Noninvasive Quantification of Solid Tumor Microstructure Using VERDICT MRI

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Abstract

There is a need for biomarkers that are useful for noninvasive imaging of tumor pathophysiology and drug efficacy. Through its use of endogenous water, diffusion-weighted MRI (DW-MRI) can be used to probe local tissue architecture and structure. However, most DW-MRI studies of cancer tissues have relied on simplistic mathematical models, such as apparent diffusion coefficient (ADC) or intravoxel incoherent motion (IVIM) models, which produce equivocal results on the relation of the model parameter estimate with the underlying tissue microstructure. Here, we present a novel technique called VERDICT (Vascular, Extracellular and Restricted Diffusion for Cytometry in Tumors) to quantify and map histologic features of tumors in vivo. VERDICT couples DW-MRI to a mathematical model of tumor tissue to access features such as cell size, vascular volume fraction, intra- and extracellular volume fractions, and pseudo-diffusivity associated with blood flow. To illustrate VERDICT, we used two tumor xenograft models of colorectal cancer with different cellular and vascular phenotypes. Our experiments visualized known differences in the tissue microstructure of each model and the significant decrease in cell volume resulting from administration of the cytotoxic drug gemcitabine, reflecting the apoptotic volume decrease. In contrast, the standard ADC and IVIM models failed to detect either of these differences. Our results illustrate the superior features of VERDICT for cancer imaging, establishing it as a noninvasive method to monitor and stratify treatment responses. Cancer Res; 74(7): 1902–12. ©2014 AACR.

Introduction

Classical histology provides the gold standard for diagnosis of cancer, offering the sole method to access specific information on the cellular architecture of tissue such as the viability of cells, their size, and packing density. However, histology requires invasive extraction of the tissue, typically through biopsy, and is generally limited to small sampling regions. Noninvasive techniques, such as MRI and computed tomography, are widely used in clinical research for tumor detection and for measuring progression and regression via gross volumetric changes. However, such changes in tumor size can take many weeks or months to occur, and more sensitive imaging techniques for probing the tumor microenvironment before and following therapy are urgently required.

Diffusion-weighted MRI (DW-MRI) of tumors is increasingly used as a research tool, both for diagnostic imaging (1) and measuring response to therapy (2). It is sensitive to the displacement of water particles at length scales much smaller than the image resolution and as water mobility is influenced by interactions with cellular structures, DW-MRI can be used to infer information about the local tissue architecture for the whole tumor. Most studies have used the technique in its simplest form by summarizing water mobility via the apparent diffusion coefficient (ADC; refs. 1–4) often assuming that reduced ADC reflects increased cellularity. However, while some studies have found a negative correlation between tumor ADC and cellularity (1, 3), others have found no such correlation (4, 5). The inconsistency most likely arises because ADC is a gross measurement that conflates various physiologic parameters, including cell density, size, shape, permeability, subcellular architecture, and vascular perfusion effects. These biophysical mechanisms affect the ADC in different ways that can compete and even cancel each other, confounding overall specificity.

Model-based DW-MRI approaches can potentially separate these effects to provide estimates of specific histologic features and thus offer a much richer description of the underlying tissue on which to base a diagnosis or grade assessment. This has proven successful in brain imaging, where geometric models of brain tissue predict the measured signal more accurately (6). However, the challenges of modeling tumor tissue are considerably greater.

