Comparison of Dynamic Susceptibility-weighted Contrast-enhanced MR

Methods: Recommendations for Measuring Relative Cerebral Blood Volume in Brain Tumors¹

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Purpose:

To investigate whether estimates of relative cerebral blood volume (rCBV) in brain tumors, obtained by using dynamic susceptibility-weighted contrast material-enhanced magnetic resonance (MR) imaging vary with choice of data acquisition and postprocessing methods.

Materials and Methods:

Four acquisition methods were used to collect data in 22 high-grade glioma patients, with informed written consent under HIPAA-compliant guidelines approved by the institutional review board. During bolus administration of a standard single dose of gadolinium-based contrast agent (0.1 mmol per kilogram of body weight), one of three acquisition methods was used: gradient-echo (GRE) echo-planar imaging (echo time [TE], 30 msec; flip angle, 90°; $n = 10$), small-flip-angle GRE echo-planar imaging (TE, 54 msec; flip angle, 35°; $n = 7$), or dual-echo GRE spiral-out imaging (TE, 3.3 and 30 msec; flip angle, 72°; $n = 5$). Next, GRE echo-planar imaging (TE, 30 msec; flip angle, 90°; $n = 22$) was used to collect data during administration of a second dose of contrast agent (0.2 mmol/kg). Subsequently, six methods of analysis were used to calculate rCBV. Mean rCBV values from whole tumor, tumor hot spots, and contralateral brain were normalized to mean rCBV in normal-appearing white matter.

Results:

Friedman two-way analysis of variance and Kruskal-Wallis one-way analysis of variance results indicated that qualitative rCBV values were dependent on acquisition and postprocessing methods for both tumor and contralateral brain. By using the non-parametric Mann-Whitney test, a consistently positive (greater than zero) tumor-contralateral brain rCBV ratio resulted when either the preload-postprocessing correction approach or dual-echo acquisition approach ($P < .008$ for both methods) was used.

Conclusion:

The dependence of tumor rCBV on the choice of acquisition and postprocessing methods is caused by their varying sensitivities to T1 and T2 and/or T2* leakage effects. The preload-correction approach and dual-echo acquisition approach are the most robust choices for the evaluation of brain tumors when the possibility of contrast agent extravasation exists.

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Supplemental material: <http://radiology.rsna.org/cgi/content/full/2492071659/DC1>
<http://radiology.rsna.org/cgi/content/full/2492071659/DC2>
<http://radiology.rsna.org/cgi/content/full/2492071659/DC3>

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In 1990, Rosen et al (1) demonstrated that if a bolus of a gadolinium-chelated contrast agent is administered and images of the brain are acquired during this administration, a transient decrease in signal intensity occurs. This approach is based on the principle of susceptibility-weighted contrast material-enhanced magnetic resonance (MR) imaging, for which a bolus of a gadolinium-chelated contrast agent that is confined to the vasculature induces a difference in susceptibility between the vessel and the tissue. This signal intensity decrease can be converted into a concentration-time curve ($\Delta R2^*[t]$), from which cerebral blood volume can be computed for each image voxel, resulting in relative cerebral blood volume (rCBV) image maps.

Several groups have applied this dynamic susceptibility-weighted contrast-enhanced MR imaging method to evaluate primary brain tumors such as gliomas. It is well known that the most aggressive gliomas are characterized by the formation of new blood vessels (angiogenesis), which is essential for their progression from low grade to high grade, with a clear correlation between increased vascularity and malignancy (2–5). The feasibility and relevance of using

dynamic susceptibility-weighted contrast-enhanced MR imaging to measure rCBV in brain tumors has been supported with studies that show that rCBV may help in better differentiation of histopathologic tumor types than conventional MR imaging, may provide information to help predict tumor grade (6–11), and may better help to predict survival than histopathologic findings (12). In addition, rCBV maps may aid in the differentiation of posttreatment changes from tumor recurrence (13,14) and may be predictive of early local recurrence or malignant transformation (15,16).

Despite these promising results, it has long been known that use of dynamic susceptibility-weighted contrast-enhanced methods for the determination of rCBV can be complicated by a leaky blood-brain barrier, as is often present with brain tumors. Under these conditions, contrast agent leaks out of the vessels into the extravascular-extracellular space, which can result in shortening of the tissue water T1. The resulting signal increases compete with the susceptibility-induced signal decreases (1,17), thus confounding the rCBV estimates obtained with dynamic susceptibility-weighted contrast-enhanced MR imaging. This “leakage effect” has been variably considered. Some perform the study without accounting for leakage effects (7,10,15,18). Others process data only from regions that do not demonstrate contrast enhancement (15). Some use small-flip-angle gradient-echo (GRE) (6,19,20) or dual-echo (21) acquisitions to minimize the T1 leakage effects. Alternatively, a

loading dose of contrast agent may be administered prior to acquiring the dynamic susceptibility-weighted contrast-enhanced data to minimize subsequent changes in T1 that might occur during the first-pass dynamic susceptibility-weighted contrast-enhanced study (9,22). Postprocessing mathematic correction algorithms may also be applied (9,22–26).

The purpose of this study was to investigate whether estimates of rCBV in brain tumors, obtained by using dynamic susceptibility-weighted contrast-enhanced MR imaging, vary with the choice of data acquisition and postprocessing methods. The approach was to compare several of the most commonly used methods for acquiring and computing rCBV in patients with high-grade gliomas. The study was designed so that a direct comparison among several of the techniques, in the same patient at the same time, could be performed. To our knowledge, this question has not been previously addressed in such a direct and comprehensive manner.

