# Quantification of perfusion change with carbogen breathing in a subcutaneous rat tumour model using ASL and comparisons with T2* change, ADC and IAUC

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**Introduction** Several studies have shown a relationship between tumour oxygenation and outcome from radiotherapy [1]. Therefore the effect of tumour oxygenating agents (TOX) agents such as carbogen on tumour physiology is of great interest. BOLD MRI can be used to give an indication of tumour oxygenation and BOLD imaging has been previously used to estimate changes in tumour oxygenation due to carbogen inhalation [1-4]. However, the BOLD signal does not give a direct measurement of tumour oxygenation as the $T_2$* response is susceptible to changes in perfusion and blood volume as well as oxygenation levels.

ASL is a well-established method for measuring perfusion (*F*) but measurements have not been previously reported in subcutaneous tumour models. Here we investigate the feasibility of using ASL in such tumours to assess the utility of the technique to aid in understanding the origin of the BOLD response to carbogen by supplying a quantitative estimate in the changes in perfusion due to carbogen inhalation. This could then be directly compared with changes in $T_2$* to supply more information on the origin of the $T_2$* change.

**Methods and Results** $T_2$*, FAIR ASL, and DWI data were acquired in 6 subcutaneous rat tumours while breathing air and carbogen. All *in-vivo* procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986. *In vivo* experiments were performed on 6 rats implanted with LoVo colorectal subcutaneous tumours (mean volume 1.8 $cm^3$) on a Varian 9.4 T system using a 6 cm diameter quadrature birdcage RF coil. $T_2$* and FAIR ASL data were acquired in all of the rat tumours with air and carbogen breathing. At the end of the protocol DCE-MRI data and DWI data were acquired in 5 of the tumours with the rat returned to breathing air for further comparisons. Anaesthesia was induced with 2% isofluorane in air and maintained with 1-1.5% isofluorane throughout the duration of the scan. Breathing rate and temperature were monitored throughout the experiment and body temperature maintained at 37 °C. All images were acquired with a 6 x 6 cm FOV and matrix = 128(frequency) x 64(phase). DCE-MRI data were acquired using multi slice spoiled GE sequence with TE = 1.3 ms, TR = 150 ms. 4 non-contiguous 2 mm slices were acquired through the tumour every 2.1 s. $IAUC_{10\text{ and }60}$ (IAUC calculated over the first 10 and 60 s post enhancement) maps were generated for each tumour (given in units of mM.s). $T_2$* data were acquired using a multi slice GRE sequence with TE = 2.2, 3.2, 4.4, 5.6, 6.8, 8.0, 9.2, 10.4, 11.6 and 12.8 ms, TR = 300 ms and flip angle = 20°. 8 non-contiguous 1 mm slices were acquired. $T_2$* maps were generated and the slices were added together to match the DCE-MRI slices for analysis. Diffusion data were acquired from 4 non contiguous slices using a multi slice pulsed gradient spin echo imaging sequence with TE = 14 ms, TR = 1000 ms, nt = 2, δ= 2.5 ms and b = 0, 36, 143, 322, 572 and 894 s/$mm^2$. ADC maps were generated. FAIR ASL data were acquired on a single 2 mm slice, which was selected to correspond with the DCE-MRI and $T_2$* images. A gradient spoiled centric phase encoded turboFLASH sequence was used for the imaging acquisition with: a sech180 inversion pulse, slice thickness ratio (STR) =3, flip angle = 10°, TE= 3 ms, TR=5 ms and nt=4 at inversion times of TI = 0.6, 1, 2, 3, 15 s with TR = 15 s. For each tumour ROIs were selected and the mean $T_2$* for air and carbogen breathing recorded along with ADC, $IAUC_{10}$ and $IAUC_{60}$. The ASL signal curve was also recorded with air and carbogen breathing and *F* was quantified in units of ml blood/min/100 ml tissue by fitting the ASL time course data from the region with the two compartment model [5] using a Levenberg-Marquardt fitting routine (Microcal Origin 7.5, Microcal Inc, California, USA). To look for correlation between parameters scatter plots were generated and Spearman's rank correlation coefficient (r) and p were calculated, the results of which are presented below.