VERDICT couples DW-MRI to a mathematical model of tumor tissue to access features such as cell size, vascular volume fraction, intra- and extracellular volume fractions, and pseudo-diffusivity associated with blood flow. To illustrate VERDICT, we used two tumor xenograft models of colorectal cancer with different cellular and vascular phenotypes. Our experiments visualized known differences in the tissue microstructure of each model and the significant decrease in cell volume resulting from administration of the cytotoxic drug gemcitabine, reflecting the apoptotic volume decrease. In contrast, the standard ADC and IVIM models failed to detect either of these differences. Our results illustrate the superior features of VERDICT for cancer imaging, establishing it as a noninvasive method to monitor and stratify treatment responses.
accurately than simple ADC models and provide estimates of specific tissue features, such as fiber orientation distribution, diameter distribution, and packing density (6–9). A few early attempts to model the diffusion signal in a similar way in tumors exist. The intravoxel incoherent motion (IVIM) model (10) assumes that tissue water resides in two nonexchanging compartments: vascular (pseudo-diffusing water inside blood vessels) and nonvascular (diffusing water in and around cells). IVIM has been used to study various cancer types, such as breast (11), prostate (12), pancreatic (13), and kidney (14) tumors, showing improvement in data description compared with the ADC. However, while the IVIM model acknowledges and separates out the additional signal from vascular water, its description of diffusion in the cellular component of the tissue remains simplistic; just as for the ADC, it does not account for cellular geometry and compartmentalization. Xu and colleagues (15) used a geometric tissue model of cancer cells with computer simulations to demonstrate the limitation of considering ADC alone, but made no attempt to estimate the model parameters from measured data.

Here, we propose a noninvasive imaging paradigm, called VERDICT (Vascular, Extracellular and Restricted Diffusion for Cytometry in Tumors), for quantifying microstructural features of tumors, in vivo, and demonstrate its use in two well-characterized tumor xenograft models of colorectal cancer. The VERDICT model includes the three primary components: (i) vascular, (ii) extracellular–extravascular space (EES), and (iii) intracellular water that influence the DW-MRI signal and exist in a wide range of tumors. Fitting the model to DW-MRI datasets with various diffusion times and diffusion weightings (see Materials and Methods) provides estimates of the size and packing density of the cells, the vascular and EES volume fractions as well as the pseudo-diffusion coefficient associated with blood flow. The aims of the study are: (i) to compare the VERDICT model with current models (ADC and IVIM); (ii) to investigate the accuracy of VERDICT parameter estimates in representative tissue samples (colorectal xenograft models) by comparison with histology; (iii) to evaluate the sensitivity of the VERDICT framework to differences in tumor pathophysiology; (iv) to assess the ability of VERDICT to detect response to therapy. Two experiments were performed. The first addressed aims 1, 2, and 3 by fitting VERDICT, IVIM, and ADC models to DW-MRI signals from both cell lines. The second experiment addressed aim 4 by assessing the acute response to a chemotherapeutic agent (gemcitabine). For this experiment, we aimed to detect small, acute changes in cell size induced by gemcitabine.

Materials and Methods

Animal models

All experiments were performed in accordance with the local ethical review panel, the UK Home Office Animals Scientific Procedures Act 1986, and United Kingdom Co-ordinating Committee on Cancer Research guidelines (16). A total of \(5 \times 10^6\) human colorectal adenocarcinoma cells (LS174T and SW1222) were injected subcutaneously into the flanks of female nude MF1 NU/NU mice (2–3 months, 20–25 g). Mice were scanned at 5 weeks following inoculation, when tumors were 8 to 12 mm in diameter. Subcutaneous xenografts formed from these cell lines exhibit markedly different phenotypes: SW1222 tumors form a well-differentiated cellular structure featuring regular gland-like structures and dense vasculature, while the LS174T is moderately to poorly differentiated, with tightly packed cells and low vascular perfusion (17, 18).

The VERDICT model

The mathematical model of the VERDICT framework characterizes water diffusion in vascular, EES, and intracellular compartments in tumors. These three tissue components are common to a wide range of different types of tumors, although their precise nature may vary significantly. Mathematically, VERDICT is the sum of three parametric models, each describing the diffusion magnetic resonance signal in a separate population of water from one of the three components:

- Signal \(S_1\) comes from intracellular water trapped inside cells.
- Signal \(S_2\) comes from EES water adjacent to, but outside cells and blood vessels.
- Signal \(S_3\) arises from water in blood undergoing microcirculation in the capillary network.

