In vivo characterisation of tumour microstructure with VERDICT MRI

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Target Audience Biophysical modellers, diffusion MRI and cancer imaging researchers.

Purpose Diffusion-weighted (DW) MRI data, acquired with multiple gradient durations, spacings and directions, were used to probe the microstructure of two different human colorectal tumour xenograft models, non-invasively. For this purpose, a new mathematical tissue model was developed, which we named Vascular, Extracellular and Restricted DIffusion for Cytometry in Tumours (VERDICT).

Methods Two human tumour xenograft models were used as a system to explore differential tumour pathophysiology[1]. We defined a DW-MRI protocol to cover the whole measurement space and then fit the VERDICT model to the data to suggest which features of the cellular architecture cause the differences in the raw signals. We also compare our model's performance with the apparent diffusion coefficient (ADC) and intravoxel incoherent motion (IVIM)[2] models.

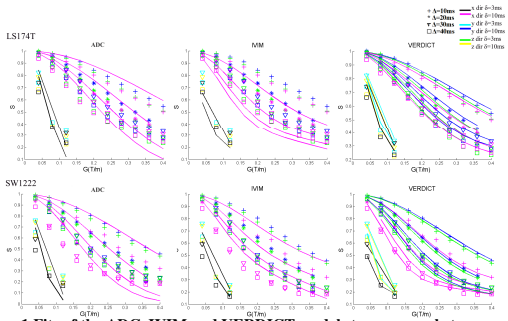

Figure 1 Fits of the ADC, IVIM and VERDICT models to an example tumour sample from each cell line. The symbols represent the scan data and the lines show the fit by the models. The normalised signal S is plotted for all Δ, δ as a function of |G| for all diffusion directions.

VERDICT Model The model characterises water diffusion in a vascular, extracellular-extravascular space (EES) and intracellular (IC) compartments in tumours. These three tissue components are common to a wide range of different types of tumours, although their precise nature may vary. VERDICT is the sum of three parametric models, each describing the diffusion MR signal in a separate population of water from one of the three components: 1) Signal S_1 comes from IC water trapped inside cells. 2) Signal S_2 comes from EES water adjacent to, but outside cells and blood vessels. 3) Signal S_3 arises from water in blood in the capillary network. The total diffusion MR signal is the weighted sum of the signals from each compartment, with weights summing to 1. To model signal for the IC compartment we use spheres[3]. The model for the EES compartment uses a diffusion tensor (DT) model[4]. Here we constrain it to be isotropic. The vascular pseudo-diffusion model also uses a DT model. Preliminary experiments[5] suggest a high degree of anisotropy in this component in our data, so we use a DT that assumes perfusion in the vascular space is oriented along a single direction. The final set of parameters of the VERDICT model to be estimated in this study was 7: f1 (vascular volume fraction), f2 (EES volume fraction), f3 (IC volume fraction), P (pseudo-diffusion), R (cell radius), κ, λ (define the main orientation of the anisotropic DT).

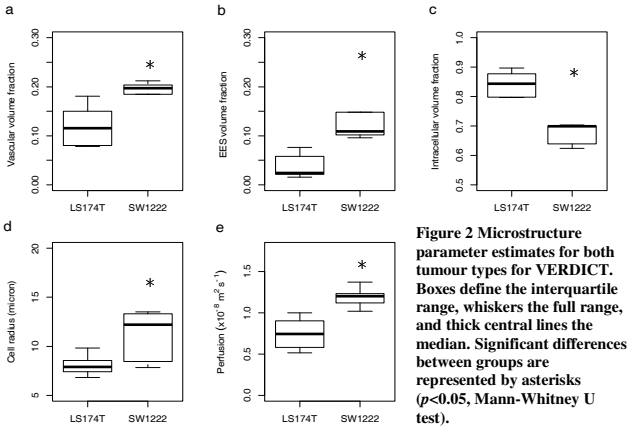

Figure 2 Microstructure parameter estimates for both tumour types for VERDICT. Boxes define the interquartile range, whiskers the full range, and thick central lines the median. Significant differences between groups are represented by asterisks (p<0.05, Mann-Whitney U test).

Biological Tumour Models We used two human colorectal cell lines: SW1222 (n=6) and LS174T (n=6). Xenografts formed from these cell lines exhibit markedly different phenotypes: SW1222 tumours form a well differentiated cellular structure with regular gland-like structures and dense vasculature, whilst LS174T is moderately to poorly differentiated, with tightly packed cells and low vascular perfusion[1].

