Novel microCT Imaging Techniques for *in vivo* Quantification of Vascular Volume in Murine Tumor Models

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Abstract

Growing interest in anti-angiogenic therapies has created a need for more sophisticated means to evaluate treatment response. This novel method of minimally-invasive quantification of vascular volume in murine tumor lines utilizes CT imaging of a blood pool contrast agent. Transgenic and xenograft mouse lines were imaged to monitor growth of tumor and vascular volumes and response to angiogenic treatments. Image sets of treatment response were analyzed by three expert and four untrained observers to determine reliability of the vascular segmentation process. In conclusion, observer training had a measurable impact on the statistical significance of volume measurements. Despite a fairly large variation in absolute volume measurements, fractional changes in volume were highly correlated in the expert group and shows a consistent ratio of tumor to vasculature volume. Ultimately, contrast μCT imaging can be an effective technique monitor tumor growth and response to anti-angiogenic therapy.

Introduction

Contemporary methods of evaluating cancer therapy (CT tumor size, clinical outcome) may be inadequate to understand treatment response to new molecular therapies[1]. In particular, understanding the vascular response to antivascular therapies may prove key to the preclinical development of such agents and in the clinical evaluation of effectiveness. Unfortunately, current methods of evaluating vascular dynamics in tumors are inadequate for longitudinal studies. Histological staining can provide highly resolved images of vascular density and angiogenesis[2,3] but is a destructive technique. CT or MR perfusion techniques provide in vivo measurements of vascular characteristics[4] but may have poor resolution and are unavailable on many preclinical scanners. *In vivo* measurement of vascular volume through contrast μCT may prove a useful tool to preclinical development of such agents and in the clinical evaluation of effectiveness.

Materials and Methods

A transgenic adenocarcinoma mouse prostate tumor model (TRAMP) was imaged with a blood pool contrast agent (Fenestra VC) over a period of three weeks to test the quantification method and to evaluate the relative growth in tumor volume and vascular volume. In addition, two xenograft mice (X1 and X2, line SCC-1483) were imaged several times while receiving antivascular treatment with panitumumab (Vectabix) and bevacizumab (Avastin).

Scans were performed using a Siemens Inveon μPET/CT scanner and analyzed in Amira, a 3D visualization and segmentation package. Images were manually segmented into tumor and vascular volumes using a threshold-based region-growing tool. In the prostate model, vasculature surrounded the outer surface of the tumor, so a vascular mask was created by expanding the tumor volume (Fig 1,2). Error analysis in the segmentation of the prostate model was performed by using Amira's grow function to expand the region for an upper bound, using the shrink function to contract the region for a lower bound, and taking the average.

In the xenograft models, only vasculature within the tumor boundary was considered. Pre- and post-treatment scans were segmented by seven observers into tumor and vascular volume. Three of the seven observers were considered experts (had experience with mouse μCT and Amira) and four were considered untrained. Statistical analysis was performed to determine interobserver reliability of the Fenestra segmentations and to estimate the error of vascular segmentation for treatment response.

[1]: Miller 2005 J Natl Canc Inst
[2]: Eberhard 2000 Canc Research
[3]: Hlatky 2002 J Natl Canc Inst
[4]: Provenzale 2007 Am J Roent

Fig. 1: Initial prostate tumor (yellow), tumor mask (green), and vasculature (red)

Fig. 2: Prostate tumor, tumor mask, and vasculature at week three

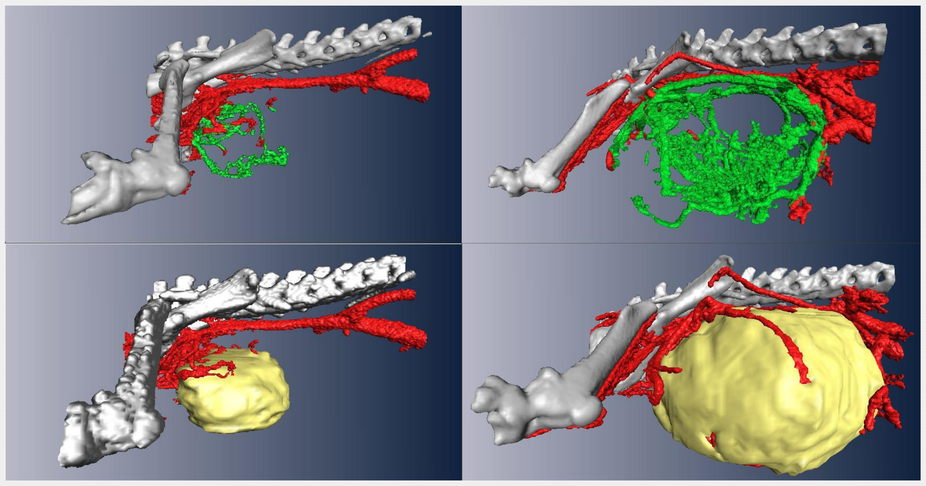


Table 1: Interobserver segmentation results for xenograft mice. Absolute and relative volume changes are shown for the expert, untrained, and entire groups.