The model does not incorporate exchange between the three water populations. The total magnetic resonance signal for the multi-compartment VERDICT model is:

\[
S = \sum_{i=1}^{3} f_i S_i
\]

where \(f_i\) is the proportion of signal with no diffusion weighting \((b = 0)\) from water molecules in population \(i\), \(0 \leq f_i \leq 1\), and \(\sum_{i=1}^{3} f_i = 1\).

To model the signal for the intracellular compartment, we can use any one of multiple models for restricted diffusion in domains of various shapes (19, 20). Here, we use spheres because the cells in our tumors are quite isotropic, but ellipsoids or cylinders are other possibilities. The model for the EES compartment uses a diffusion tensor model (21). Here, we constrain it to be isotropic, but if the cells and their arrangement are anisotropic, this diffusion tensor should also be anisotropic. The vascular pseudo-diffusion model also uses a diffusion tensor model. Preliminary experiments (22) suggest a high degree of anisotropy in this component of our data, so we used a diffusion tensor that assumes pseudo-diffusion in the vascular space is oriented along a single direction, but more isotropic models may be appropriate in situations in which the capillaries have less coherent orientation (see Supplementary Data). In total, seven parameters of the VERDICT model were estimated in this study: \(f_1, f_2, f_0, P, R, \theta, \varepsilon\). where \(f_1\) is the volume fraction of the intracellular compartment, \(f_2\) is the volume fraction of the EES compartment, \(f_0\) is the volume fraction of the vascular compartment, \(P\) is the pseudo-diffusion coefficient, \(R\) is the cell radius parameter, which we refer to as cell radius index, as it relates to the distribution of actual radii in a nontrivial
way (7), and \( \theta \) and \( \psi \) define the main orientation of the anisotropic diffusion tensor.

**In vivo MRI**

DW-MR images were acquired in vivo, using a 9.4T scanner (Agilent) with maximum gradient strength 400 mT/m. We used a pulsed gradient spin echo (PGSE) sequence (23) with 46 diffusion weightings: diffusion times \( \Delta = 10, 20, 30, \) and 40 milliseconds, gradient durations \( \delta = 3 \) milliseconds for all \( \Delta \) and \( \delta = 10 \) milliseconds for \( \Delta = 30 \) and 40 milliseconds; gradient strength \( |G| \) varied from 40 to 400 mT/m in 10 steps of 40 mT/m for \( \delta = 3 \) milliseconds and \( |G| = 40, 80, \) and 120 mT/m for \( \delta = 10 \) milliseconds. Separate images were acquired with diffusion gradients placed along each of the three imaging coordinate axes. We used minimum echo times (TE) for each \( \delta \) and \( \Delta \) combination to maximize the signal-to-noise ratio (SNR) and chose repetition times (TR) to minimize gradient heating effects. For each combination of diffusion weighting parameters, we acquired \( b = 0 \) images to correct for \( T_1 \) and \( T_2 \) dependence. A separate diffusion tensor imaging (DTI) acquisition was also performed using a 42-direction scheme with \( b = 1,000 \) s/mm\(^2\) and six unweighted \((b=0)\) measurements. Imaging parameters for the diffusion-weighted imaging and DTI acquisitions were: in-plane field of view 25 mm \( \times \) 25 mm, matrix size 64 \( \times \) 64, with 5 mm \( \times \) 0.5 mm slices. Total acquisition time for one animal was 2.5 hours.

