Precise correlation of relative cerebral blood volume and von Willebrand factor stained histology using the HistToMRI toolbox

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INTRODUCTION Correlation of in vivo brain MRI with ex vivo tissue analysis is important for the characterization of brain tumors, as MR biomarkers of tumor infiltration need histological validation prior to adoption. We have developed a technique to precisely quantify and compare histology to MRI on a voxel-wise basis. We demonstrate its utility in a U87 brain tumor model, where we compare relative cerebral blood volume (rCBV) as measured with dynamic susceptibility contrast (DSC) MRI to ex vivo von Willebrand factor (vWF) expression.

METHODS *Animal Subjects* The care of the animals before and during the experimental procedures was conducted in accordance with the policies of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Our institutional animal care and use committee approved all protocols. Five rats were injected with $2x10^5$ U87MG cells (adult glioblastoma, American Type Culture Collection, Manassas, Virginia) in the right frontal lobe at a depth of 3 mm relative to the dural surface[1].

Imaging At 16 days after tumor cell inoculation, MRI studies were performed on a 9.4T Bruker AVANCE scanner. The rats were anesthetized with 1.5% isoflurane and immobilized with a fiberglass bite-bar. A T1-weighted spin-echo image was acquired (TE/TR = 12.6 ms/1500 ms; matrix = 256 × 256; FOV = 3.5 cm; slice 2 mm). Five axial (rat coronal) imaging slices were collected. A loading dose of 0.1 mmol/kg Gadodiamide (Gd) was administered 10 min before the DSC (dynamic susceptibility contrast) scan, in order to diminish confounding contrast agent leakage effects on the determination of rCBV[2-4]. A GRE-EPI (gradient-echo echo planar imaging) sequence (TE = 18.8 ms, TR = 1000 ms, 5 NEX, $\alpha = 52.7^o$) was used to acquire the DSC data. Specifically, GRE-EPI images were collected continuously for a total of 2 min, for 1 min before, and then during a bolus injection of a 0.1 mmol/kg Gd contrast agent. Finally, a T1-weighted spin-echo image was acquired (TE/TR = 12.6 ms/1500 ms; matrix = 256 × 256; FOV = 3.5 cm; slice 2 mm) to delineate enhancing tumor[1].

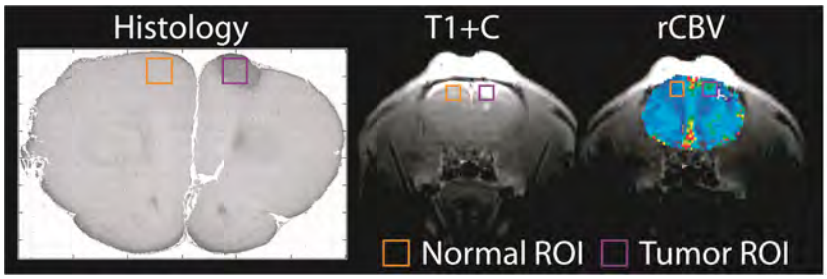

Figure 1. Demonstration of the ROI generation. Two ROIs were created for each rat with a tumor, one within the tumor, and another in contralateral normal tissue, each 10x10 voxels

Histology Processing Each rat's histological samples were stained with vWF to highlight endothelial cells. 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 combined with other photos from the same sample to create one composite image using custom software written in Matlab (Mathworks, Natick, MA). The process began with a white background correction. The RBG photos were then reduced to the red component alone giving the best black and white contrast among the tissue types for the vWF stained slides. In order to best separate vWF stain, unstained cytoplasm and extracellular fluid the images were contrast optimized by manipulating the thresholds for each segment. Static intensity thresholds were applied to each photo for segmentation of vWF (darkest black), cytoplasm (medium gray), and extracellular fluid (white). A representative vWF segmentation of an entire slice is shown in Figure 1 (Left) where darker values indicate regions of heightened vWF. *Histology to MRI Correlation* We developed additional custom software (HistToMRI toolbox) to precisely relate histology to the MRI acquired prior to tissue excision. The software is a Matlab (Mathworks, Natick, MA) toolbox. Each digital stain image was matched with its corresponding MRI and rCBV scan. The HistToMRI toolbox was used to draw regions of interest (ROIs) matched with a section of stained tissue corresponding to the MRI. Two ROIs were drawn in each rat, one in each hemisphere (Figure 1). The HistToMRI toolbox then calculated the contribution of each tissue segmentation as a percentage within the space equivalent to each MR voxel. This created a one to one direct correspondence. Histological segmentation values from each voxel, along with the MRI values within each voxel were then extracted and combined across all samples for comparison. Values from the tumor and contra-lateral normal brain were then compared statistically using an unpaired student's T-test. A Pearson correlation was also performed on the combined rCBV and vWF data.

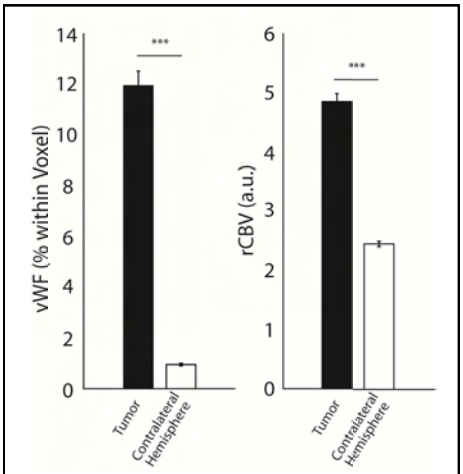

Figure 2. Bar charts showing heightened vWF **(Left)** and rCBV **(Right)** within tumor compared to normal (*** Indicates p<0.00001)

RESULTS Figure 2 shows the results from the mean comparison of the vWF and rCBV in the three groups. There is a significant difference in vWF concentration between tumor (N=5, mean=11.58%) and contra-lateral normal tissue (N=5, 0.89%) p<0.00001 (Figure 2 Left). Similar results were found in the rCBV comparison (Figure 2, Right). Pearson correlation showed significance when comparing rCBV and vWF (p<0.00001). **DISCUSSION** This study shows the utility of the HistToMRI toolbox in comparing histology and MRI precisely. We find a correlation of vWF and rCBV in U87 rat model of brain cancer. The HistToMRI toolbox processing method has the potential to improve the characterization of brain tumors and aid in the evaluation and Ivalidation of MRI biomarkers.

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