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Intra-Tumor Distribution and Test-Retest Comparisons of Physiological Parameters quantified by Dynamic Contrast-Enhanced MRI in Rat U251 Glioma

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Abstract

The distribution of dynamic contrast enhanced MRI (DCE-MRI) parametric estimates in a rat U251 glioma model was analyzed. Using Magnevist as contrast agent (CA), 17 nude rats implanted with U251 cerebral glioma were studied by DCE-MRI twice in a 24 h interval. A data-driven analysis selected one of three models to estimate either: 1) CA plasma volume (v_p), 2) v_p and forward volume transfer constant (K^{trans} ; or 3) v_p , K^{trans} , and interstitial volume fraction (v_e), constituting Models 1, 2 and 3, respectively. CA interstitial distribution volume (V_D) was estimated in Model 3 regions by Logan plots. Regions of interest (ROIs) were selected by model. In the Model 3 ROI, descriptors of parameter distributions – mean, median, variance and skewness – were calculated and compared between the two time points for repeatability. All distributions of parametric estimates in Model 3 ROIs were positively skewed. Test-retest differences between population summaries for any parameter were not significant ($p > 0.10$; Wilcoxon signed-rank and paired t tests). This and similar measures of parametric distribution and test-retest variance from other tumor models can be used to inform the choice of biomarkers that best summarize tumor status and treatment effects.

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Keywords

distribution volume; interstitial volume; K^{trans} ; Logan plot; Magnevist; Patlak plot

INTRODUCTION

Pharmacokinetic analyses of dynamic contrast enhanced MRI (DCE-MRI) are being widely used for evaluating vascular properties in solid tumors *via* such parameters as the plasma volume fraction (v_p), forward volume transfer constant (K^{trans}), and, if the reverse transfer constant (k_{ep}) can be estimated, interstitial volume fraction (v_e) (1–3). Estimates of K^{trans} and k_{ep} (and thus v_e , calculated as the ratio K^{trans}/k_{ep}) depend on dynamically changing concentration-time data, but the distribution volume (V_D), defined as the sum of plasma volume and interstitial volume fraction, can also be estimated from equilibrating DCE-MRI data using a Logan graphical approach (4). It has recently been demonstrated that V_D and tumor cellularity are well correlated (5). Thus, estimates of microvascular function using T_1 -weighted DCE-MRI can produce measures of tumor physiology, and such measures may be useful as tumor biomarkers (6,7).

Our approach to the analysis of DCE-MRI data is best described as data-driven model selection (8). In this work we apply the 3-parameter standard model (SM) (2,3), or nested subsets of this model, voxel-by-voxel to DCE-MRI studies in orthotopic U251 cerebral tumors in athymic rats. DCE-MRI estimates of vascular parameters require a concentration-time trace of an indicator in both arterial blood and tissue. Standard practice uses the dynamic change in longitudinal relaxation rate ($R_1=1/T_1$) to estimate the dynamic change in contrast agent (CA) tissue concentration. A recent paper from this laboratory (9) has examined the DCE-MRI experiment in detail, with particular attention to identifying sources of systematic error. Notably, it was shown that, in a 7 Tesla MRI experiment, a dual-echo gradient-echo (2GE) sequence was required so that the analysis for the change in R_1 after CA injection in this experiment designed to assess the concentration-time trace of CA concentration could adjust for the strong effect of T_2^* dephasing.

Once a time trace of R_1 is calculated, the pharmacokinetic model that best accounts for the variation of the signal can be selected using a data-driven, F-test selected model selection paradigm. It has been demonstrated that, in cerebral tissue and tumors, the three parameters of the SM, at most, are sufficient for accounting for the concentration-time behavior of the DCE-MRI data in both animal models of cerebral tumor (9), and in humans (10). In normal brain, and in the tumor's peripheral regions, one or two parameters suffice to describe temporal variation in the data. In the present study, a model selection paradigm (5,9–11) was employed to characterize the best model to account for the concentration-time behavior of the DCE-MRI data in each voxel, and the available model parameters were then employed as a summary of tissue microvascular characteristics for that voxel. Given the model selection paradigm, essentially contiguous regions of interest (ROIs) with a common model could be defined for further analysis. Two studies were carried out in a 24 h time interval to examine the operating characteristics of the DCE-MRI experiment and analysis.