**Response to gemcitabine**

For the therapy study, a shortened version of the imaging protocol was used with 13 diffusion weightings, as well as the DTI scan. The protocol was chosen to fit within a scanning time constraint of 1 hour that we impose to achieve sufficient temporal resolution for the experiment while allowing investigation for an effect at just 5 hours postdosing. The 13 weightings are a subset of the 46 in the full protocol including long and short diffusion times and a range of gradient strengths chosen to cover the range of signal attenuations evenly, and thus support a good fit of the model to the data. Five mice bearing LS174T xenografts were scanned before and 5 hours after intraperitoneal administration of 120 mg/kg of gemcitabine (Gemzar; Eli Lilly and Company) in 0.2 mL saline (24). A control group \((n = 5)\) LS174T was administered 0.2 mL saline and underwent an identical imaging protocol. The specific imaging parameters for the response study were \( \Delta = 10, 30, \) and 40 milliseconds with \( \delta = 3 \) milliseconds for \(|G| = 120, 280, \) and 360 mT/m and \( \Delta = 30 \) and 40 milliseconds with \( \delta = 10 \) milliseconds for \(|G| = 40 \) and 120 mT/m. The DTI acquisition had 15 directions and one \( b = 0 \) measurement. Total acquisition time for each animal was 1 hour, although the same number of measurements typically requires around 15 minutes on a human system.

**Model fitting**

VERDICT uses a similar iterative optimization procedure (8) for model fitting that accounts for local minima and Rician noise. First, the fitting was performed with data averaged over all voxels within a tumor region of interest (ROI); subsequently, the fitting was repeated in each voxel. A set of model parameters was constrained in the optimization using transformations to limit the range of each parameter to biophysically meaningful values. The cell radius index \( R \) was constrained so that \( 0.1 \mu m \leq R \leq 20.1 \mu m \), the pseudo-diffusion coefficient \( P \) was constrained to be larger than free water diffusion \([P \geq 3.05 \mu m^2/\text{ms} (25)]\), and the volume fractions of all compartments were constrained to \([0,1]\) and to sum to 1. To increase the stability of the fitting, we fix the diffusion coefficient of the intracellular and EES compartments to a value that provides the best fit to the data: \( P_{\text{best}} = d_{\text{B}} \| = 9 \times 10^{-10} \mu m^2/\text{s} \). The implementation will be available in the open source Camino toolkit at http://cmic.cs.ucl.ac.uk/camino/.

**Statistical analysis**

This was performed using the R software platform. Results were presented as box-and-whisker or ladder plots. Significance for discriminating the two cell lines was assessed by Mann–Whitney \( U \) test, and response to therapy by a paired Mann–Whitney \( U \) test. \( A < 0.05 \) was considered to be significant.

**Histologic analysis**

To validate the estimates we obtain from VERDICT, we used histologic analysis to acquire independent measurements of cell size, cell density, and detection of apoptotic cells. For the assessment of apoptosis in vitro at 24 and 72 hours, we use flow cytometry. Details of each method are in Supplementary Materials and Methods.

**Results**

**Experiment 1: characterization of tumor types**

The aim of the first experiment was to characterize differences between two tumor xenografts SW1222 and LS174T, using different models (ADC, IVIM, and VERDICT). Figure 1 shows that VERDICT captures the broad trends in the data for both cell lines by comparing the measured and predicted normalized diffusion signal \( S \) as a function of the imaging parameters (gradient strength \(|G|\), diffusion time \( \Delta \), pulse width \( \delta \), and gradient orientation; Supplementary Fig. S1 shows the VERDICT fit to all the samples). Conversely, also in Fig. 1, the ADC and IVIM models exhibit clear departures from the data for both cell lines (note that both the ADC and IVIM models are inherently isotropic so the predicted signals for the \( x, y, \) and \( z \) gradient directions overlap). These departures illustrate an inability of ADC and IVIM to model the variation in the signal; results are similar for all the tumor samples. These results show that VERDICT explains the DW-MRI data for both cell lines better than current models (aim 1). Supplementary Fig. S2 shows an example of DW-MRI data averaged over a ROI in the tumor and magnetic resonance images for various diffusion weightings (\( b \) values), to illustrate data quality in both datasets.

