Validation of functional diffusion maps (fDMs) as a biomarker for human glioma cellularity

B. M. Ellingson[1,2], M. G. Malkin[2,3], S. D. Rand[1,2], J. M. Connelly[2,3], C. Quincey[3], P. S. LaViolette[2,4], D. P. Bedakar[1,2], and K. M. Schmainda[1,2]

[1]Dept. of Radiology, Medical College of Wisconsin, Milwaukee, WI, United States, [2]Translational Brain Tumor Program, Medical College of Wisconsin, Milwaukee, WI, United States, [3]Dept. of Neurology and Neurosurgery, Medical College of Wisconsin, Milwaukee, WI, United States, [4]Dept. of Biophysics, Medical College of Wisconsin, Milwaukee, WI, United States

Introduction

In neoplasms, a decrease in apparent diffusion coefficient (ADC) is believed to reflect an increase in tumor cellularity[1-6], and an increased ADC is believed to reflect necrosis or decreases in cellularity as a result of successful treatment[2,7]. Functional diffusion maps (fDMs) were developed to exploit these principles on a voxel-by-voxel basis, and may be a useful tool for predicting the effect of chemotherapy and radiotherapy[8-11]. Despite positive initial results and clinical enthusiasm, a comprehensive validation of the assumptions made when performing human fDM analysis has not been performed. Perhaps most importantly, the current threshold used for human fDMs was originally chosen using a small sample size over a short follow-up interval, chosen without defining the biological detection sensitivity (minimum change in cell density required for voxel classification), and chosen without examining the impact on clinical sensitivity and specificity to disease progression. The purpose of the current study was to comprehensively validate fDMs as a biomarker for brain tumor cellularity and introduce the *rate of change* in hypercellular volume as a new fDM metric for detecting disease progression.

Methods

Sixty-nine patients with gliomas were enrolled in the current retrospective study approved by the Institutional Review Board at our institution. Clinical MRI scans included 3D-SPGR anatomical, pre- and post-contrast T1-weighted, and FLAIR sequences on a 1.5-T MR scanner (GE Medical Systems, Waukesha, WI). ADC was calculated from diffusion weighted images acquired with b=0 and b=1,000 s/mm^2, using all gradients applied equally (isotropic). All images for each patient were registered to their own pre-treatment baseline SPGR anatomical images using a mutual information algorithm and a 12-degree of freedom transformation using FSL (FMRIB, Oxford, UK). After registration, voxelwise subtraction was performed between ADC maps acquired at subsequent time points and the baseline ADC maps to create ΔADC images. Individual voxels were stratified into three categories: voxels where ADC increased beyond a ΔADC threshold ("hypocellular", red), voxels where ADC decreased beyond a ΔADC threshold ("hypercellular", blue), and voxels with no change in ADC beyond the chosen ΔADC threshold (green).

Hypothesis 1: Glioma cell density is inversely proportional to ADC measurement Seventeen patients with a variety of glioma grades (WHO grades II-IV) who underwent closed diagnostic stereotactic biopsy via StealthSystem™ surgical navigation were retrospectively examined. ADC measurements corresponding to the precise spatial regions of the biopsy samples were extracted from the pre-operative ADC maps, after registration to post-operative anatomical MR images. Cell density measurements were taken from biopsy specimens using MetaMorph™image analysis software. Linear regression was performed between the mean ADC measurement spatially matched to the biopsy site and the mean cell density obtained from the biopsy specimen for each patient.

Hypothesis 2: ADC variability across scan days must be determined to properly set ΔADC thresholds The 95% C.I.s for different mixtures of normal-appearing white matter (NAWM), gray matter (NAGM), and cerebrospinal fluid (CSF) were calculated for 69 patients with post-baseline times ranging from 1 week to 1 year. If the data had equal variance over time, according to Bartlett's test for equal variance, voxels from all patients and all time points were pooled to provide an overall distribution for calculation of ΔADC C.I.s for each tissue type examined: NAWM, NAGM, NAWM+NAGM, NAWM+NAGM+CSF (Fig.1B).

Hypothesis 3: fDMs created with different ΔADC thresholds reflect different sensitivity and specificity to brain tumor progression Five randomly chosen patients with recurrent glioblastoma having statistically similar total abnormal FLAIR volumes were used to test whether the 95% C.I.s for ΔADC (*Hypothesis 2*) produced physically different fDMs to those produced using the current ΔADC threshold of 0.55x10^{-3} mm^2/s. In addition, we closely examined 33 patients who eventually showed disease progression. Each session for each patient was categorized as either stable disease (SD, no change or improvement in neurological status *and* radiographic presentation) or progressing disease (PD, defined as having either neurological decline *or* radiographic progression). Receiver-operator characteristic (ROC) analysis was used to determine the sensitivity and specificity of the *rate of change* in hypercellular volume (in uL/day) for different ΔADC thresholds to progressive disease.