MATERIALS AND METHODS

The model equation

Since the basic approach to the pharmacokinetics of DCE studies has been previously published (5,9,10), we proceed to the model equation associated with the full SM used for parametric estimates (9), as shown below:

$$\Delta R_{1t}(t) = K^{trans} \int_0^t e^{-k_{ep}(t-\tau)} \Delta R_{1p}(\tau) d\tau + v_p \Delta R_{1p}(t), \quad [1]$$

where: R_{1p} is the longitudinal relaxation rate of all protons in the blood plasma, R_{1t} is the longitudinal relaxation rate of all protons in the tissue, K^{trans} is the unidirectional volume transfer rate of the indicator from plasma across the vascular endothelium and blood-brain barrier into the interstitial space, k_{ep} is the transfer rate constant from the interstitial compartment to the vascular compartment, v_p is the fractional volume of the CA's vascular distribution space, usually thought to be the plasma distribution space. The plasma fractional volume and the blood fractional volume are related via the following relationship:

$v_b = \frac{v_p}{1-Hct}$, where Hct is the average hematocrit in the vessels of the voxel. The application of this model equation, and subsets of this model equation, to DCE-MRI data is described below in the section on Numerical Methods.

Similarly, the model equation for the Logan plot estimates of distribution volume in DCE-MRI data, equation 4 in Aryal et al. (5), is given by:

$$\frac{\int_0^t \Delta R_{1t}(\tau) d\tau}{\Delta R_{1t}(t)} = v_D \frac{\int_0^t \Delta R_{1p}(\tau) d\tau}{\Delta R_{1t}(t)} + \text{Constant} \quad (2)$$

where $R_{1p}(t)$ and $R_{1t}(t)$ refer to the subtraction of the pre-contrast relaxation rate from its post-contrast value in the plasma and tumor regions, respectively, as a function of time.

If there is a time after which the plot of $\frac{\int_0^t \Delta R_{1t}(\tau) d\tau}{\Delta R_{1t}(t)}$ versus $\frac{\int_0^t \Delta R_{1p}(\tau) d\tau}{\Delta R_{1t}(t)}$ yields a straight line, the slope of the fitted straight line gives the distribution volume (note that both axes plot with the units of time). The linearity of the Logan plot signals that the concentration of CA in the plasma has equilibrated with the concentration of CA in the interstitial space.

R_1 calculation

Under the conditions of the experiment, i.e. using a dual-echo gradient echo sequence (the mgems sequence in the Agilent VNMRj library), and assuming that the flip angle is known across the brain of the rodent, an estimate of the time trace of R_1 can be calculated from the following:

$$S(t) = \frac{M_0 \sin(\theta) (1 - e^{-TR * R_1(t)}) e^{-TE * R_2^*(t)}}{1 - \cos(\theta) e^{-TR * R_1(t)}} \quad (3)$$

where $S(t)$ is the signal intensity at time t , M_0 is the equilibrium magnetization of the protons, θ is the flip angle, TR is the repetition time between pulses, TE is the echo time (the time between the center of the excitation pulse and the readout gradient), and $R_2(t)$ is the transverse relaxation rate. Equation (3) can be solved for $R_1(t)$:

$$R_1(t) = \frac{1}{TR} \ln \left[\frac{1 - \left(\frac{S(t) \cos(\theta)}{M_0 e^{-\frac{TE}{T_2^*}} \sin(\theta)} \right)}{1 - \left(\frac{S(t)}{M_0 e^{-\frac{TE}{T_2^*}} \sin(\theta)} \right)} \right] \quad (4)$$

The U251 rat model of cerebral glioma

The experimental procedures were approved by the Institutional Animal Care and Use Committee. Nude rats (N=18) were intracerebrally implanted as follows: animals were anesthetized with 80 mg/kg ketamine and 15 mg/kg xylazine given intramuscularly. The scalp was swabbed with Betadine and alcohol, the eyes coated with Lacri-lube and the head immobilized in a small animal stereotactic device (Kopf, Cayunga, California). After draping, a 1 cm incision was made 2 mm right of the midline and the skull exposed. A burr hole was drilled 3.5 mm to the right of bregma, without penetrating the dura. A #2701 10 μ L Hamilton syringe with a 26 gauge needle containing U251MG tumor cells freshly harvested from log phase growth (5×10^5 in 10 μ L of PBS) was lowered to a depth of 3.0 mm and then raised back to a depth of 2.5 mm. Cells were then injected at a rate of 0.5 μ L/10 s until the entire volume was injected. The needle was gently withdrawn, the burr hole sealed with sterile bone wax and the scalp sutured (5).

Between 21–24 days after tumor induction, each animal was anesthetized with isoflurane (4% for induction, 0.75 to 1.5% for maintenance, balance $N_2O:O_2 = 2:1$) and allowed to spontaneously respire. A tail vein was cannulated for CA administration. Body temperature was maintained at 37 °C with warm air and monitored via an intrarectal type T thermocouple. Identical procedures were carried out in the two MRI studies conducted 24 h apart for each animal.