	Entire mean	Entire std dev	Expert mean	Expert std dev	Untrained mean	Untrained std dev
Tumor volume 2-21-07 (mm^3)	162.9	51.8	125.6	29.7	190.9	48.3
Tumor volume 3-01-07 (mm^3)	118.3	41.6	82.7	18.5	145.0	32.1
Post-treatment volume / Pre-treatment volume	0.72	0.08	0.66	0.08	0.76	0.05
Vascular volume 2-21-07 (mm^3)	16.4	5.0	16.9	2.7	16.0	6.8
Vascular volume 3-01-07 (mm^3)	13.6	7.1	11.1	1.3	15.6	9.5
Post-treatment volume / Pre-treatment volume	0.79	0.32	0.66	0.03	0.88	0.42

Fig. 3: Visualization of author's pre- and post-treatment segmentation of tumor and vasculature in mouse X1

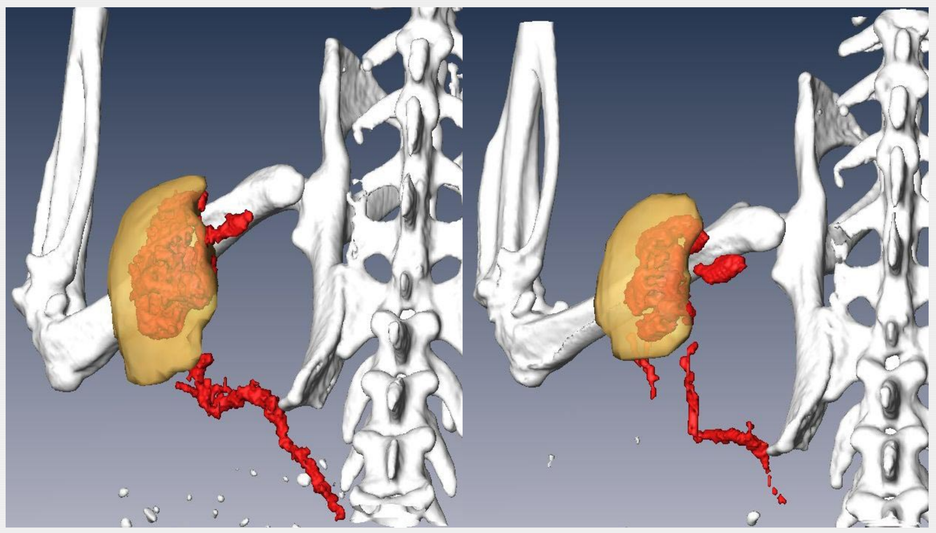
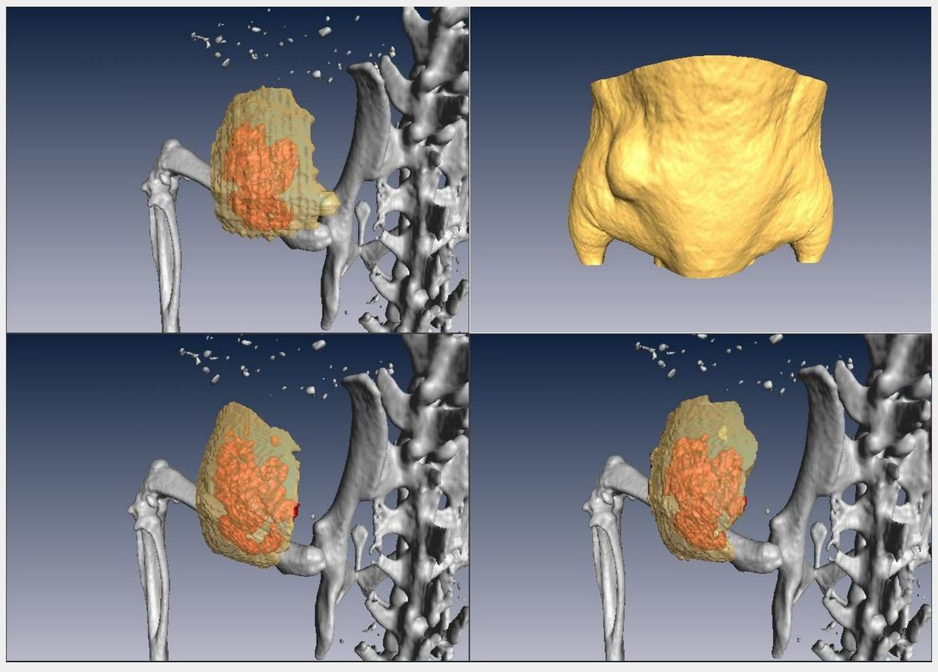