**Parameter estimation and comparison with histology**

Table 1 shows the model parameter estimates with the reference values from histology and other MRI methods. The cell radius indices \( R \) for SW1222 tumors were significantly
greater than those for LS174T by a factor of 1.4 as in histology. The absolute size estimates took physiologically realistic values, ranging from 7 to 14 μm (cell diameter of 14–28 μm; Fig. 2). As expected, because of shrinkage during preparation, cell diameters measured on histologic sections were on average smaller (12.3%) than VERDICT estimates.

Of each of the volume fraction parameters, VERDICT ascribed the greatest value to the intracellular space, with

Table 1. Microstructure parameters measured by VERDICT compared with histologic (or other MRI) methods

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Ratio (SW/LS)</th>
<th>VERDICT</th>
<th>Ratio (SW/LS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS174T</td>
<td>SW1222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell diameter, μm</td>
<td>16.1 ± 0.8a</td>
<td>22.5 ± 2a</td>
<td>1.4</td>
<td>18.5 ± 0.9</td>
</tr>
<tr>
<td>Intracellular volume</td>
<td>0.79 ± 0.01a</td>
<td>0.80 ± 0.02a</td>
<td>1.0</td>
<td>0.84 ± 0.02</td>
</tr>
<tr>
<td>Vascular volume</td>
<td>0.08b</td>
<td>0.25b</td>
<td>3.1</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Pseudo-diffusion (perfusion for reference)</td>
<td>0.15 ± 0.11 mL/g/min</td>
<td>0.28 ± 0.16 mL/g/min</td>
<td>1.9</td>
<td>(7.4 ± 0.8) × 10⁻⁹ m²/s</td>
</tr>
<tr>
<td>EES volume</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

NOTE: The ratio of the value of each parameter measured in each tumor type is also shown. No histologic values for the EES volume were available for either tumor type.
°Measured from histologic sections in this study.
°Folarin and colleagues (18).
°Walker-Samuel and colleagues (26).
values from LS174T on an average 1.2 times greater than from SW1222 tumors (mean volume fraction 0.84 ± 0.02 and 0.68 ± 0.02, respectively). The analysis of equivalent histologic sections from the tumors found a measured intracellular volume fraction ratio of 1.0 between the SW1222 and LS174T tumors (SW/LS). For the EES volumes, the estimates in SW1222 tumors were larger than those in LS174T. VERDICT also distinguished between the two tumor types based on their vascular characteristics. Both the vascular volume fraction and pseudo-diffusion coefficient $P$ were significantly greater in SW1222 tumors than in LS174T tumors. This characteristic difference between the two cell lines has been previously described by Folarin and colleagues (18) in a study conducted at the same laboratory and using the same stock of cells as used in this study. The absolute values for mean vascular volumes were 0.12 and 0.22 for LS174T and SW1222 tumors, respectively, consistent with previous measurements of 0.08 and 0.25 (18), again, performed at the same laboratory with the same samples as the current study, even though the ratio for VERDICT was 1.8 and the measured vascular volume fraction ratio of the reference was 3.1. Moreover, our own measurements using arterial spin labeling MRI (26) gave perfusion estimates that were 1.9 times larger in SW1222 than LS174T tumors, and this ratio from VERDICT estimates was 1.6. This experiment explored the accuracy of VERDICT estimates in comparison with histology, satisfying aim 2.

The comparison between the two cell lines found all VERDICT parameter estimates significantly different (Fig. 3), distinguishing the microstructure of the two tumor types (aim 3). Figure 3F–J displays parameter estimates for the ADC and the IVIM models. None of the parameter estimates from either the ADC or the IVIM models show significant differences between the two tumor types, which highlights the additional discriminatory power of the VERDICT model. We also present, for completeness, the orientation angle estimates for all the samples in Supplementary Table S1.

**Parameter maps**

Figure 4 shows microstructural parameter maps for the volume fractions of each model component, the cell radius index $R$ and the perfusion coefficient $P$ from a voxel by voxel fitting of VERDICT. As with the whole ROI analysis, spatial mapping allows clear differences between the two cell lines to be discerned, particularly via the intracellular volume fraction, the radius index, and the pseudo-diffusion coefficient (aim 3). Also they reveal similar trends to the averaged signal results in Fig. 3A, for all parameters. This demonstrates the ability of VERDICT to investigate tumors at a regional basis to reveal potential heterogeneity.

