Assessing Breast Cancer Angiogenesis *In Vivo*: Which MRI Biomarkers are Relevant?

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INTRODUCTION: Angiogenesis is a hallmark of breast cancer and essential for tumor invasion and metastasis [1]. Although various anti-angiogenic drugs have been developed, none are currently approved for treating breast cancer [2]. Therefore, there is a crucial need for noninvasive biomarkers of breast cancer angiogenesis to evaluate the efficacy of new anti-angiogenic therapies *in vivo*. Susceptibility contrast MRI is a non-invasive, *in vivo* technique capable of providing quantitative information about tumor angiogenesis [3]. The purpose of this study was to determine how accurately *in vivo* steady-state susceptibility contrast (SSC)-MRI biomarkers of angiogenesis in an orthotopic human breast cancer model predicted the appropriate 3D vascular correlates derived from high-resolution micro-CT (μCT).

Table 1 SSC-MRI biomarkers and LOOCV analysis

μCT parameter	*In vivo* MRI parameter		LOOCV: NRMSE
Fractional blood volume ($FBV_{\mu CT}$)	**ΔR2**	-	**0.196**
	FBV_{MRI}* [4]	$\frac{3}{4\pi}\frac{\Delta R_2^*}{\gamma\Delta\chi B_0}$	0.263
Vessel radius ($VSI_{\mu CT}$)	**R**	$\frac{\Delta R_2^*}{\Delta R_2}$	**0.336**
	VSI_{MRI}* [4]	$0.425\left(\frac{D}{\gamma\Delta\chi B_0}\right)^{1/2}\left(\frac{\Delta R_2^*}{\Delta R_2}\right)^{3/2}$	0.453
Vessel density	Q [5]	$\frac{\Delta R_2}{(\Delta R_2^*)^{2/3}}$	0.187
	N* [5]	$\frac{Q^3}{D}$	**0.097**

*: "Absolute" MRI parameter; **BOLD**: indicates smaller NRMSE

METHODS: Human MDA-MB-231 breast cancer cells were inoculated into the mammary fat pad of athymic NCr-*nu/nu* mice mice. Tumors were imaged *in vivo* on a 9.4 T horizontal bore scanner (Bruker BioSpin) with SSC and diffusion-weighted MRI at post-inoculation week (PIW) 3 (n=10) and PIW 5 (n=7). SSC MRI involved multi-echo gradient echo (GE) and spin echo (SE) scans before and after an injection of ferumoxide (25mg Fe/kg body weight, AMAG). *In vivo* images were acquired with in-plane resolution=100 μm and slice thickness=1 mm. Whole tumor *ex vivo* 3D μCT angiography was conducted by Numira Biosciences on a subset of excised tumors (PIW 3 n=5, PIW 5 n=3) at 8 μm isotropic resolution. *In vivo* biomarkers of tumor fractional blood volume, vessel radius and vessel density were computed on a voxel-wise basis (**Table 1**), and corresponding maps were computed from the segmented μCT-derived vasculature on the same *in vivo* spatial grid (**Fig. 1**). MRI biomarkers were validated against their μCT analogs using linear regression and leave-one-out cross-validation (LOOCV) analyses. The normalized root mean square error (NRMSE) was computed for each *in vivo* biomarker to measure its relative predictive value. The sensitivity of each biomarker to temporal changes in angiogenesis was assessed by statistically comparing their values at PIW 3 vs. PIW 5 using a Mann-Whitney U test.

RESULTS: With the exception of VSI_{MRI}, all *in vivo* MRI biomarkers displayed significant correlations (p<0.05) with their μCT analogs. According to their respective concordance correlation coefficients (ρ_C) [6], FBV_{MRI} and $FBV_{\mu CT}$ were in good agreement whereas VSI_{MRI} and $VSI_{\mu CT}$ were not (**Fig. 2A-B**). This was also reflected in the linear regression models: the intercept and slope of the FBV model were not significantly different from zero and one, respectively, whereas those of the VSI model were (p<0.05). In addition, the median tumor FBV values measured from the two imaging modalities were not significantly different, whereas the VSI values were (**Fig. 2C-D**). Similar analyses could not be performed for vessel density because N is a measure of 2D histological vessel density (per unit area), while the μCT vessel density is a 3D measurement (per voxel). Based on LOOCV analysis, ΔR_2 was a better predictor of $FBV_{\mu CT}$ than FBV_{MRI}; R was a better predictor of $VSI_{\mu CT}$ than VSI_{MRI}; and N was a better predictor of vessel density than Q (**Table 1**). $VSI_{\mu CT}$, VSI_{MRI} and R all indicated that the median tumor vessel radius increased significantly from PIW 3 to PIW 5. In contrast, all three measures of vessel density (μCT vessel density, N and Q) decreased significantly from PIW 3 to PIW 5. Neither $FBV_{\mu CT}$ nor FBV_{MRI} were significantly different between the two time points, but ΔR_2 was significantly lower at PIW 5 than at PIW 3.