MRI studies

All studies were performed in a Varian (now Agilent, Santa Clara, California), 7 Tesla, 20 cm bore system with a Direct Drive spectrometer and console. Gradient maximum strengths and rise times were 250 mT/m and 120 μ s. All MRI image sets were acquired with a 32 $\times$ 32 mm² FOV.

For the purpose of co-registering images with post-mortem histopathology, high-resolution T_1 -weighted images were acquired pre- and post-CA with the following parameters: matrix: 256 $\times$ 192, 27 slices, 0.5 mm thickness, no gap, number of echoes (NE) = 1, number of averages (NA) = 4, TE/TR = 16/800 ms. For the purpose of producing voxel-by-voxel maps of tissue T_1 prior to, and immediately after the 2GE DCE-MRI study, prior to the 2GE sequence, and immediately after, two Look-Locker (LL) sequences were run. LL sequence parameters were as follows: matrix: 128 $\times$ 64, five 2.0 mm slices, no gap. NE=24 inversion-

recovery echoes, TR=2000 ms. The 2GE sequence that was run between the paired LL acquisitions had the following parameters: 150 acquisitions at 4.0 sec intervals: matrix: 128×64, three 2.0 mm slices, no gap, flip angle = 27°, NE= 2 NA=1 TE = 2.0, 4.0 ms, TR = 60 ms, SW=150 kHz. CA (Magnevist, Bayer Healthcare Pharmaceuticals, Wayne, New Jersey) bolus injection was performed by hand push at image 15. Total run time was 10 minutes.

The arterial input function used in parameter estimations was calculated based on a previous radiotracer-sampling based method (12) as described in Aryal et al. (5). Briefly, as part of a quantitative autoradiography validation of MRI measures of vascular parameters, Nagaraja et al. (12) produced a group-averaged radiotracer-based model arterial input function which, when normalized and tested as a model arterial input function (AIF) in the MRI experiments produced very stable estimates of vascular parameters. With this result in mind, and to normalize the radiological input function to the $R_1(t)$ measures of CA concentration, the average $R_1(t)$ signal from the caudate putamen contralateral to the tumor was integrated over the latter two-thirds of the time trace, as was the radiological input function. It was assumed that: 1) no vascular leakage of Gd-DTPA occurred in the caudate putamen region contralateral to the tumor; 2) the plasma volume of the caudate putamen was 1%; and 3) hematocrit was 0.45. The integrated area of the radiological AIF was scaled so that its area was 100 times that of the integrated area of the average value of $R_1(t)$ in the caudate putamen contralateral to the tumor.

Numerical methods – processing

Using data from the LL-MRI studies performed before and after the 2GE DCE-MRI study, voxel-by-voxel maps of T_1 pre- and post-contrast were generated using previously published methods (11,13). Given the T_1 maps and dynamic 2GE data prior to and following the CA injection, the concentration-time curve of the CA was approximated by the calculated R_1 variation in time, using Equation 3. As noted, the scaled radiological input function was used as the arterial AIF in all studies. A global starting point was selected, usually one or two time points after the arrival of the indicator in the vasculature, and the next 90 points (6 minutes) of data was fitted by minimizing the Sum Squared Error (SSE).

Consider Equation 1. For any one voxel of DCE-MRI data, there are four possible descriptions. 0) The voxel contains little or no perfused tissue. Consequently, there is no detectable change in R_1 and all parameters are set to 0. 1) The vasculature in the voxel does not detectably leak contrast agent across the period of observation. Consequently, $v_p = 0$, $K^{trans} = k_{ep} = 0$. This is Model 1 and the case in most normal brain. 2) The vasculature in the voxel detectably leaks contrast agent, but there is no evidence of reflux from the tissue to the vasculature. Consequently, $v_p = 0$, $K^{trans} = 0$, $k_{ep} = 0$. This is Model 2 and the case described in the original Patlak graphical method (2). 3) The vasculature detectably leaks contrast agent, and there is evidence of reflux from the tissue to the vasculature. Consequently, $v_p = 0$, $K^{trans} = 0$, $k_{ep} = 0$. This is Model 3, equivalent to full model described by the SM.

One set of parameters per voxel was reported. The choice of model to report was driven by a model comparison performed via an F-test of alternative models (9,10). Only if the higher-order model had a sufficiently high probability of being required by the data was it accepted.

The effect of this procedure was to prevent the overfitting of data, with its deleterious effects on stability of estimates. In the brain, the maps produced were: a map of model selection, a nearly complete map of plasma volume, a partial map of K^{trans} , and a smaller map of v_e , calculated as K^{trans}/k_{ep} . See Figure 1.