**Experiment 2: response to gemcitabine**

Flow cytometry of LS174T cells treated with 5 μmol/L gemcitabine for 24 hours showed a >5-fold increase in apoptosis, compared with untreated controls (15.5% ± 3.2% and 2.4% ± 2.3%, respectively). This confirms the sensitivity of the cell line to gemcitabine. *In vitro* microscopy of LS174T cell cultures revealed a significant decrease in cell size from 5 hours following exposure to gemcitabine, relative to controls, which is consistent with the time course of apoptotic volume decrease (AVD) reported previously (Fig. 5F; ref. 27).

For *in vivo* VERDICT data analysis, parameter estimation pre- and post-gemcitabine therapy was performed by fitting the model to the averaged signal over the whole tumor ROI for each dataset. No significant change in any parameter estimate was observed in a control group injected with saline solution ($P > 0.05$). The ADC and IVIM model estimates showed no significant change in treatment and control groups. At 5 hours following gemcitabine administration, VERDICT showed a significant increase in vascular volume fraction and a significant decrease in intracellular volume fraction ($P < 0.05$, Mann–Whitney paired $U$ test) in Fig. 5A–E, identifying response to therapy (aim 4). Most samples also showed a small increase in cell radius (mean 17% in three samples) and in pseudo-diffusion coefficient (mean 11.6% in three samples), but the overall change was not significant.

Histologic assessment showed no significant increase in staining for caspase-3 (a marker of apoptosis) at 5 hours following gemcitabine administration; however, a significant increase in the percentage caspase-stained cells was measured at 24 and 72 hours, relative to controls (Fig. 5G; control, 18% ± 3%; 24 hours, 33% ± 5%; 72 hours, 27% ± 5%; $P < 0.05$). No change in cell size was visible on histologic sections at 5 hours.
Discussion

Through its use of the dispersion of endogenous tissue water, DW-MRI in combination with an appropriate mathematical model has a promising role as a quantitative imaging technique for probing complex tumor microstructure. Such noninvasive approaches are urgently needed in the clinic for grading disease and assessing response to therapy, and to replace invasive and often practically challenging techniques such as needle biopsy. Previous studies using DW-MRI in tumors have concentrated on the use of ADC and IVIM techniques and, despite numerous attempts to establish a correlation between tumor microstructure and their grading.
parameter estimates, the results remain equivocal. VERDICT provides a more effective probe of microstructure than ADC and IVIM, significantly enhancing DW-MRI tumor studies. We expect the technique to be broadly applicable in a wide variety of biologic tissue and adjustable for different types of tumors, although adjustments to the models for each individual compartment are likely to be necessary for best performance in different applications.

This study investigated the human colorectal carcinoma cell lines SW1222 and LS174T, whose markedly differing phenotypes provide a useful comparative model system. VERDICT provided a better fit to in vivo data than both the standard ADC and IVIM models. The parameter estimates of VERDICT were broadly consistent with histology and arterial spin labeling MRI, performed either during the study, or taken from previously published reports in the literature from our own laboratory, using the same stock of cells as used in this study. Our results also showed that, unlike ADC and IVIM, VERDICT identified statistically significant differences between the two tumor types, such as greater blood volume, pseudo-diffusion coefficient, cell size, EES volume fraction, and lower intracellular volume fraction in SW1222 tumors, compared with LS174T tumors.

Some discrepancies between VERDICT estimates and our reference values do appear. Minor disagreement was observed for the intracellular volume fraction, which VERDICT predicted to be larger for LS174T than SW1222, while histology did not. These differences may arise for various reasons, for example, due to limitations of the model (discussed later), imperfect parameter estimates from histology [sectioning and/or fixation can significantly alter the original state of the tissue and the water it contains (28)], and bias and inaccuracy in the use of stereologic techniques to infer three-dimensional structure.

