Title

CELLULAR INVASION, MOTILITY, AND PROLIFERATION LEVEL ESTIMATES (CIMPLE MAPS): APPLICATION TO TEMOZOLOMIDE- AND BEVACIZUMAB-TREATED PATIENTS

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CLINICAL STUDY - PATIENT STUDY

Cell invasion, motility, and proliferation level estimate (CIMPLE) maps derived from serial diffusion MR images in recurrent glioblastoma treated with bevacizumab

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Abstract Microscopic invasion of tumor cells and undetected tumor proliferation is the primary reason for a dismal prognosis in glioblastoma patients. Identification and quantification of spatially localized brain regions undergoing high rates of cell migration and proliferation is critical for improving patient survival; however, there are currently no non-invasive imaging biomarkers for estimating proliferation and migration rates of human gliomas in vivo. To accomplish this, we developed CIMPLE (cell invasion, motility, and proliferation level estimates) image maps using serial diffusion MRI scans and a solution to a glioma growth model equation. CIMPLE represent a novel method of quantifying the level of aggressive malignant behavior. In the current pilot study, we demonstrate the utility of CIMPLE maps to predict progression free survival (PFS) and overall survival (OS) in 26 recurrent glioblastoma patients treated with bevacizumab from our Neuro-Oncology database. Voxel-wise estimates of cell proliferation rate predicted spatial regions of contrast enhancement in 35% of patients. A linear correlation was found between the mean proliferation rate and progression-free survival (PFS; $P < 0.0001$) as well as overall survival (OS; $P = 0.0093$). Similarly, the mean proliferation rate was able to stratify patients with early and late PFS as well as OS.

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Introduction

Glioblastoma multiforme (GBM) is a malignant brain tumor with a very poor patient prognosis. Currently, the standard of care consists of surgical resection, concurrent radiotherapy and chemotherapy, followed by adjuvant chemotherapy [1]. Despite an aggressive approach to tumor control, all GBMs eventually progress. This eventual tumor progression is thought to be due to microscopic invasion of tumor cells undetected by standard imaging techniques [2]. Thus, there is a significant need for non-invasive imaging biomarkers that are sensitive to the presence and activity of invading tumor cells.

Recently, a new magnetic resonance imaging biomarker was developed termed cell invasion, motility, and proliferation level estimates (CIMPLE maps) [3, 4]. The approach is to use serial diffusion-weighted MRI (DWI) data in the same patient, then solve a partial differential equation describing tumor growth dynamics, assuming the apparent diffusion coefficient (ADC) is a direct reflection of tumor cell density [5]. This partial differential equation, referred to generically as the glioma growth or diffusion–reaction equation, has been used in a number of simulation studies meant to describe the macroscopic growth and invasion of gliomas under a variety of treatment conditions [6–8]. These simulations typically ascribe singular values for "cell diffusion rate" and "cell proliferation rate" to describe growth and invasion of the tumor as a whole. Alternatively, CIMPLE maps utilize serial DWIs to solve the glioma growth equation on a voxel-wise basis for microscopic growth and invasion, creating image maps of

"cell diffusion rate" and "cell proliferation rate." Because of the known spatial heterogeneity in human GBM, image maps of cell proliferation and invasion calculated from CIMPLE maps may be valuable for isolating the most malignant portions of the tumor, or identifying the portions of the tumor not responding to treatment. Preliminary studies have shown a strong linear correlation between CIMPLE map estimates of proliferation rate and MR spectroscopy estimates of choline-to-N-Acetylaspartate (Cho/NAA) ratio obtained within similar image voxels, as well as significant differences in cell migration and proliferation rates between low and high grade gliomas [4], suggesting that CIMPLE maps may provide a unique estimate of tumor growth dynamics in vivo.

Despite promising initial findings, the prognostic value of CIMPLE maps has not been explored. Additionally, CIMPLE maps are sensitive to changes in tumor dynamics that are independent of changes in vascular permeability (i.e. contrast-enhancement), therefore CIMPLE maps may be able to identify patients who do not respond to anti-angiogenic agents earlier than conventional tumor assessments. In the current pilot study, we explore whether CIMPLE maps are predictive of progression free survival (PFS) and overall survival (OS) in recurrent GBM patients treated with bevacizumab.