The practice of model selection also influenced the selection of ROIs for Logan plot estimates of V_D . Since the estimates of distribution volume can only be made when CA leaks from the capillary into the interstitial space in an amount sufficient to measure its back flux to the vasculature, Logan plot estimates of distribution volume were generated only in Model 3 regions. For fitting the Logan plot, the graph was visually inspected in each study to select the starting time, and then the data, plotted as a Logan plot, was fitted to a straight line from the starting point to the end of the data, ten minutes after the start of the DCE-MRI sequence, and about nine minutes after CA injection.

The slice with the largest cross-section of tumor was selected for study. To reflect common practices in the field, two paths to data summary in the Model 3 ROIs were taken: in the first (the temporal-spatial, or T-S, path), the temporal change in $R_1(t)$ was analyzed voxel-by-voxel (14,15), and the resulting parametric maps were summarized by the group properties of the parameters in the Model 3 ROIs. In the second (the spatio-temporal, or S-T, path), the change in $R_1(t)$ was summed across the Model 3 ROI frame-by-frame (16,17), and then analyzed for model parameters, producing one set of summary model parameters per study for the ROI.

Statistical practices

Our intent in presenting this data is to describe the distribution of the DCE-MRI parametric estimates in a typical model tumor, and to demonstrate their test-retest properties. There are three presentations to be made – characterizing the voxel-by-voxel distribution of T-S vascular parameters in each ROI and generating appropriate summary statistics of that sample, characterizing the sampling of summary statistics across animals, and characterizing the stability of summary statistics within animals via test-retest sampling.

Two sets of values from Model 3 regions, one from S-T and the other from the T-S paths, were available for the two studies in each animal, producing voxel-by-voxel (T-S) or one summary (S-T) estimate of v_p , K^{trans} and v_e . Only an S-T estimate for V_D was available.

Data acquired via the T-S path were examined for parametric distribution characteristics, with the median, mean, variance and skewness in the Model 3 ROI calculated for each vascular parameter (v_p , K^{trans} , and v_e). In order to give an idea of the stability of the statistics (median, mean, variance, and skewness of each vascular parameter in the T-S sample), the sample variance across animals was computed for each statistic. In parameters with non-normal distributions, Wilcoxon signed rank tests were applied for test-retest analysis. Summary estimates (means and medians) were compared between the two methods (T-S and S-T) and between test and retest studies for each method using paired t-tests and/or Wilcoxon rank-sign tests. We note again that the intent behind these analyses was to generate a relatively complete sense of the characteristics of DCE-T1 estimates of vascular parameters, and to relate the summary measures to each other.

For Model 2 region, only the mean values from T-S methods were estimated and the test-retest analysis was performed using paired t-test.

In the T-S group, a Bland-Altman plot (18) was first used to examine, for each summary vascular parameter reported, whether the sample of 17 animals demonstrated a trend in the test-retest difference versus the parameter's mean value. A Kendall-tau test was used to formally test for such a trend. In the event that the Kendall-tau test demonstrated a dependency of variance on the value of the parameter in the sample as a whole, the parameter was log-transformed and tested again.

RESULTS

Of the 18 rats, images from one animal suffered from movement artifacts in one of the sessions and, thus, 17 pairs of studies were available for analysis. All tumors were visible in the post-contrast T_1 -weighted imaging, which was begun about 12 minute, after CA injection. At 21–24 days after tumor implantation, the typical largest diameter of the tumor on a coronal image was ~5 mm.

For each test-retest examinations in a 24 h interval, two sets of maps of model parameters were produced in three 2 mm slices centered on the tumor. An example is shown in Figure 1 from test (top row) and retest (bottom row) for T_1 , plasma volume, v_p (column 1), K^{trans} in pixels where either Model 2 or Model 3 was accepted (column 2), and v_e (column 3), where Model 3 was accepted; a map of model choice is shown in column 4. We shall continue to use this study throughout as an example.

Figure 2 shows the graphical analysis of the data for Logan plot in a test-retest study from the same rat. In this example, the graph is linear after 49 time points (abscissa = 40 min) in the test group study (Figure 2A); the straight-line fitting was made in the range 40:75 min with a slope 0.1915, equivalent to the distribution volume 19.15%. For the retest study (Figure 2B), the straight-line fitting began at point number 52 (abscissa = 45 min); the straight-line fitting was made in the range 45:80 min with slope 0.1728, yielding a distribution volume 17.28%. Mean ($\pm$ sd) V_D values were: 14.72 ± 4.89 for test and 13.82 ± 3.58 for retest ($p=0.16$).

