**Vascular mapping by MRI and histology in a rat brain metastasis model with radiotherapy**

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**Introduction and objectives**

Disrupted vasculature is a key feature of tumours and understanding this disruption is clinically useful as altered blood flow influences tumour treatment. However, clinical imaging using MRI does not give direct information on vasculature, but rather indirect approximations. Here we use a rat model of brain metastasis to identify correlations between MRI measurements of tumour vasculature and gold-standard post-mortem histological analysis. Our objective was to allow prediction of histology using MRI, thus providing more detailed information than currently available.

**Methods**

Metastatic colonies were induced in BD-IX rats (n=12) by intracerebral injection of 1000 ENU-1564 cells. At week 3, half the rats received a single dose of 25Gy tumour irradiation. All animals underwent MRI at 9.4T at weeks 3 and 4, and the brains subsequently collected for histology. MRI included perfusion by MP-pCASL[1], cerebral blood volume (CBV), diffusion (ADC, 3 directions), anatomical imaging (pre- and post-Gd) and $T_1$ and $T_2$ mapping.

Brains were sectioned and processed histologically to produce stains for CD31 (vessels), pimonidazole (hypoxia), and cresyl violet (nuclei). Vessel stains were processed to produce maps of vessel density, percentage of area occupied by vessels, mean vessel cross-sectional area and mean vessel diameter. Pimonidazole stains were processed to produce maps of histologically hypoxic tissue. Dense cresyl violet staining (>2 SD above contralateral mean) was used to show tumour. Histology was co-registered with MRI by using a perspective transform to align individual sections with their corresponding MRI slice followed by combination of multiple 10µm histological sections to give a mean representation of the 1mm MRI section. Co-registered histological parameter maps were compared to MRI data. Regions were considered to be tumour if they were either hypoxic or contained a high ipsilateral cell density.

**Results**

Combining MRI modalities greatly increased the sensitivity for detecting histologically-identified tumour regions, whether or not they were hypoxic (0.80 for combined imaging *vs.* 0.54 for post-Gd $T_1$-weighted imaging alone, or 0.40 mean for any single MR modality). This improvement was particularly pronounced in the post-radiotherapy tumours (0.74 for combined imaging *vs.* 0.35 for post-Gd alone), a timepoint when accurate identification of tumour is more critical. This result is likely attributable to the marked increase in biological heterogeneity of the radiotherapy-treated tumours, with regions of decreased blood flow and volume, as well as cystic regions developing (high ADC, $T_1$ and $T_2$ values). The common compromise for increased sensitivity is decreased specificity but despite the marked increase in sensitivity here, specificity for tumour was only marginally decreased using the combinatorial approach (0.87 *vs.* 0.93 using post-Gd alone).

In the radiotherapy group, the outer rim of post-Gd enhancement was characterised by generally normal histological vessel parameters, with normal blood flow and volume. However, within the tumour core, differing pathologies were present. The outer portion of the tumour core exhibited fewer vessels, each larger. Both size and density dropped towards zero as regions became cystic.

**Discussion**

Multi-modal MRI is more sensitive for detecting the complete extent of a tumour than any single MR modality alone, particularly after radiotherapy. Further work is required to elucidate the exact relationships between vascular parameters as measured by histology and by MRI.

1. Larkin *et al*, JCBFM 2018