6 Week Project

Developing a Statistical Method of Quantifying Vascular Response after Radiotherapy
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Introduction

The survival for patients with malignant glioma tumors is very low even after radiotherapy, chemotherapy and surgery. The survival rate for both genders is very low compared to other common cancers (1). Studying brain tumors gives valuable information in improving diagnosis and treatment. One of the methods used to treat brain tumors, in combination with other methods, is radiation therapy. With new radiation technology advancements, a single high dose of radiation can be administered to the tumor using a technique called radiosurgery. A high dose of radiation is capable of inducing vascular damage. Malignant glioma invade through the process of angiogenesis, or the growth of tumor blood vessels (2). This results in a higher microvascular density in the tumor than normal tissue. It is important to understand the impact radiation therapy has on the perfusion of tumor vasculature, specifically in this study, malignant glioma. This can be done performing CT perfusion scans before and after radiosurgery. With the recent use of CT perfusion, studies have shown that increased contrast enhancement correlates with high microvascular density, giving physiological information of the region (3). CT perfusion allows researchers to measure hemodynamic properties and determine the vascular response after treatment in a noninvasive manner. The purpose of this study is to develop a method called parametric response map (PRM) to evaluate the vascular response of malignant glioma treated with radiotherapy. When assessing tumor response, PRMs are a good predictor of patient survival in patients treated with malignant glioma (4). The specific purpose of this project is to determine a statistical approach in mapping out which voxels have significantly increased or decreased in the tumor region-of-interest. It is hypothesized that tumor regions will show lower vascular function after radiotherapy.
Theory

CT perfusion is based on tracer kinetics, studying the wash-in and wash-out of a contrast agent in blood vessels (5). It is a noninvasive imaging technique that acquires repeated CT images as a bolus of iodinated contrast is injected into the vein of a subject. Iodinated contrast agents are used because they have a larger attenuation of x-rays than soft tissue and produce a higher intensity measured in Hounsfield units (6). This is because to the higher atomic number of iodine due to the photoelectric effect. When the contrast agent is injected intravenously into the subject, CT scans of a single section are taken as a function of time to examine the distribution of contrast agent as it passes through a tissue region-of-interest. The intensity change as a function of time is measured voxel, to produce a time-enhancement curve. An example of two time-enhancement curves is shown below.

![Time-enhancement curves for a voxel in the tumor region and in the normal brain.](image)

**Fig. 1** Time-enhancement curves for a voxel in the tumor region and in the normal brain.

Since CT enhancement is proportional to contrast concentration (7), tracer kinetics analysis can be used to determine blood volume (BV), blood flow (BF) and capillary permeability surface area product (PS) for each voxel. BF is estimated to be the peak value of the curve measured in
ml/min/100g of contrast agent (8). BV is estimated to be the area under the curve measured in ml/100g of contrast agent. PS is measured using the following equation.

\[
PS = -BF \times \ln(1 - \text{extraction fraction})
\]

[1]

The extraction factor is at the minimum transit time when the enhancement decays with time. A study by Miles et al. proposes that an increase in blood flow (BF), blood volume (BV) and capillary permeability surface area product (PS) are found in tumors (9). A region with high PS is known to be very leaky and therefore the contrast remains in the region for a longer time. This is one of the ways to clearly see tumors since tumor vasculature is very leaky. This is due to disruption of the blood-brain barrier, causing tumor vessels to function abnormally. Knowing the physiological parameters for each pixel in the brain, parametric maps can be attained for pre and post treatment, as shown below.

![Parametric maps before (upper row) and after (lower row) treatment. The parameters shown are blood flow (A), blood volume (B) and PS (C).](image)

**Fig 2.** Parametric maps before (upper row) and after (lower row) treatment. The parameters shown are blood flow (A), blood volume (B) and PS (C).
Methods

The CT perfusion images analyzed were of Wistar rat subjects (n=13) injected with C6 glioma in the right side of the brain. A baseline CT perfusion scan was done when the tumor developed, 12 days on average after implantation. Radiosurgery treatment was applied with a single high dose of radiation and CT perfusion images were again taken ranging from 4 to 7 days after treatment. The parametric maps were calculated for both pre and post treatment images using the time-enhancement curve. A GUI-based MATLAB (Mathworks Inc.) program was previously written for voxel-by-voxel analysis of CT perfusion images and this was used to create the PRMs. After co-registration using the 3D slicer software, the pre and post images were imported into the GUI program. Only slices that showed tumors were included in the analysis. Tissue outside the rat brain were excluded from the analysis. Contours were made around the normal brain to check for difference. No difference was expected because radiation was not applied to that half of the brain. Subtracting the PS values of the corresponding voxels between pre and post gave a difference map. An example is shown below.