Methods

Patients

All patients gave informed written consent to have their data in a database according to the guidelines approved by the Institutional Review Board at our institution. Data acquisition and storage was performed in compliance with all applicable Health Insurance Portability and Accountability Act (HIPAA) regulations and the principles expressed in the Declaration of Helsinki. A total of 26 patients were selected retrospectively from our Neuro-Oncology database spanning from 8/1/2006 through 8/5/2010. Inclusion criteria included: (1) pathology confirmed GBM with recurrence based on MRI, clinical data, and/or histology, (2) radiation and temozolomide at initial diagnosis, (3) treatment with bevacizumab at least 3 months post-radiotherapy to reduce the probability of pseudoprogression and treatment-induced necrosis, and (4) a minimum of 3 follow-up MRI scans after bevacizumab treatment initiation not spanning more than 8 months, all with good quality diffusion MRI data. No tumor resection surgery was performed between the tumor recurrence and initiation of bevacizumab. It is important to note that pre-treatment (i.e. pre-bevacizumab) ADC maps were not used in this analysis due to known alterations in ADC when comparing pre- and post-treatment ADC maps. Instead, the first three ADC maps acquired after initiation of bevacizumab treatment were used for analysis. It is also important to note that our inclusion criteria was biased toward long-term survivors, since patients needed a minimum of three scans after initiation of treatment. The average time period over which CIMPLE maps were constructed with respect to initiation of bevacizumab treatment was 124 ± 6.2 days standard error of the mean (SEM) (median interval = 126.5 days, maximum interval = 239 days). All patients were regularly treated every 2 weeks per cycle with bevacizumab (Avastin, Genentech, South San Francisco, CA; 5 or 10 mg/kg body weight). A total of 20 patients were on steroids at the time of imaging (dose ranges 0.125–16 mg dexamethasone), and 6 patients were not on steroids. All patients were treated with radiation therapy (typically 6000 cGy) and biospsy or tumor resection at time of initial tumor presentation. At the time of last assessment (August, 2010) all 26 patients had progressed and all but one patient had deceased. A summary of patient characteristics can be found in Table 1.

Magnetic resonance imaging

Data was collected on a 1.5T MR system (General Electric Medical Systems, Waukesha, WI) using pulse sequences supplied by the scanner manufacturer. Standard anatomical MRI sequences included axial T1 weighted (TE/TR = 15 ms/400 ms, slice thickness = 5 mm with 1 mm inter-slice distance, NEX = 2, matrix size = 256 9 256, and

Table 1 Patient characteristics

Total patients	26
Histology	
de novo GBM	23
Secondary GBM	3
Age	
Mean (years)	60
Range (years)	26–81
First surgery	
Gross-total resection	12
Sub-total resection	11
Biopsy	3
Cycles of adjuvant temozolomide	
Mean	7.3
Range	1–24
Recurrences before bevacizumab	
First recurrence (# Patients)	23
Second recurrence (#Patients)	2
Third or more recurrences (#Patients)	1
KPS at start of bevacizumab	
Median	70
Range	50–90

FOV = 24 cm), T2 weighted FSE (TE/TR = 126–130 ms/4000 ms, slice thickness = 5 mm with 1 mm interslice distance, NEX = 2, matrix size = 256 9 256, and FOV = 24 cm), and fluid-attenuated inversion recovery (FLAIR) images (TI = 2200 ms, TE/TR = 120 ms/4000 ms, slice thickness = 5 mm with 1 mm interslice distance, NEX = 2, matrix size = 256 9 256, and FOV = 24 cm). DWIs were collected with TE/TR = 102.2 ms/8000 ms, NEX = 1, slice thickness = 5 mm with 1 mm interslice distance, matrix size = 128 9 128 (reconstructed images were zero-padded and interpolated to 256 9 256), and a FOV = 24 cm using a twice-refocused SE-EPI preparation [9]. ADC images were calculated from acquired DWIs with b = 1,000 s/mm^2 and b = 0 s/mm^2 images. Additionally, gadopentetate dimeglumine enhanced (Magnevist; Berlex, Wayne, NJ; 0.1 mmol/kg) axial and coronal T1 weighted images (coronal: TE/TR = 15 ms/400 ms, slice thickness 3 mm with 1 mm interslice distance, NEX = 2, a matrix size of 256 9 256, and FOV = 24 cm) were acquired immediately after contrast injection.