MRI Acquisition DW-MR images were acquired *in vivo* with a 9.4T scanner. We used a pulse-gradient spin-echo sequence with 46 diffusion weightings: diffusion times Δ=10, 20, 30, 40ms, gradient durations δ=3ms for all Δ and δ=10ms for Δ=30, 40ms; gradient strength |G| varied from 40-400mT/m in ten steps of 40mT/m for δ=3ms and |G|= 40, 80,120mT/m for δ=10ms. Diffusion gradients were placed along the three imaging coordinate axes. We normalised the data for T_2 dependence. We also performed a separate diffusion tensor imaging (DTI) acquisition of 42 directions (b value 2.2x10^{9}s/m²). The acquisition time was 2.5 hours/animal. Field of view: 25x25mm, matrix size: 64x64 with 5x0.5mm slices.

Response to gemcitabine LS174T tumours were scanned using a shortened DW-MRI acquisition that maintained the main characteristics of the full protocol, but lasted 1 hour. Mice were then administered gemcitabine (120mg/kg, n=5) or saline (n=5) and scanned again 5 hours later.

Model fitting We manually segmented the whole tumour region of interest and used similar iterative optimization procedure to [6] that accounts for local minima and Rician noise. We fixed the diffusion coefficient to a value that provided the best fit to the data: $d_{IC}=d_{EES}=9\times10^{-10}$m^2/s.

Table 1: Microstructure parameters measured by VERDICT, compared with histological (or other MRI) methods. The ratio of the value of each parameter measured in each tumour type is also shown, for comparison. No histological values for the EES volume were available for either tumour type.[+] Measured with histology, [†] 1, [*] 7

	Reference			VERDICT		
	LS174T	SW1222	Ratio (SW/LS)	LS174T	SW1222	Ratio (SW/LS)
Cell diameter (μm)	16.1 ± 0.8*	22.5 ± 2*	1.4	18.5 ± 0.9	25.5 ± 1.2	1.4
Intracellular volume	0.79 ± 0.01 [+]	0.80 ± 0.02 [+]	1.0	0.84 ± 0.02	0.68 ± 0.02	0.8
Vascular volume	0.08 [†]	0.25 [†]	3.1	0.12 ± 0.02	0.22 ± 0.01	1.8
Pseudo-diffusion (Perfusion for reference)	0.15 ± 0.11 mL g^{-1} min^{-1}	0.28 ± 0.16 mL g^{-1} min^{-1}	1.9	(7.4 ± 0.8) × 10^{-4} m^2 s^{-1}	(11.8 ± 0.5) × 10^{-4} m^2 s^{-1}	1.6
EES volume	-	-	-	0.03 ± 0.01	0.19 ± 0.01	1.6

Results The VERDICT model captured broad trends in the data for both cell lines, while ADC and IVIM exhibited clear departures from the data, indicating that these simple models were unable to capture the variation in the signal (Fig.1). Results were similar for all samples. All VERDICT parameter estimates were significantly different between the two cell lines (Fig.2). We found good agreement between histological and VERDICT estimates especially for the cell diameter and the perfusion coefficient (Table 1). At 5 hours following gemcitabine dosing, IC fraction significantly decreased and vascular volume significantly increased (P<0.05, Mann-Whitney U-test). VERDICT estimates from control tumours did not change significantly. No difference between ADC and IVIM parameters was found between tumour types or following gemcitabine dosing.

Discussion & Conclusions This study proposes a mathematical model to noninvasively quantify microstructural parameters in tumours, which we evaluated in two differing colorectal tumour xenograft models. We found that VERDICT parameter estimates accurately reflected known differences in the microstructure of the two tumour types. The reduction in IC volume fraction observed following gemcitabine therapy is consistent with apoptotic volume decrease caused by a reduction in cell water content, which has previously been identified as a precursor to apoptosis[8]. These trends were not found in ADC or IVIM parameter estimates. Thus VERDICT could offer a powerful new set of biomarkers for differentiating between tumours and assessing treatment response.

References & Acknowledgements 1 Folerin et al, Microvasc Res (2010) **2** Le Bihan et al, Radiology (1988) **3** Murday and Cotts, JChemPhys, (1984), **4** Basser et al, Biophys J, (1994), **5** Panagiotaki et al, ISMRM 2013 **6** Panagiotaki et al NeuroImage (2012) **7** Walker-Samuel et al, ISMRM (2013) **8** Kasim et al, Apoptosis (2013).
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