VERDICT was also used to detect changes induced by a chemotoxic agent. Flow cytometry demonstrated sensitivity of LS174T cells to gemcitabine, with a significant increase in apoptosis at 24 hours following dosing, which is in agreement with previous studies (29). At 5 hours following administration of gemcitabine, VERDICT estimates of intracellular volume significantly decreased by 8.5%. An in vitro experiment also revealed a significant decrease in LS174T cell size from 5 hours after gemcitabine dosing, while the size of control cells remained constant. In contrast, no change in cell size could be detected on histologic sections, although the tissue processing associated with histology could well mask this effect. No
significant increase in immunohistochemical staining of tumor sections for caspase ("executioner" proteins that are upregulated during apoptosis) or DNA double strand breaks was found at 5 hours. However, biochemical effects would not necessarily be expected at this early time point. Caspase staining was significantly increased at 24 and 72 hours. The change in cell size at 5 hours following dosing identified with in vivo VERDICT and during in vitro experiments, before caspase upregulation, are consistent with previous descriptions of AVD (27). This raises the exciting possibility that in vivo detection of AVD with VERDICT could offer an early response biomarker that is identifiable before histologically measurable effects.

Less anticipated was the significant increase in blood volume that was also measured. Vascular effects caused by chemotherapy have been reported previously, such as in breast cancer at 2 months (30) and 7 days (31) following therapy. This acute effect could also potentially be due to antivascular effects caused by apoptosis of endothelial cells (32, 33). Gemcitabine has been found to induce a transient pulmonary constriction within 30 minutes of administration into an isolated lung perfusion model, which is further suggestive of a direct vascular effect (34). Thus, the increase in pseudo-diffusion measured by VERDICT could either be due to a tumor-specific effect or due to systemic vascular toxicity.

The key features of the VERDICT model that enable it to fit the data better than ADC or IVIM are: (i) signal anisotropy, which simple ADC and IVIM models do not capture, but VERDICT does (here in the vascular compartment); and (ii) restricted diffusion, which neither ADC nor IVIM model incorporate, but VERDICT does with the intracellular compartment. Signal anisotropy is evident in Fig. 1 and Supplementary Fig. S2 from the separation of the signals with the same b value but different direction. However, anisotropic versions of the ADC model (the diffusion tensor model (21)) and the IVIM model (a two-tensor model), as well as an isotropic version of VERDICT (with an isotropic vascular compartment), are also inferior to the VERDICT model (Supplementary Fig. S3). Thus, both additional features of the VERDICT model are necessary to explain the signal in this application.

Wider use and acceptance of the VERDICT technique will rely on its implementation in the clinic, which places limits on acquisition times required for good patient compliance.
The acquisition protocol of the first DW-MRI experiment was purposefully designed to establish the best form for the model, and for supporting feasible and stable microstructure parameter estimation rather than for use as a working protocol. The reduced protocol used in the second experiment is representative, in terms of number of measurements, of what might be feasible to acquire clinically. This protocol returned parameter values comparable with those found in the first experiment, which suggests that prospects are good for finding an economical protocol for clinical acquisitions using, for example, experiment design optimization, as in ref. 35.

An important issue in translation of the VERDICT framework to clinical applications is the limited gradient strength available on clinical magnetic resonance systems, which typically have 40 to 60 mT/m rather than 400 mT/m in the animal scanner we used in this study. Cells in many human tumors have a size similar to the cells in the xenograft models we used here, that is, around 15 μm. The standard PGSE sequence with a gradient strength of 60 mT/m is sensitive to pore sizes with diameters of 5 to 25 μm (35, 36), although smaller pores are still detectable but can be identified only as “small” rather than sized accurately (7, 35). Thus, VERDICT may be best suited to applications where the cell size fits within the 5 to 25 μm range. Limited gradient strength means that SNR is lower in data from clinical systems compared with animal systems. Although voxels are larger, which mitigates the effect to some extent, we can expect a reduction of the SNR of 20 in the data presented here to as low as 10 in clinical applications. We believe such data can still support estimation of the VERDICT parameters, as models of similar complexity, such as the diffusion tensor model, are routinely fitted to data of similar SNR in human brain imaging applications. Nevertheless, applications will require careful experiment design to maximize the precision and utility of the recovered parameters. Once applications are established, the stability of the VERDICT parameter estimates across centers is also likely to be similar to that of the diffusion tensor model, although we would hope that through the use of experiment design optimization during the early translation phases, we can agree on common imaging protocols from the outset to minimize cross-center variation.