In the sample of 17 animals, the average number of pixels with Model 3 acceptance was 153 ± 102 for test and 149 ± 87 for retest ($p=0.46$; $n=17$; paired t-test). The distribution of each parameter within a given ROI (T-S path) demonstrated the intra-sample variations. Each parameter in the T-S sample exhibited a positive skewness: 0.41 for v_p , 0.97 for K^{trans} , and 1.51 for v_e (all measures of skewness were different from 0 by a Wilcoxon rank-sign test, with the highest p-value of 5×10^{-4} for v_p , and the lowest 3×10^{-7} for v_e). An example of such positively skewed data for K^{trans} values is shown in Figure 3, with a skewness of 0.85. None of the measurements, median, mean, variance, and skewness, of the three parameter values in Model 3 regions were significantly different in test-retest studies. The skewness in the T-S sampling of vascular parameters suggests, as others have indicated (19), that the median may be a better summary statistic for single-point reporting of trends in the sampled parameters. The test-retest difference of median values was not significantly different from

those of mean values ($p = 0.15$ for all three parameters). These data summaries along with the results of statistical analyses are given for these three parameters in Tables 1, 2 and 3, respectively.

The paired tests were analyzed for differences. Differences between the two time points of study were not statistically different for any parameter, either by Wilcoxon signed rank- or paired t-tests. This suggests that stable estimates of vascular parameters over a 24 h period are available in this model at this stage of tumor progression. For all parameters, there was no evidence that the sample of *differences* in summary statistics was not normally distributed, and no parameter showed any significant dependency between absolute mean *difference* and their combined mean, suggesting that an experimental design that generates within-subject paired studies is sound practice for the detection of systematic effects due to treatments that might intervene between the two studies. In Figure 4 are Bland-Altman plots, where the differences between the mean values of the three parameters in the two studies is plotted against their combined mean for each parameter in the Model 3 region, along with the mean difference and the 95% limit of agreement for the mean difference. No significant trends are visible, and none of these show a dependency *via* a Kendall-tau test. A similar plot (Figure 5) for median values of the parameters, however, indicated a dependency of differences in median over combined median values for v_e ($p \sim 0.017$, Kendall-tau test). Therefore, a log-transformed data was used to plot the difference vs. combined median for v_e , whereas, the difference being independent to combined values, original data was used for v_p and K^{trans} as shown in Figure 5. The dependency of the difference in test-retest K^{trans} median on the value of the parameter itself somewhat dampens enthusiasm for its use as a summary statistic for that parameter.

Model 2 regions were relatively larger in total area than were Model 3 regions with number of pixel size: 219 ± 63 for test and 225 ± 77 retest. The mean values of K^{trans} in Model 2 regions were about 8 times lower than those values in Model 3 regions, and estimates of plasma volume were about 20% lower. For K^{trans} , the test group mean ($5.49 \pm 1.93 \times 10^{-3} \text{ min}^{-1}$) moved downward, but not significantly ($p = 0.59$, $n = 17$), to the retest group mean ($5.28 \pm 1.73 \times 10^{-3} \text{ min}^{-1}$). For v_p , the test group mean ($0.64 \pm 0.52\%$) increased, but not significantly ($p = 0.09$, $n = 17$), to the retest group mean (0.90 ± 0.55). Although the test-retest analysis looks stable, there is strong evidence that, in many parts of the Model 2 region, the plasma volume estimate is biased low and also that, while the estimate of K^{trans} is fairly precise, its interpretation as a vascular transfer function must be altered (9).

Although no significant difference was detected between test and retest for all parameters in each of the methods (T-S and S-T), an important difference emerged when the T-S estimates of v_p and K^{trans} were compared to those from the S-T analysis. For v_p , values from the S-T method had a higher estimate than pixel-based method for both test [mean difference between two methods = -0.6309 , 95% CI = $(-0.9040, -0.3578)$, p -value = 0.0002] and retest [mean difference between the methods = -0.8843 , 95% CI = $(-1.1751, -0.5936)$, p -value < 0.0001]. For K^{trans} , the T-S method had a higher estimate than S-T method for both test [mean difference between the methods = 0.0340 , 95% CI = $(0.0279, 0.0401)$, p -value < 0.0001] and retest [mean difference between the methods = 0.0332 , 95% CI = $(0.0269,$

0.0395), p -value < 0.0001]. v_e showed no differences between the two methods of estimation.

DISCUSSION

The yield of this study, and the significance, lie in a description of parameter distributions and sampling statistics in an untreated group of animals – a matter of primary importance when considering tests for *changes* in treated groups of animals, and a reasonable starting point when designing studies in humans.