Definition of disease progression

Disease progression was defined by both the standard Macdonald criteria [10], indicated by a 25% increase in enhancing tumor, as well as a modified criteria that includes progression of a non-enhancing tumor evident by an increased mass effect and/or architectural distortion such as blurring of the gray–white interface [11].

CIMPLE mapping

As outlined in a previous publication [4] and in Appendix, an expression for cell diffusion rate, D, and proliferation rate, q, can be described for each image voxel by using the Methods of Characteristics [12] applied to solve the glioma growth equation applied to ADC of water molecules. Specifically, using three ADC maps (water diffusion) collected on sequential days, the proliferation rate, q, and cell motility (cell diffusion) D, can be directly estimated.

Image registration

All images for each patient were independently registered to a high-resolution (1.0 mm isotropic), T1-weighted brain atlas (MNI152; Montreal Neurological Institute) using a mutual information algorithm and a 12-degree of freedom transformation using FSL (FMRIB, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl/). Fine registration (1–2 degrees and 1–2 voxels) was then performed using a Fourier transform-based, 6-degree of freedom, rigid body registration algorithm [13] followed by visual inspection to ensure adequate alignment.

Implementation and analysis of CIMPLE maps

The creation of CIMPLE maps was incorporated into an AFNI pipeline (AFNI, Analysis of Functional NeuroImages; http://afni.nimh.nih.gov/afni) using a combination of bash and AFNI calculation commands. Nearest neighbor interpolation was implemented in AFNI and used to estimate the spatial gradients of ADC. Resulting cell diffusion coefficient maps, D, and proliferation rate maps, q, were smoothed using a 3 9 3 median filter to eliminate erroneous spikes in the image maps.

The volume of T2 weighted signal abnormality on pre- and post-treatment T2 weighted and/or FLAIR images, along with pre- and post-treatment T1-weighted contrast enhancing volumes were defined using a semi-automated ROI region growing technique consisting of (1) manually defining the relative region of tumor occurrence, (2) thresholding either FLAIR or post-contrast images within these regions using an empirical threshold combined with a region-growing algorithm, and then (3) manually editing the resulting masks to exclude any obious radiation-induced changes or leukoaraiosis. For CIMPLE maps, regions of interest (ROIs) were created for the entire T2 weighted signal abnormality on pre-treatment T2 weighted and/or FLAIR images. Additionally, The final quantitative maps of proliferation rate, q, were generated using a minimum contiguous cluster size of 0.2 ml, thresholded above 2 year^{-1}, in order to eliminate erroneous voxels and better isolate the region(s) of proliferative tumor. The total retained cluster volumes, along with the average and maximum proliferation rate within these clusters, were recorded as biomarkers for PFS and OS. In addition to proliferation rates, we also recorded mean cell invasion (diffusion) rates within the proliferative clusters chosen as ROIs.

Hypothesis testing and statistical analysis

Linear regression was performed between the volume of proliferative tissue, mean proliferation rate, and maximum proliferation rate and PFS or OS to test whether patients having high volumes of proliferative tumor, regions of high cell proliferation rates, or regions of high cell migration rates, as measured with CIMPLE maps, will have a significantly shorter PFS and OS compared with patients having lower volumes or rates of proliferative tumor. Additionally, we stratified patients based on the group mean volume of proliferative tissue, mean proliferation rate, and maximum proliferation rate in order to determine if these metrics could stratify short and long-term PFS and OS using a log-rank statistical analysis on Kaplan–Meier data. Similarly, we performed linear regression between the mean cell migration (diffusion) rates within regions of

proliferative tissue and PFS or OS. In order to limit selection bias, PFS and OS were calculated from the time of CIMPLE map calculation.