Results

Results support the hypothesis that tumor cell density is inversely correlated with ADC measurements in human gliomas (*Fig. 1A, r^2=0.7933, P<0.0001*) with a biological sensitivity of 2.14x10^{-5} [mm^2/s]/[nuclei/high-power-field(HPF)]. Results suggest no significant statistical differences in ΔADC variability over time (*Bartlett's test, P>0.05*); however, ΔADC variability was dependent on the tissue type analyzed (Fig.1C). The 95% C.I. for NAWM=0.25x10^{-3}mm^2/s, NAGM=0.31x10^{-3}mm^2/s, NAWM+NAGM=0.40x10^{-3}mm^2/s, and NAWM+NAGM+CSF=0.75x10^{-3}mm^2/s. The volumes of hyper- and hypocellularity calculated using the ΔADC threshold defined as the 95% C.I. for NAWM was significantly different than the 95% C.I. for NAWM+NAGM+CSF, otherwise all other thresholds were statistically similar. ROC curves classifying progressive disease from stable disease using the *rate of change* in hypercellular volume suggested all ΔADC thresholds were significantly better than chance (Fig. 1D, *Area Under Curve(AUC), P<0.001*). A minimum increase in cell density of 12 nuclei/HPF are required when using the 95% C.I. for NAWM, 15 nuclei/HPF are required when using the 95% C.I. for NAGM, 19 nuclei/HPF are required when using the 95% C.I. for NAWM+NAGM, 35 nuclei/HPF are required when using the 95% C.I. for NAWM+NAGM+CSF, and 26 nuclei/HPF are required when the current threshold is used for fDM classification.

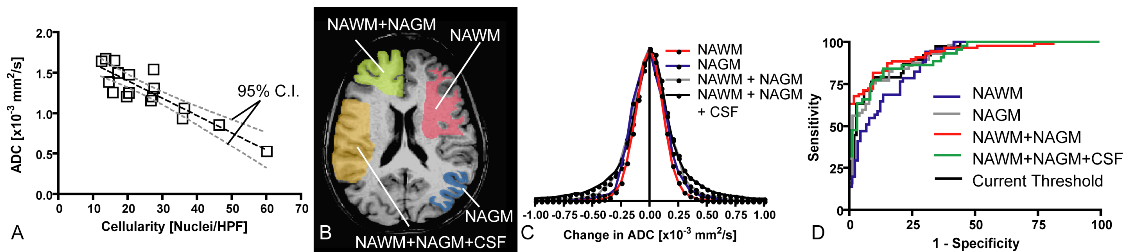

Figure 1

Discussion

This was the first study to comprehensively examine, validate, clarify, and calibrate the biological and clinical implications of the ΔADC thresholds used for human fDM analysis. The current study suggests fDMs be constructed using a ΔADC threshold of 0.40x10^{-3} mm^2/s based on better clinical performance on ROC analysis compared to the other thresholds. This threshold physically corresponds to the 95% C.I. for NAWM+NAGM taken from a large patient population (n=69) and a biological sensitivity of approximately 19 nuclei/HPF, or approximately half the sensitivity of a trained histopathologist[12,13]. Using this threshold, a rate of change in hypercellularity of more than 11.2 uL/day results in a 87% sensitivity and 85% specificity to PD during standard treatment, suggesting the *rate of change* in hypercellular volume is a sensitive, novel fDM metric that can be used as a tool for patient monitoring. In summary, the current study provides comprehensive evidence validating fDMs as a biomarker for brain tumor cellularity. ***Acknowledgements*** NIH/NCI R21-CA109820; MCW Advancing Healthier Wisconsin/Translational Brain Tumor Program; MCW Cancer Center Fellowship ***References*** [1]Sugahara T, JMRI, 1999. [2]Lyng H, MRM, 2000. [3]Chenevert TL, J Natl Cancer Inst, 2000. [4]Hayashida Y, AJNR, 2006. [5]Manenti G, Radiol Med, 2008. [6]Gauvain KM, AJR, 2001. [7]Chenevert TL, Clin Cancer Res, 1997. [8]Moffat BA, Proc Natl Acad Sci, 2005. [9]Moffat BA, Neoplasia, 2006. [10]Hamstra DA, J Clin Oncol, 2008. [11]Hamstra DA, Proc Nat Acad Sci, 2005. [12]Swanson KR, Cell Prolif, 2000. [13]Swanson KR, J Neurol Sci, 2003.