Fig. 4: Comparison of expert observer segmentations (pretreatment scan of mouse X1)



Results

- **Growth in the prostate model was measurable (Fig. 1,2)**
  - Tumor volume increased from 940 ± 50 mm^3 to 4000 ± 200 mm^3 over the span of three weeks
  - Vascular volume increased from 20 ± 5 mm^3 to 320 ± 70 mm^3
  - Ratio of vascular volume to tumor volume increased from 1.5% to 6%
- **Volume decreases in treated xenograft mice were measurable (Fig. 3,5) and showed a linear relationship (Fig. 6)**
  - *Ratios of post-treatment tumor volume to pre-treatment volume:* 66% ± 8% in expert group, 72% ± 8% in entire group, 76% ± 5% in untrained group.
  - *Ratios of post-treatment vascular volume to pre-treatment volume:* 66% ± 3% in expert group, 79% ± 32% in entire group, 88% ± 42% in the untrained group.
- **Observer training had a measurable impact on statistical significance and standard deviation**
  - *Paired t-test for tumor volumes:* **p=.04** in expert group, **p=0.0003** for entire group, p=.74 for untrained group
  - *Paired t-test for vascular volumes:* **p=.02** in expert group, p=.13 for entire group, p=.18 for untrained group
- **Fractional changes in volumes were highly correlated in all groups (less than 10% standard deviation)**

Fig 5: Tumor and vascular segmentation volumes

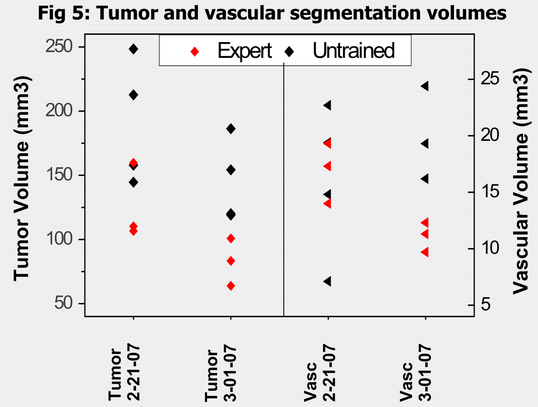
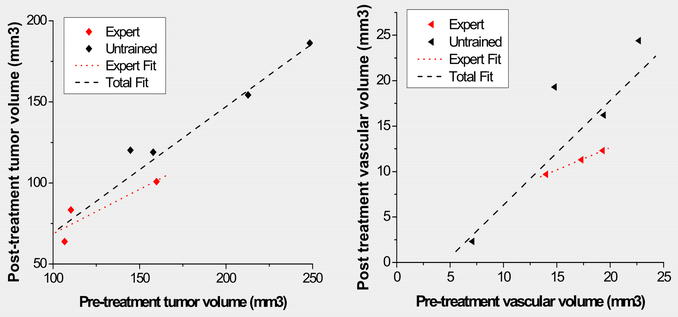


Fig 6: Linear response of treatment volumes in tumor and vasculature



Conclusions

Contrast μCT imaging was shown to effectively monitor tumor growth and response to anti-angiogenic therapy. While individual interpretation of the tumor and vascular volumes can vary dramatically, comparisons show that trained observers can measure consistent response. A single observer might expect a 25% error in absolute segmentation volume and a 10% error in relative segmentation volumes. Preliminary results suggest that the ratio of tumor to vascular volume remains constant during angiogenic therapy. Ultimately, when care is taken in the segmentation process, contrast μCT imaging can be an effective way to measure statistically significant changes in tumor and vascular volume over time.

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