Fig. 3 Pre (A) and post (B) PS maps being subtracted to produce a difference map (C). A legend is shown for the difference map with the same units (mL/100g). Pre and post images show the contour of the normal brain. The difference map shows the contour of the tumor region-of-interest.

To quantify statistical difference between pre and post treatment, the distribution of PS change in the normal brain was used to check for significance. If there is no large difference in the normal
brain based on the histogram, the thresholds would be based on 5th and 95th percentiles of the normal brain histogram. These percentiles were used as the upper and lower thresholds to determine which voxels have changed significantly. By editing the MATLAB code, these thresholds were used in creating the PRMs to assess tumor response. Those pixels having a PS value lower than the 5th percentile were given a value of negative one. Those higher than the 95th percentile were given a value of positive 1. The pixels that had a value between the thresholds, i.e., have not significantly increased or decreased in PS, were given a value of zero.

An example of such a map is shown below in Figure 4.

**Fig. 4** Parametric response map, using the pre and post images from Figure 3, displaying pixels that have significantly increased, decreased or showed no difference in PS. A value of negative two was given to distinguish the background from the brain.

**Results**

The normal brain, based on the contour in the difference map, showed similar values before and after treatment. Figure 5 shows an example from one subject showing most of the pixels having zero difference in BV after treatment.

This validated the work in quantifying tumor response. The
upper and lower thresholds were based on the upper 95th and lower 5th percentiles, respectively. There was a large variance between subject thresholds as shown in Figure 6.

![Fig. 6 PS average and standard deviation for upper and lower thresholds in normal brain for all subjects.](image)

Applying specific thresholds to the difference maps for all subjects can quantify the number of pixels in the tumor region-of-interest that have a significant change in PS. Figure 7 shows the response of tumors based on the PS change.

![Fig. 7 Pixel distribution of change in PS of the tumor region-of-interest based on PRM.](image)
Below are visual examples of some of the PRMs attained using the thresholds.

![Fig. 8 Tumor response for three subjects: SRS14 (D), SRS12 (E) and D4 (F). Pre-treatment map is shown as column A, post-treatment map as column B and the parametric response map as column C. For the PRMs, red pixels correspond with a significant increase, yellow corresponds with no change and cyan corresponds with a significant decrease.](image)

**Discussion**

We have developed a method for quantifying the heterogeneous vascular response observed between subjects as shown in Figure 7. The various subject responses can be categorized into groups based on their vascular response in the tumor region-of-interest (ROI).

This first group consists of subjects that show a large PS decrease in the tumor ROI. An example of this group is shown in Figure 8(D) in the following subjects: SRS11, SRS13, SRS14, A1a and A1c. The group corresponds to the regular response of irradiation (10). The second group shows a large PS increase in the tumor ROI. An example is shown in Figure 8(F). This is a rare occurrence that is not well explained. However, there is evidence that this group may have a
higher number of pericytes around the capillaries. Pericytes are known to protect blood vessels from injury by radiation (11). The hypothesis that PS will decrease in the tumor region is not necessarily going to be always true. The third group shows various responses. Some subjects have slightly decreased in PS, but largely have not changed significantly. Technically, they did show more decrease than increase in PS after radiotherapy, and therefore could be assessed as part of the first group. Some subjects, however, show more heterogeneity in tumor response with both equal percentages of PS increase and decrease. This can be seen in Figure 8(E). The PS in the tumor ROI has decreased on the outside, but has also increased on the inside. This is a good example of how PRMs are great at assessing tumor response. Using the averaging method would simply show no difference in the tumor region while clearly change occurred after radiotherapy, even if in different regions of the tumor. In some cases, such as in Figure 8(F), there is a large PS increase around the original location of the tumor ROI. This is known as the peri-tumoral region. Future work involves using the same method to analyze this affected region. Also, expanding this work to include BV and BF PRMs is important. The PRM method takes into account the heterogeneity of glioma tumor response to quantify the response effectively and, as shown by Galbán et al., be a good predictor of patient survival. The single high dose or radiation does have significant consequence on the tumor vasculature. In most cases it decreased the vascular PS, but cases of increase also occurred.

Conclusion

The purpose of this project was to determine a statistical approach in mapping out which voxels have significantly increased or decreased in the tumor region-of-interest using parametric
response maps. It was hypothesized that tumor regions will show lower vascular function, specifically PS, after radiotherapy. It was found that the PRM method using subject specific thresholds can be used to quantify heterogenous vascular response.
References