Results

The majority of GBM patients treated with bevacizumab upon recurrence in the current pilot study showed a significant reduction in both peritumoral edema and contrast enhancement (92% 24/26 patients). No obvious relationship between the reduction in edema and/or contrast enhancement and PFS/OS was observed (Table 2). Specifically, in the 26 patients examined in the current study, we found no difference in PFS or OS between patients having an initial T2/FLAIR lesion volume higher than the group average and those with a lower T2/FLAIR lesion volume (PFS: Log-Rank, P = 0.2909; OS: Log-Rank, P = 0.7226); no difference in PFS or OS between patients having a residual (post-treatment) T2/FLAIR lesion volume higher than the group average and those with a lower residual T2/FLAIR lesion volume (PFS: Log-Rank, P = 0.8385; OS: Log-Rank, P = 0.6421); no difference in PFS or OS between patients having a change in T2/FLAIR lesion volume higher than the group average and those with a lower change in T2/FLAIR lesion volume (PFS: Log-Rank, P = 0.1624; OS: Log-Rank, P = 0.5882); no difference in PFS or OS between patients having a contrast enhancing volume larger than the group average and those having a lower volume of contrast enhancement (PFS: Log-Rank, P = 0.3142; OS: Log-Rank, P = 0.7963); no difference in PFS or OS between patients having a residual (post-treatment) contrast enhancing volume higher than the group average and those having a lower volume of residual contrast enhancement (PFS: Log-Rank, P = 0.6914; OS: Log-Rank, P = 0.5547); and no difference in PFS or OS between patients having an increase contrast enhancing lesion volume after treatment and those with a decrease in contrast enhancing volume (PFS: Log-Rank, P = 0.0760; OS: Log-Rank, P = 0.4392). Mean PFS for all patients included in the current study was 145 ± 36 days SEM (median PFS = 144 days) and mean OS was 550 ± 58 days SEM (median OS = 521 days) from the time the first CIMPLE map was generated. Note that these survivals were with respect to calculation of the first CIMPLE map (i.e. 3rd DWI scan) and not form the start of bevacizumab treatment. CIMPLE maps were generated approximately 695 ± 58 days SEM (median 515 days) after initial diagnosis.

Table 2 Prediction of patient survival using traditional MR estimates of tumor volume in n = 26 patients

Tumor ROI	PFS (P-value)	OS (P-value)
Pre-treatment T2 volume	0.2909	0.7226
Post-treatment T2 volume	0.8385	0.6421
Change in T2 volume	0.1624	0.5882
Pre-treatment CE volume	0.3142	0.7963
Post-treatment CE volume	0.6914	0.5547
Change in CE volume	0.0760	0.4392

CIMPLE map estimates of proliferation rate predict contrast enhancement

As illustrated in Fig. 1, CIMPLE map estimates of proliferation rate generated at the 3rd scan session post-treatment appeared to predict spatial regions of contrast enhancement in some patients. Visual observations of overlap between CIMPLE map estimates of proliferation rate and new contrast enhancement occurred in 9 of the 26 patients examined (35%). Mean PFS was 122 days from the time of the first CIMPLE map for these patients, compared with 160 days for patients where CIMPLE maps were not predictive of contrast enhancement. In another 4 of the 26 patients examined (15%), tumor progression occurred in the form of diffuse T2 abnormality without obvious changes in contrast enhancement. When taking only the patients who had contrast enhancement at recurrence into consideration, CIMPLE map estimates of proliferation rate predicted spatial regions of contrast enhancement in 9 of 22 patients (41%). Additionally, CIMPLE maps did not detect any clusters of proliferative tissue larger than 0.2 ml and having a proliferation rate[2 $year^{-1}$ in 3 of the 26 patients (11.5%). Interestingly, these patients had a PFS of more than 1 year and an OS of more than 2 years, suggesting patients exhibiting no detectable proliferative clusters at the beginning of treatment may have very slow growing tumors. In the remaining patients, CIMPLE map clusters of proliferative tissue did not appear useful for predicting spatial changes in contrast-enhancement or non-enhancing tumor.

CIMPLE maps estimates of proliferation rate predict progression free survival

A significant linear correlation was observed between mean proliferation rate within FLAIR abnormal regions and PFS (Fig. 2b; Pearson's correlation coefficient, R^2 = 0.5461, P \ 0.0001); however, no significant linear correlations were observed between the volume of proliferative tissue (Fig. 2a; Pearson's correlation coefficient, R^2 = 0.0953, P = 0.1249) or maximum proliferation rate within FLAIR abnormal regions (Fig. 2c; Pearson's correlation coefficient, R^2 = 0.0895, P = 0.1375) and PFS. Log-rank analysis of Kaplan–Meier data suggested no statistical difference in PFS between patients having a volume of proliferative tissue greater than 7.26 ml (group