

An investigation of DCE-MRI as a non-invasive measure of angiogenesis in rectal cancer

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Introduction: Dynamic contrast-enhanced MRI (DCE-MRI) may be used in colorectal cancer to differentiate benign from recurrent malignant disease and to monitor the response of primary rectal cancer to neoadjuvant therapy^{1,2}. DCE-MRI measurements have been investigated as potential non-invasive surrogate markers of tumour angiogenesis in breast and cervical carcinoma^{3,4}. In this study we correlated DCE-MRI kinetic parameters with the histological markers of tumour angiogenesis (CD31 and vascular endothelial growth factor (VEGF) immunostaining) in primary rectal cancer.

Methods: 15 patients (median age 59 year old; range, 47-77) with proven primary rectal adenocarcinoma awaiting surgical resection underwent preoperative DCE-MRI. Spoiled gradient-echo (SGE) sequences with 8 different TE [5-75ms], TR=100ms, $\alpha=40^\circ$, 1 slice were used for R_2^* measurement using an IDL[®] least-squares fitting routine. Following this, T_1W DCE-MRI images were acquired using another SGE sequence (TE=4.7ms, TR=11ms, $\alpha=35^\circ$, 4 slices). 40 images were acquired every 12 seconds for 8 min 5s. An injection of 0.1mmol/kg Gd-DTPA was given using a power injector at 4ml/s during the 5th data acquisition point. The data was fitted to the Tofts and Kermod model⁵ using methods previously described⁶ and quantitative kinetic parameters were calculated; transfer constant [K^{trans}], leakage space [v_e], maximum contrast medium uptake [Gd-DTPA]. The proportions of enhancing pixels failing the modelling process were also recorded. Following this, a T_2^* -weighted GRE sequence was used to acquire data every 2 seconds over 2 minutes (TE=20ms, TR=30ms, $\alpha=40^\circ$, 1 slice) with 0.2mmol/kg Gd-DTPA injected at 4ml/s after 20s. These data were used to calculate relative blood volume (rBV), relative blood flow (rBF) and mean transit time (MTT) using the central volume theorem by applying a gamma variate fit function⁵. All calculations were performed using in-house software (Magnetic Resonance Imaging Workbench – Institute of Cancer Research, London). Regions of interest (ROI) were drawn around the tumour edge by a single experienced observer. Histograms of pixel data were obtained and median values were correlated with immunohistochemistry. Histological sections of the resected specimen in a similar (but not exact) orientation to the MRI were used for immunohistochemical analysis of CD31 and VEGF expression. CD31 score was derived by Chalkley counting of microvessels within tumour hot spots, whereas VEGF immunostain intensity was derived using spectral imaging (where the amount of light absorption by the histological section was proportional to the amount of immunostain present). Visual grading of the sections was also performed to generate a VEGF score (product of intensity [0-3] and percentage of immunostain [0-3]). In addition, preoperative serum VEGF levels were measured using the QuantiGlo[®] Human VEGF Immunoassay kit (R&D Systems Europe, UK). Spearman's rank correlation coefficient, r_s , was used to determine the correlation between MRI kinetic and immunohistochemical markers; the statistical significance was the 2-tailed p-value for rejecting the hypothesis of zero correlation.

Results: The median CD31 score was 5.6 microvessels (range, 2.9-8.5), VEGF stain intensity was 0.42 a.u (range, 0.34-0.53) and the VEGF score was 3 (range, 2-9). Mean VEGF immunostain intensity measured by spectral imaging correlated well with visual VEGF grading ($r_s=0.84$; $p<0.001$). There was no correlation between T_1 and T_2^* weighted DCE-MRI kinetic parameters. No correlation between T_1 - and T_2^* -weighted DCE-MRI kinetic parameters with mean CD31count was seen except for K^{trans} , which correlated inversely with the mean CD31 count ($r_s= -0.65$; $p<0.05$). In general, VEGF expression did not correlate with T_1 - and T_2^* -weighted DCE-MRI kinetic parameters except for [Gd-DTPA] which approached significance ($r_s=0.57$; $p=0.055$). R_2^* correlated inversely with mean VEGF immunostain intensity ($r_s=-0.58$; $p<0.05$) but not with CD31 immunostaining. Circulating serum VEGF levels did not correlate with any MRI parameter.

Discussion: Spectral imaging is an appropriate method of quantifying VEGF protein expression which correlates well with the traditional subjective grading method of stain intensity. We were surprised to note a lack of correlation between T_1 - and T_2^* -weighted DCE-MRI kinetic parameters which we have observed in ovarian and breast cancers. There was also a poor correlation overall between T_1 - and T_2^* -weighted DCE-MRI kinetic parameters and recognised tissue immunostains or serum markers of angiogenesis; we did not confirm a correlation between T_1 -weighted DCE-MRI parameters and serum VEGF as previously noted by George *et al*². A relatively small patient sample size, imperfect registration between the imaging and histological planes, global analysis method and the well recognised discrepancy between visible and perfused vessels in tumours may have contributed to the lack of correlations seen. Clearly, the relationship between imaging and histologic methods of assessing angiogenesis is not a simple one and our results underpin the need for further validation of DCE-MRI and R_2^* as non-invasive indicators of rectal cancer angiogenesis.

References

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