A number of important results emerge from an examination of the parametric distribution and test-retest variability of DCE-MRI measures used to summarize tumor vascular physiology in this rat model of cerebral glioma. We first note that, when temporally analyzed and then spatially summed (T-S path), all parameters presented with right-skewed distributions in the ROIs chosen. Thus, median values of parametric distributions in a well-chosen ROI might be considered, rather than mean values. The appearance of a typical skewness strongly suggests that some measure that reflects the non-normal distribution of parameter values in an ROI should be stated, particularly because the higher values of K^{trans} are probably related to regions of aggressive angiogenesis. Herein, skewness is reported, but a report of the 95% upper confidence interval (19) may also be useful. The relatively common practice of stating a “mean maximum value” (20–22) in some parameter in a tumor region probably should be avoided, since it is essentially an estimate of range, which in many instances will vary upward with sample size, i.e., with the size of the ROI chosen: skewness and the 95% confidence interval do not vary with sample size.

Estimates of vascular permeability parameters appeared sufficiently reproducible to support their use as biomarkers, particularly when used as paired comparisons. For instance paired comparisons of the 17 median values of K^{trans} in the T-S analysis produced a mean difference of ~2% of the grouped mean values of that statistic.

A brief survey of the literature of glioma and DCE-MRI shows heterogeneity in analysis and data summary. As an illustration of variation in reporting results, consider data summary for K^{trans} . In one approach, after a region-of-interest (ROI) is selected, usually by referring to a post-contrast image set, the DCE-MRI data treatment follows the S-T path. The temporally varying mean (S-T path) (23), or median (24) of the spatially summed DCE-MRI data is used to form a summary statistic of K^{trans} in the ROI selected. In other approaches, the T-S path is followed (16,17). For summary statistics, the mean $\pm$ SEM of the map values in an ROI is often reported (20,25,26), although it has been noted (19) and the results herein agree, that K^{trans} , among other parameters, is typically not normally distributed in enhancing tumors.

Note that the DCE-MRI measures examined were strongly influenced by the application of a model selection paradigm to DCE-MRI data. Thus, these results may differ from those in which the ROI to be summarized was selected by visual inspection of post-contrast T₁-weighted MRI images, particularly if areas of necrosis were included. We strongly recommend, in order to maintain data uniformity in summary measures of vascular

parameters, that a data-driven analysis with some objective method of model selection (e.g. F-test, log-likelihood ratios test, Akaike Information Criterion, Bayesian Information criterion) be employed for the selection of ROIs.

A few studies have reported the test-retest analysis in DCE-MRI parameter measurements in tumor as well as in normal tissue (25,27,28). In one study, Padhani et al. (28) reported reproducibility of DCE-MRI parameters (global mean of ROIs, i.e., a T-S study) in normal tissues (muscle, marrow, and fat) with quite long (months) test-retest intervals. These were studies in patients with prostate cancer who, in the interval between tests, had undergone anti-androgen treatment. In another study with a very similar analysis, test-retest DCE-MRI studies conducted (25) with a one-week interval in 16 patients with lower body carcinomas and sarcomas. This study reported that test-retest differences in normal tissue were stable enough to detect changes greater than 14–17%. Similarly, in a study on newly presenting glioma, Jackson et al. (27) reported the high reproducibility of (T-S) mean values of K^{trans} and v_e measurements imaged 2-days apart by DCE-MRI in nine patients. High values of parameters were excluded, and no measure of the upward spread of K^{trans} values was presented.

Even disregarding differences in methods for choosing ROIs, there are a number of reservations about comparing our results with that of the papers just cited. While our model employed a three-parameter fit with a characteristically high value for the coefficient of determination (R^2) (9,10), the model employed in the papers cited above was a 2-compartment Tofts model; the values for K^{trans} were much higher than our 3-parameter model, leaving an uncertainty as to whether the comparison of the reproducibility of two-parameter models to that of a three-parameter model is appropriate.

Because quite large samples of tissue, with typically hundreds of voxels of data, were taken to form a single mean estimate of the parametric estimates of the (3- or 2-parameter) model summarized herein, it is quite likely that the random-like errors due to MRI signal sampling are negligible. In a carefully executed experiment that eliminates the major sources of systematic error (e.g., T_2^* dephasing), the errors we are sampling in the summary measures of parametric estimates are not ‘errors’ in the sense that they differ from the mean of the MRI signal sample, but ‘errors’ in the sense that they differ from the mean of the tissue physiology sampled across animals. While these errors may be distributed according to some rule, their origins almost certainly contain effects that are systematic in nature. That is to say, the physiologies of the tumors in these separate animals differ from each other, possibly because the tumors differ in some undetected relationship to the host. When summarized in a group, the ‘variance’ of the group incorporates the undetected systematic errors (differences from the mean) of the group. Thus, since paired tests tend to null systematic differences between animals (tumor size, host-tumor interactions, etc.) that affect the parameter values themselves we strongly recommend experimental designs where a baseline study can be followed by treatment and retest. However, it is not trivial that the varied vascular abnormalities segmented by the three models in each tumor may point to differing pathologies in terms of local vasculature and its associated microenvironment.