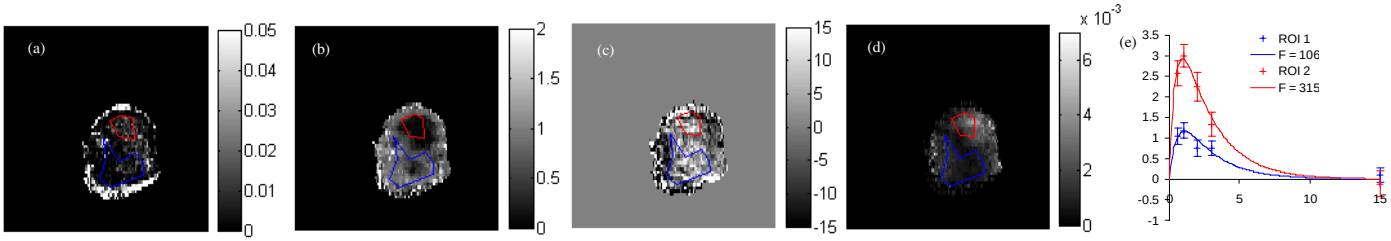


***Fig 1:*** *(a) $IAUC_{10}$ map, (b) $IAUC_{60}$ map (c) $T_2$* % change due to carbogen inhalation (d) ADC map (e) ASL signal curves and fit from two regions shown in blue and red with fitted F (ml blood/min/100ml tissue).*

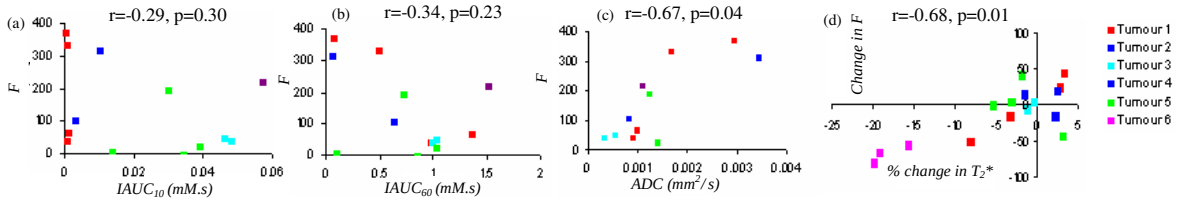


***Fig 2:*** *Mean values of (a) $IAUC_{10}$, (b) $IAUC_{60}$ and (c) ADC plotted against mean F (air). (d) % change in $T_2$* plotted against absolute change in F following carbogen breathing.*

**Discussion** FAIR ASL was shown to be a feasible method for measuring *F* in a subcutaneous tumour model and sensitive enough to quantify changes resulting from carbogen breathing. ASL measurements have not previously been reported in such a model and demonstrate how, with careful implementation, ASL can be used in tumours outside the brain in animals and potentially in humans. ASL may supply important additional information for understanding tumour vasculature as the more commonly used IAUC measurements are dominated by CA leakage and do not correlate with *F*. The effect of carbogen inhalation on $T_2$* varied quite dramatically within each tumour with different regions showing different behaviour (fig 1(d)). However the change in $T_2$* and change in *F* showed positive correlation as would be expected (fig (2(d)) (r = 0.68 p = 0.01). A positive correlation was also shown between ADC and *F* (fig (2(c)) (r = 0.67, p= 0.04 ); a link between diffusion, via the intravoxel incoherent motion mechanism, and perfusion has been previously reported [6]. In summary, a novel and informative application of the ASL technique has been demonstrated that may have application in future oncology studies in animal and human tumours outside the brain.

**References** 1. Dunn et al., *JMRI*, 2002 **16**:511; 2. Taylor et al., *JMRI* 2001 **14**:156; 3. Howe, et al., *JMRI*, 1999 **17**:1307; 4. Fan, et al.,. *IJROBP*, 2002 **54**:1202; 5. Parkes & Tofts, *MRM*, 2002 **48**:27; 6. Le Bihan & Turner *MRM* 1992 **27**:171.

**Acknowledgements** This work was supported by AstraZeneca and EPSRC under an industrial CASE award CASE/CNA/03/25.