

Ex-vivo infiltrative brain tumor histologically correlates with decreased ADC and increased rCBV as measured with functional diffusion and perfusion maps

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INTRODUCTION Restricted diffusion, represented as a decrease in signal intensity on MRI derived apparent diffusion coefficient (ADC) maps, has been shown to correlate with increased cellularity in brain tumors. As a result, functional diffusion maps (fDMs) have been developed to aid in identification of temporal changes. Relative cerebral blood volume (rCBV) maps contribute vascular information about these tumors as well. Logically then temporal changes in rCBV can be quantified using functional perfusion maps (fPMs). The aim of this study was to examine areas of overlap among the fDMs (decreasing ADC only) and fPMs (increasing rCBV only) to histologically gathered ex-vivo tumor tissue. **METHODS** One patient with high-grade glioma was recruited for this study. Patient A underwent biopsy followed by radiation, chemotherapy⁴ and eventually bevacizumab at recurrence. **Imaging** The patient underwent routine clinical imaging at our institution using a 1.5T GE MRI scanner (GE, Waukesha, WI) on 20 separate occasions throughout treatment. The typical imaging sequence consisted of a conventional pre and post-contrast T1-weighted acquisition, along with diffusion weighted imaging gathered 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 $b=1000\text{s/mm}^2$. ADC maps were then calculated from these images. 15 of the 20 scanning sessions also included dynamic susceptibility contrast (DSC) perfusion imaging. 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 imaging²⁻⁴. 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 injected at a rate of 3 mL/s. Typically, 13 slices of DSC data were acquired with the following parameters: 5mm, skip 1.5mm slice prescription, fat suppression, TE: 30ms, TR: 1.0-1.65s, field of view: 220 x 220mm, matrix size: 128 x 128, and voxel size: 1.72x1.72x5mm. Axial FLAIR imaging was also gathered. **Functional Diffusion Maps** FLAIR abnormalities were manually segmented from each of 20 imaging studies and voxels were included if heightened FLAIR had been present at any time point. The final scan was 17 days prior to death. Voxels were considered “hypercellular” if ADC temporally decreased by more than 0.00044¹ when compared to the baseline imaging study. **Functional Perfusion Maps** The standardized^{5,6} rCBV maps from 15 different time points were co-registered to the baseline SPGR image, and percent change differences of greater than 100% within the same FLAIR mask were considered hyper-vascular. The overlap of the fDM and fPM at the final imaging study was then calculated as voxels with decreasing ADC and increasing rCBV. A region of interest was defined by a cluster sampled for histology. **Histology Processing** A brain only autopsy was performed. The brain was fixed in formalin and then sliced axially giving careful attention to the sagittal and coronal orientation to best slice it in the same axial plane as the most recent MRI. Samples were taken from 8 different regions of interest. This analysis concentrated on 4 of them. Each sample was H&E stained to reveal cell nuclei, and

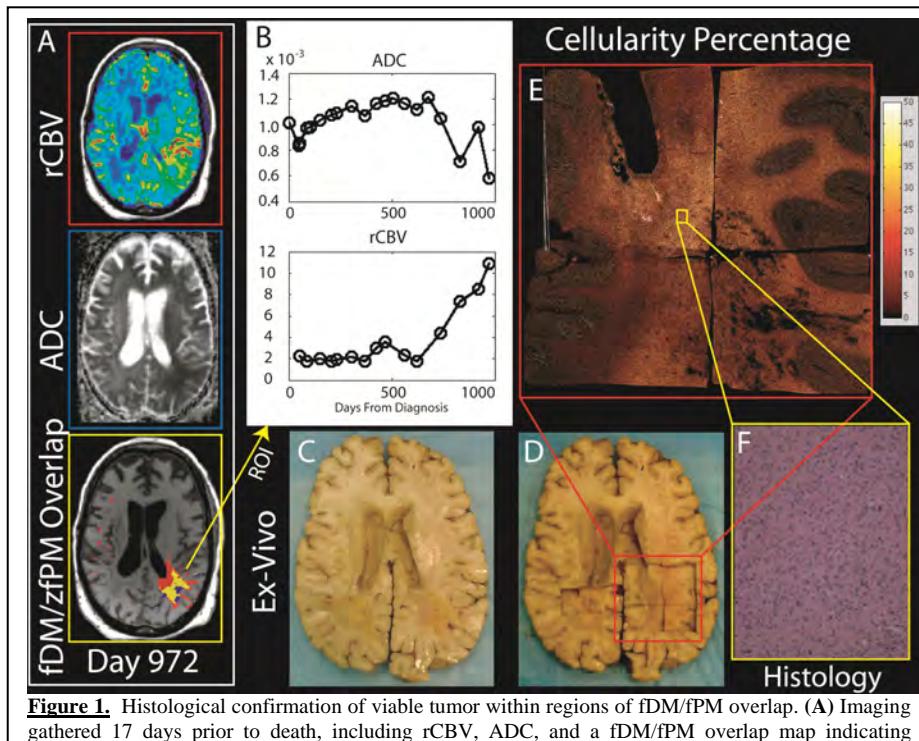


Figure 1. Histological confirmation of viable tumor within regions of fDM/fPM overlap. (A) Imaging gathered 17 days prior to death, including rCBV, ADC, and a fDM/fPM overlap map indicating regions of decreased ADC (blue), increased rCBV (red) and overlap (yellow). (B) Time series of ADC and rCBV within region of overlap at the final imaging study. (C) Ex-vivo slice aligned to region highlighted in imaging. (D) Sampled regions within ex-vivo slice. (E) Histological map of cellularity as defined by a percentage of the represented pixel occupied by nuclei. (F) Example of an H&E stained highly cellular region as seen on histology.

slides were photographed at 10x magnification across the entire sample using a motorized microscope stage and Nikon Instruments software (Melville, NY). Each photo was segmented 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 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). Cellularity was then visualized by plotting the percentage alone across each sample (Figure 1E). **RESULTS** The patient's final imaging study 17 days prior to death showed heightened rCBV and darkened ADC within a region which overlapped on fDMs and fPMs. Over time the region showed progressively decreasing ADC and increasing rCBV (Figure 1B). The region was sampled histologically and dense tumor cellularity was found (Figures 1E and 1F). **DISCUSSION** Regions of fDM/fPM overlap showed dense tumor infiltration in one case ex-vivo. This overlap biomarker shows promise for detecting regions of aggressive infiltrating tumor.

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