Reduced diffusion and perfusion in bevacizumab-induced diffusion restricted necrosis versus brain tumor hypercellularity

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INTRODUCTION Recent conflicting reports suggest that bevacizumab induced restricted diffusion and perfusion in regions suspicious for brain tumor infiltration, are both the result of neoplastic hypercellularity and necrosis with restricted diffusion. This study compares the ADC and relative cerebral blood volume (rCBV) MRI values within regions of restricted diffusion containing both hypercellularity and neighboring necrosis in a patient ex-vivo. METHODS Patient Population One patient with infiltrative high-grade glioma, underwent a biopsy followed by radiation, chemotherapy and eventually bevacizumab at recurrence. At autopsy, the patient was diagnosed with glioblastoma, WHO grade 4. Imaging Twelve days prior to death, the patient underwent MR imaging at our institution using a 1.5T GE MRI scanner (GE, Waukesha, WI). The imaging sequence consisted of a conventional pre- and post-contrast T1-weighted acquisition. Perfusion weighted images were gathered including two b-values b=0 and b=1000s/mm2. ADC maps were then calculated from these images. Prior to DSC, a 0.05 to 0.1 mmol/kg (pre-load) dose of gadolinium (Gd) contrast agent was administered for clinical post-contrast T1-weighted imaging. Single shot gradient-echo (GE) echo-planar imaging was used to collect images during a second 0.1 mmol/kg bolus injection of Gd contrast. An rCBV map was calculated as previously published and standardized. Histology Processing A brain only autopsy was performed on the patient. The brain was 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 (Figure 1). Samples were taken from the brain on multiple slices. Each sample was H&E stained to reveal cell nuclei and processed with VEGF immunohistochemistry to determine regions expressing VEGF. 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 individually and then stitched together with all other photos using custom software written in Matlab (Mathworks, Natick, MA). The process began with a white background correction followed by a contrast optimization and segmentation of cell nuclei. One sample contained both radiation necrosis and hypercellularity within a region of decreased ADC on the MRI. For the purpose of this study, this sample was extensively studied.

RESULTS ADC and rCBV values were compared between regions of necrosis and adjacent hypercellularity. An additional ROI was generated in a region of normal white matter (WM) far from the tumor for extraction of MRI values for comparison. Statistical Comparisons ADC and rCBV values were compared between the three tissue segments: necrosis, hypercellularity, and normal WM using a one-way ANOVA. P-values < 0.0001 were considered significant. RESULTS ADC within diffusion restricted necrosis was higher than ADC within hypercellularity and normal WM. rCBV was highest in hypercellularity, but still higher in the necrosis than normal WM (Figure 2). Restricted diffusion necrosis also had high expression of VEGF (Figure 3).