Errors in parametric estimates may have two sources – random-like errors in measurement whose source (e.g. the white noise of MRI signal sampling) is itself randomly distributed, and systematic errors whose source lies in some property of the system that is not accounted-for in the model. Random-like errors produce increases in the variance of the estimators, and systematic errors produce bias in the estimators. It should be recognized that significant sources of bias exist in the DCE-MRI experiment itself, and in the subsequent analysis. These sources have been previously reviewed (9), but we note that a correction for T_2^* dephasing effects in saturated gradient-echo sequence experiments at high fields is necessary. The often-employed usage of the signal intensity alone to estimate $R_1(t)$ (7) will not generate an unbiased estimate of that quantity in the high fields that are routinely employed for small-animal imaging (9). We also note that a reliable estimate of the ‘arterial’ input function is an intractable problem, particularly at high fields, one that we have dealt with by employing a group-averaged AIF, normalized to plasma concentrations in non-leaky areas. Thus, it is a virtual certainty that a hand-pushed bolus produced some variation in both the input and the tissue response, a variation that could not be accounted for without a case-by-case estimate of the AIF. Nevertheless, the very good fits observed in Model 3 regions (R^2 typically larger than 0.98) support this approach.

Few studies have examined compared the T-S and S-T approaches to transvascular transfer indices. The differences observed herein between the two methods were unexpected and highlight the importance of such a comparison. It may be that an error-weighted sum of T-S values might serve as a better estimate of means in the T-S approach, though very few computational packages provide the necessary estimates of the errors of the parametric estimates. Note the very high stability of S-T estimates, compared to those of any of the measures of T-S estimates. If means and mean differences are all that are required in a study, it might be most reasonable to identify an ROI and sum data across that ROI before analyzing for the kinetic parameters.

To summarize, test-retest measures of the physiological state of the tumor and its surround, as categorized by the chosen model selection paradigm, show a relatively low variability in both Model 3 and Model 2 regions, with a Logan Plot analysis in Model 3 regions generating an additional stable parameter of physiological interest, V_D . Model selection paradigms generate stable and reproducible ROIs for data summary. Experimental designs that generate intra-subject test, retest measures are highly recommended. Pixel-by-pixel estimates (the S-T path) generate strongly right-skewed estimates in each of the parameters of the SM. Therefore, in generating summaries of vascular parameters, some measure of upward variation should be stated. If only a summary statement of the overall behavior of vascular parameters in an ROI is required by the experimental design, the T-S approach may be the best summary descriptor because it is the most stable. Given an experimental design that responds to the sampling characteristics described herein, the effects of an intervention that changes the physiology of the tumor and its surroundings should be detectable with good sensitivity. It is noteworthy in this context that the present test-retest variations are within the range of such values reported from several previous studies using DCE-MRI (25,27,28) and also using other imaging modalities (29–31). Thus, the present data strongly support the use of DCE-MRI with appropriate model selection and experimental design for tumor characterization and assessment of acute response to therapy.

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List of abbreviations

DCE-MRI	dynamic contrast enhanced MRI
CA	contrast agent
v_p	plasma volume
K^{trans}	forward volume transfer constant
v_e	interstitial volume fraction
V_D	interstitial distribution volume
Hct	hematocrit
ROI	region of interest
SM	standard model
FOV	field of view
2GE	dual-echo gradient-echo
NE	number of echoes
NA	number of averages
LL	Look-Locker
R_1	longitudinal relaxation rate
sd	standard deviation
SSE	sum squared error
TS	temporal-spatial
ST	spatio-temporal

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Figure 1.

Test-retest T_1 -weighted images acquired by a dual echo gradient echo sequence and corresponding parametric maps from one animal. Top row: test; Bottom row: retest. Columns from left to right: T_1 image, v_p , K^{trans} , v_e , and model selection maps. Value ranges for each parameter are shown in the color bar to the right of each map. For the Model selection map, white is Model 3 acceptance, dark blue is Model 2 acceptance, light blue is Model 1 acceptance and light green/yellow is Model 0.

Figure 2.

Graphical analysis of the data for Logan pot estimates of distribution volume. The solid line shows the linear fitting of the data, the slope of the fitted straight line gives the distribution volume. In this particular example, the straight-line fitting was made in the range 40:75 min for test study (left) with slope 0.1915 and 45:80 min for retest study (right) with slope 0.1728 equivalents to the distribution volume of 19.15% and 17.28% respectively.

