MRI correlates of tumour grade and hypoxia status in human breast cancer

A. R. Padhani[1], M-L. W. Ah-See[2], N. J. Taylor[1], J. J. Stirling[1], F. M. Daley[2], P. I. Richman[2], J. A. d'Arcy[3], S. Walker-Samuel[3], A. L. Harris[4], D. J. Collins[3], M. O. Leach[3], A. Makris[2]

[1]Paul Strickland Scanner Centre, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, United Kingdom, [2]Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, United Kingdom, [3]CRUK Clinical MR Research Group, Institute of Cancer Research, Sutton, Surrey SM2 5PT, United Kingdom, [4]Weatherall Institute, John Radcliffe Hospital, Oxford, United Kingdom

Synopsis: Histological tumour grade and oxygenation status (CA-IX immunohistochemistry) of breast cancers have been correlated with BOLD and dynamic MRI. An inverse correlation of R2* and blood volume (r=0.6; p=0.003) was shown. Strong relationships between breast cancer blood volume/flow and tumour grade are noted. An inverse relationship between blood volume/flow and CA-IX staining was unexpected and paradoxical. R2* did not correlate with CA-IX staining.

Background: Tumour grade and oxygenation are important prognostic features in breast cancer[1, 2]. The purpose of the current study is to see whether BOLD or dynamic contrast enhanced MRI (DCE-MRI) are predictive of tumour grade and hypoxia status of breast cancers.

Methods:
32 untreated patients with biopsy-proven primary breast cancer (median age 46 years old, range 33-62) were studied using a 1.5T Siemens Symphony scanner prior to any treatment. Spoiled gradient-echo [FLASH] sequences with 8 different TE [5-75ms], TR=100ms, flip=40°, 1 slice were used for R2* measurement using an IDL® least-squares fitting routine prior to the DCE-MRI studies. T1W and T2*W DCE-MRI studies were performed using methods previously described. Briefly, spoiled GRE [FLASH] sequences (TE 4.7ms, TR 11ms, flip angle=35°, 4 slices) were acquired before and after the bolus administration of 0.1 mmol/kg b.w. of Gd-DTPA with 40 time points over 8 min, through the centre of the breast cancers. T_2*W DCE-MRI sequences followed (TE 20ms, TR 30ms, flip angle 40°, single slice) with 0.2 mmol/kg b.w. Gd-DTPA being administered after the first 10 of 60 images, time resolution 2s. ROI were place around tumours to calculate pixel-by-pixel values of upslope gradient, maximum enhancement and wash-out gradient together with transfer constant (K^{trans}), leakage space (v_e) and rate constant (k_{ep}) on the T1W DCE-MRI images, using the methods of Tofts[3] on software designed for purpose (MRI Workbench, Institute of Cancer Research, London). Relative blood volume (rBV), flow (rBF) and MTT derived using gamma variate fit functions to the T2* DCE-MRI data were also obtained.

Only patients with invasive ductal cancer histology were included. Tumours were graded according to the Bloom-Richardson system. Grade assignment was done according to core biopsy or surgical specimen analysis. Where there was a discrepancy between the specimens, the final grade assignment was the highest grade seen. Low (n=1) and moderate grade tumours (n=15) were grouped as there were too few low grade tumours. Immunohistochemical staining to assess the oxygenation status of breast cancer was performed on the pre-treatment diagnostic core-biopsy samples using the carbonic anhydrase-IX (CA-IX) mouse monoclonal antibody[2] (supplied by Professor Adrian Harris, John Radcliffe Hospital, Oxford). CA-IX immunostaining was quantified in carcinoma cells as either positive or negative.

Univariate and multivariate analyses dividing morphology (size), intrinsic susceptibility (R2*) and DCE-MRI kinetics were performed. For assessment of R2*, only patients with solid masses (n=23) were included and not patients with infiltrating disease (n=5) or necrotic tumours (n=4). This is because in infiltrating/septal spreading disease, intact breast septae can cause increases in R2* and necrosis causes a paradoxical decrease in R2* [4]. Multivariate analysis of the most significant univariate variables. The statistical significance for the 2-tailed Mann-Whitney test was set at a P-value of <0.01.

	Tumour grade	**Nature of tumour grade correlation**	**CA-IX staining**
Size (n=32)			
BOLD-MRI (n=23)			
R2* Median	0.015	Higher grade tumours had lower R2*	
R2* 95 centile value	0.0001		
T1W DCE-MRI (n=32)			
Upslope gradient			
Maximum Amplitude of enhancement (% from baseline)	**0.006**	Higher grade tumours had greater enhancement	
Wash-out rate	**0.01**		
Transfer constant			
Leakage space			
Rate Constant			
T2*W DCE-MRI (n=31)			
Relative blood volume	0.002	Higher grade tumours have high rBV and high rBF	0.001
Relative blood flow	0.001		0.001
Mean transit time			
Only statistically significant correlations (p<0.01) are shown			

Results:
Univariate analysis showed that there were significant MRI correlates of tumour grade and all were related to tumour blood flow and volume (table). Multivariate analysis shows that as explanatory variables, the maximum amplitude was the dominant variable explaining tumour grade. An inverse correlation of R2* and blood volume (r=0.6; p=0.003) was also shown explaining the inverse correlation of R2* with tumour grade. Univariate analysis showed that rBV/rBF were highly correlated with CA-IX staining BUT inversely. That is, CA-IX positive patients have high blood flow and blood volume. There were no convincing correlations between R2* or T1W DCE-MRI kinetic parameters and CA-IX staining.

Conclusions:
These analyses show that there are strong relationships between breast cancer blood volume/flow and tumour grade. Note that the maximal amplitude of enhancement is also highly dependent on tumour blood flow and capillary permeability. This is expected because the levels of angiogenesis are expected to be greater in higher grade tumours[5]. The inverse correlation of tumour grade with R2* indicates that R2* contrast in breast cancers is dominated by blood volume. The inverse correlation between blood volume/flow and our marker of tumour hypoxia (CA-IX) was unexpected and paradoxical. However the paradox of large blood volume and concomitant tumour hypoxia is becoming recognized in the literature[6]. The lack of correlation of BOLD MRI (R2*) and tumour hypoxia (CA-IX) status was disappointing particularly as other studies in xenografts and human prostate cancer have show positive correlations between R2* and pimonidazole immunohistochemistry[7]. This may in part be related to the fact that CA-IX is a measure of chronic hypoxia and R2* reflects on the oxygenation within blood vessels and in the immediate surrounding tissues.

References: [1] Elston CW, *Histopathology*. 2002; 41(3A):151, [2] Chia SK, *J Clin Oncol.* 2001; 19(16):3660-8, [3]Tofts, PS and Kermode, AG. *JMRI* 1997;7:91.[4] NJ Taylor, *Proc ISMRM* 2003; 2126. [5] Hansen S, *Clin Cancer Res.* 2000; 6:139-46. [6]Kostourou V, *Neoplasia* 2004; 6,401–411. [7]NJ Taylor, *Proc ISMRM* 2003; 531.