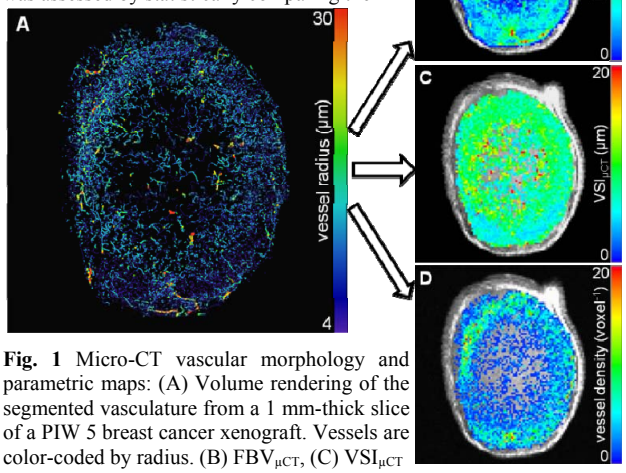

Fig. 1 Micro-CT vascular morphology and parametric maps: (A) Volume rendering of the segmented vasculature from a 1 mm-thick slice of a PIW 5 breast cancer xenograft. Vessels are color-coded by radius. (B) $FBV_{\mu CT}$, (C) $VSI_{\mu CT}$ and (D) vessel density maps computed from (A), overlaid on the raw SE image.

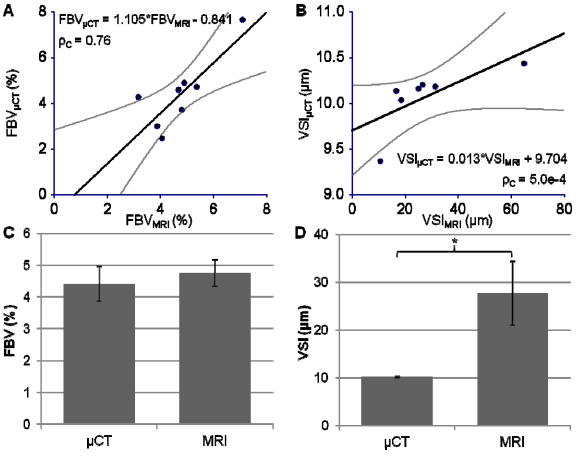

Fig. 2 Scatter plots of μCT- vs. MRI-measured FBV (A) and VSI (B). Black lines: linear regression model, gray lines: 95% confidence interval. Bar graphs (error bars indicate standard error) of median tumor FBV (C) and VSI (D). * p<0.05

DISCUSSION: The MRI biomarkers examined here can be broadly categorized as: (i) "absolute" biomarkers (FBV_{MRI}, VSI_{MRI} and N) that directly measure a vascular parameter (e.g., VSI_{MRI} measures vessel radius in μm) or (ii) "relative" biomarkers (ΔR_2, R and Q) whose values are proportional to a vascular parameter (e.g., R ∝ vessel radius). The LOOCV results suggest that the "relative" biomarkers were better predictors of the μCT-measured 3D vascular morphology, with the exception of Q and vessel density. However, Q still exhibited a lower NRMSE than any blood volume or vessel size biomarker. FBV_{MRI} was found to be in good agreement with $FBV_{\mu CT}$. However, since $FBV_{MRI} \propto \Delta R_2$*, the two may be considered equivalent if one is interested in global blood volume changes (e.g., in response to therapy). ΔR_2 (microvascular blood volume) decreased significantly between PIW 3 and PIW 5 while ΔR_2* (global blood volume) did not. This suggests the change in the angiogenic phenotype that occurred between these time points primarily involved the microvasculature. Thus, ΔR_2 may be important for early detection of therapeutic effects on angiogenic sprouting. VSI_{MRI} has been shown to overestimate the true vessel radius [4], and this is corroborated by our results. Collectively, the results from this study indicate that "relative" SSC-MRI biomarkers are better predictors of μCT-derived vessel morphology than "absolute" biomarkers, which require measuring the apparent diffusion coefficient and changes in the bulk magnetic susceptibility of blood. Additionally, the ease of computation of "relative" SSC-MRI parameters makes them promising candidates for noninvasive, *in vivo* biomarkers of breast cancer angiogenesis.

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