaging was also gathered with an inversion time of 2,200ms, TE/TR: 125.2/10,000ms, slice thickness of 5mm skip 1.5mm, flip angle of 90deg and matrix of 512x512, voxel size 0.43x0.43mm. For patients A-C imaging was acquired at 17, 12 and 4 days prior to death respectively. ADC Threshold Definition FLAIR abnormalities were manually segmented from the eight patients with meningioma, excluding voxels of T1 contrast enhancement. ADC values were then measured within and combined across patients. A 95% CI cutoff of the distribution was then calculated for application to the three GBM cases. ADC FLAIR mismatch (AFMM) was then defined in the three patients as regions within FLAIR abnormality with ADC values below said threshold. Histology Processing Brain only autopsies were performed on each GBM patient. Brains were fixed in formalin and then sliced axially giving careful attention to the sagittal and coronal orientation to best slice in the same axial plane as the most recent MRI. Samples were taken from each brain on multiple slices totaling 10 for patient A, 8 for B, and 6 for patient C. Each patient’s histological slides were H&E stained to reveal cell nuclei. Each slide was photographed at 10x across the entire sample using a motorized microscope stage and Nikon Instruments software (Melville, NY). Each photo was processed using custom software written in MatLab (Mathworks, Natick, MA). The process began with a white background correction followed by a contrast optimization of the red component in each RGB jpeg. Static thresholds were then applied to each photo for segmentation of cell nuclei (darkest black), cytoplasm (medium gray), and extracellular fluid (white). The nuclei in each photo were counted. Precise Histology to MRI Correlation Additional custom software was developed to precisely relate histology to the MRI (HistToMRI Toolbox) using MatLab (Mathworks, Natick, MA). The slides from each sample were co-registered manually to the MRI slice and region best representing the histology using photographs taken at the time of histology sampling. The high-resolution T1w SPGR images acquired was used. For patients A and B all other imaging was co-registered and resampled to this image. Patient C lacked an SPGR, so imaging was co-registered to the FLAIR. The toolbox was then used to define regions of interest within each histology slide devoid of histological artifacts. Histology cell counts were then determined within each voxel sized histological equivalent for 1-to-1 comparison (method shown in Figure 1). Histological segmentation values from each voxel, along with the MRI values within each voxel were then extracted and combined across all samples for each patient. Statistical Comparison Nuclei counts from each patient were compared between voxels of AFMM and FLAIR alone using unpaired T-tests. Results An ADC threshold of 0.0009295mm²/sec was found as the lower 95% confidence interval in the 8 cases of meningioma induced edema, and was used to define AFMM. Regions of AFMM showed heightened cellularity in all three cases compared to regions of abnormal FLAIR alone (p=0.0001) (Figures 2 and 3).

RESULTS Regions of ADC with values less than 0.0009295mm²/sec within abnormal FLAIR demonstrated hypercellularity when sampled histologically ex-vivo in three cases of invasive glioblastoma. This study shows that regions of darker ADC in both treated and untreated GBM contain hypercellularity. ACKNOWLEDGEMENTS Special thanks to the three patients and their families for participating in this study. Funding: NIH/NCI R01 CA082500 and Advancing a Healthier Wisconsin, MCW Translational Brain Tumor Research Program. REFERENCES 1. Stupp, R., et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352, 987-996 (2005).