Figure 3. Distribution of pixel-by-pixel estimates of K^{trans} [min^{-1}] values in one Model 3 region, demonstrating a positive skewness of 0.82. Because of the positive skewness, the distribution mean was larger than its median.

Figure 4.

A Bland-Altman plot of mean differences. The difference between two studies plotted against the combined mean for each parameter in Model 3 region. The mean difference between two studies (center dotted line) and 95% limits of agreement (dashed lines) are also shown.

Figure 5.

The difference in median values between two studies plotted against the combined median values for each parameter from Model 3 region. Since the original data showed some dependency between absolute differences and combined median values, the Log10 (median) values were used for v_e . The center dot line shows the average difference values for each parameter.

Summary statistics for plasma volume, v_p . Spatio-temporal (S-T) data were positively skewed during both test and retest. However, the differences were not statistically significant for any sampling statistic in the period of 24 h in both S-T and temporal-spatial O(S-T) analyses. Standard deviations (the square root of the variances) for the sampling distribution of the Statistical Parameters across animals are given in parentheses, as an indication of the characteristic spread of these parameters, with no expectation that these parameters are normally distributed.

Table 1

Statistical Parameters	Median(±SD)	Mean(±SD)	Mean(±SD)*	Variance(±SD)	Skewness(±SD)
Test values	1.26(±0.52)	1.37(±0.57)	1.46(±0.64)	1.30(±1.31)	0.60(±0.54)
Retest values	1.47(±0.63)	1.54(±0.61)	1.46(±0.58)	1.29(±1.09)	0.23(±1.12)
Mean difference	0.21(±0.47)	0.16(±0.49)	0.00(±0.49)	-0.004(±0.92)	-0.37(±1.21)
Paired t-test: p-value	0.10	0.19	0.99	0.99	0.23
Wilcoxon's sign test: p-value	0.13	0.19	0.96	0.93	0.33

Parameter values from S-T analysis were used for all examination except for * where the values were from T-S analysis.

Summary statistics for forward volume transfer constant, K^{trans} . Spatio-temporal (S-T) data were positively skewed during both test and retest. However, the differences were not statistically significant for any sampling statistic in the period of 24 h in both S-T and temporal-spatial (S-T) analyses. Standard deviations (the square root of the variances) for the sampling distribution of the Statistical Parameters across animals are given in parentheses, as an indication of the characteristic spread of these parameters, with no expectation that these parameters are normally distributed.

Table 2

Statistical Parameters	Median(±SD)	Mean(±SD)	Mean(±SD)*	Variance(±SD)	Skewness(±SD)
Test values($\times 10^{-2}$)	3.95(±1.36)	4.31(±1.47)	3.38(±1.33)	4.40(±3.96) $\times 10^{-2}$	0.85(±0.85) $\times 10^2$
Retest values($\times 10^{-2}$)	3.85(±1.37)	4.26(±1.67)	3.11(±1.16)	6.30(±10.01) $\times 10^{-2}$	1.09(±1.51) $\times 10^2$
Mean difference ($\times 10^{-2}$)	-0.10(±1.27)	-0.05(±1.50)	-0.27(±1.30)	1.90(±7.59) $\times 10^{-2}$	0.24(±0.96) $\times 10^2$
Paired t-test: p-value	0.77	0.89	0.41	0.33	0.33
Wilcoxon's sign test: p-value	0.61	0.75	0.36	0.99	0.64

Parameter values from S-T analysis were used for all examination except for * where the values were from T-S analysis.

Summary statistics for interstitial volume fraction, v_e . Spatio-temporal (S-T) data were positively skewed during both test and retest. However, the differences were not statistically significant for any sampling statistic in the period of 24 h in both S-T and temporal-spatial (S-T) analyses. Standard deviations (the square root of the variances) for the sampling distribution of the Statistical Parameters across animals are given in parentheses, as an indication of the characteristic spread of these parameters, with no expectation that these parameters are normally distributed.

Table 3

Statistical Parameters	Median(±SD)	Mean(±SD)	Mean(±SD)*	Variance(±SD)	Skewness(±SD)
Test values	12.95(±4.45)	13.63(±4.88)	12.57(±5.02)	21.74(±19.38)	1.23(±0.71)
Retest values	12.32(±3.56)	12.95(±3.74)	11.16(±3.41)	27.49(±36.84)	1.79(±2.51)
Difference	-0.63(±3.56)	-0.68(±3.65)	-1.40(±3.44)	5.74(±34.21)	0.56(±2.52)
Paired t-test: p-value	0.48	0.45	0.11	0.51	0.38
Wilcoxon's sign test: p-value	0.28	0.33	0.16	0.93	0.82

Parameter values from S-T analysis were used for all examination except for * where the values were from T-S analysis.