New applications are likely to require some adaptation of the model to suit the microstructure under investigation. We expect that the set of compartments (vascular, EES, and restricted) is likely to suit a wide variety of tumors and other tissue types. However, the precise models for each compartment are likely to require tuning depending on the shape and arrangement of cellular structures. This can require significant development informed from both histology and pilot MRI studies following a similar procedure to ref. 8, which identifies the best model from a set of candidates for white matter tissue, and which gives some detail of the similar procedure that led to the choice of model for this study (22). Once a good candidate model for a clinical application is identified, widespread adoption will require further steps involving patient-by-patient validation, for example of parameter estimates against biopsy histology, which is often available from the clinical diagnostic routine.

Although more complex than current models for DW-MRI in tumors, VERDICT is still a major simplification of real tissue microenvironment. For example, it is well known that tumor vasculature is leaky, leading to significant exchange of water between the vascular and other spin populations; VERDICT does not currently model this effect. We specifically designed the model to have the minimum number of parameters required to fit the data and found that including any additional parameters does not significantly improve the fit and destabilizes the model, increasing the variance of parameter estimates. The effects of exchange between compartments most likely manifest as changes in the estimated volume fractions of the three tissue components. Indeed, we observe that the SW1222 tumors have higher EES volume fraction than the LS174T tumors, which could potentially arise from its highly fenestrated vascular structure. Another assumption of VERDICT is that cells are perfectly spherical and nonspherical cells tend to cause the radius index to overestimate the actual radius through similar mechanisms to those discussed in ref. 7. Because of the inherent modeling limitations, it is important to remember when interpreting the parameter estimates from any model-based technique that the model is an approximation. Other effects not accounted for by the model may well influence the parameters designed to capture one particular effect.

Other useful histologic features may be accessible from other kinds of DW-MRI measurement, warranting development of more intricate models. For example, it may be possible to estimate permeability or exchange time parameters via stimulated echo measurements, as in ref. 37, or double pulsed-field gradient (dPFG) measurements, as in ref. 38. With varying echo and repetition times, we may be able to estimate compartmental relaxation (39). It may be possible to estimate pore-size distributions, as in refs. 6 and 8 with sufficient SNR, for example, by spatial smoothing of the diffusion-weighted imaging data or enforcing spatial continuity of parameter estimates. Pore shapes are potentially accessible through dPFG (40). Oscillating gradient DW-MRI provides measurements for shorter diffusion times, potentially improving sensitivity to smaller structures (41–43).

Conclusion

This study proposes the VERDICT acquisition and mathematical model to noninvasively estimate microstructural parameters in tumors, which we evaluated in two distinct human colorectal carcinoma xenografts. We found that the estimated parameters accurately reflected known differences in the microstructure and pathophysiology of the two tumor types, such as cell size, pseudo-diffusion, and cell and vascular volume fractions. This approach could potentially provide new, powerful biomarkers of tumor grade and progression, which could lead to the establishment of techniques for noninvasive treatment stratification. A key advantage of this technique is its ability to characterize whole-tumor microstructure, in contrast to traditional histology that samples only a very small region. Finally, the model detected microstructural changes, in particular a cell volume decrease, acutely after...
Noninvasive Quantification Tumor Tissue with VERDICT MRI

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References


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