Advances in Knowledge

- Determination of relative cerebral blood volume (rCBV) in brain tumors depends on the choice of dynamic susceptibility-weighted contrast material-enhanced acquisition and postprocessing methods.
- This dependence results from the fact that each approach has a different sensitivity to the effects of contrast agent leakage.
- Although effects of T1 leakage have been thought to be predominant, our results suggest that leakage can also be attributed to dipolar T2 leakage or residual T2* effects.
- Optimal tumor–contralateral brain rCBV contrast can best be achieved by using either a pre-load-postprocessing–correction approach or a dual-echo–acquisition approach.

Implications for Patient Care

- The results of this study should enable physicians to make more informed decisions in regard to their approach to perfusion MR imaging of brain tumors, as well as other pathologic findings, where there is the possibility of contrast agent extravasation.
- These results may contribute to development of a standardized approach to dynamic susceptibility-weighted contrast-enhanced perfusion imaging.

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Abbreviations:

CTI = corrected trapezoidal integration
 GRE = gradient echo
 MSD = maximum signal decrease
 NEI = negative enhancement integration
 rCBV = relative cerebral blood volume
 ROI = region of interest
 SNR = signal-to-noise ratio
 TE = echo time
 UTI = uncorrected trapezoidal integration
 PBSI = postbolus baseline subtraction integration

Author contributions:

Guarantors of integrity of entire study, E.S.P., K.M.S.; study concepts/study design or data acquisition or data analysis/interpretation, E.S.P., K.M.S.; manuscript drafting or manuscript revision for important intellectual content, E.S.P., K.M.S.; manuscript final version approval, E.S.P., K.M.S.; literature research, E.S.P., K.M.S.; clinical and experimental studies, E.S.P., K.M.S.; statistical analysis, E.S.P., K.M.S.; and manuscript editing, E.S.P., K.M.S.

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See Materials and Methods for pertinent disclosures.

See also the editorial by Sorensen in this issue.

Materials and Methods

Two years after the initiation of this study, a company (Imaging Biometrics, Elm Grove, Wis) was formed, of which one author (K.M.S.) has ownership interest. The company did not provide any support, financial or otherwise, nor was company software used to analyze the data described in this study. All data were processed and analyzed by the other author (E.S.P.) with laboratory software modified by himself for the purpose of this study. That author (E.S.P.) has no financial interest in the company. The results of this study did influence product development decisions made by the company.

Patients

From June 2005 through January 2007, patients who were suspected of having cerebral neoplasms at prior imaging studies or who had a history of a biopsy-proved neoplasm were referred for this Health Insurance Portability and Accountability Act-compliant, institutional review board-approved study and gave informed written consent. Inclusion criteria were newly diagnosed residual or recurrent high-grade (World Health Organization grade III or IV) gliomas with enhancing tumors on the contrast-enhanced T1-weighted images. The rationale for using only high-grade tumors, as opposed to low-grade tumors, is that they are known to be highly vascular, so it is reasonable to expect a positive (greater than zero) tumor-brain rCBV contrast. The tissue diagnosis was determined on the basis of tissue obtained at surgery performed within 2 months after the rCBV study.

Tumors were classified and assigned a grade according to the World Health Organization 1993 classification (27). The patient information and tumor diagnosis are provided in Table 1. The patient group consisted of 12 men and 10 women, ranging in age from 21 to 76 years, with no significant difference in age distribution between male and female patients ($P = .36$). Grade III tumors included new ($n = 1$) and recurrent ($n = 2$) anaplastic astrocytomas, recurrent anaplastic mixed gliomas

($n = 2$), new ($n = 1$) and recurrent ($n = 1$) anaplastic oligodendrogliomas, and malignant glioneuronal tumor ($n = 1$). Grade IV tumors included de novo ($n = 5$), recurrent ($n = 7$), and residual glioblastoma multiforme ($n = 2$). At the time of the rCBV study, 15 patients were being treated with steroids.

Data Acquisition Methods

All studies were performed with a 1.5-T MR unit (CVi; GE Healthcare, Milwaukee, Wis). Four approaches (methods A–D, Tables 1, 2) for acquiring dynamic susceptibility-weighted contrast-enhanced data were used (9,20,22–24,28–35). With methods A (28,29) and D (9,22,24,33), single-echo GRE echo-planar imaging, with a flip angle of 90° and TE of 30 msec, was used. With method B (20,30–32), a GRE echo-planar imaging sequence also was used but with a smaller flip angle of 35° and a

longer TE of 54 msec to minimize T1 sensitivity and maximize T2* sensitivity, respectively. With method B, only 15 baseline points were collected in an effort to match the published protocol (20,30). For method C (34,35), a custom, lipid-suppressed, single-shot dual-echo GRE spiral-out sequence was used that enables signal collection at two TEs (first TE [TE₁] = 3.3 msec, second TE [TE₂] = 42 msec), thereby allowing the determination of T2* (R2*) at each time point. The Ernst angle of 72° was chosen to maximize signal-to-noise ratio (SNR) during dual-echo acquisition. The general parameter settings used for all methods included the following: repetition time, 1000 msec; field of view, 24 cm²; matrix, 64 × 64; section thickness, 5 mm; intersection gap, 1.5 mm; number of sections, 12; and number of samples (repetitions), 180. After the dynamic susceptibility-weighted contrast-

Table 1

Patient Information, Histopathologic Diagnosis, and Acquisition Method

Patient No./ Age (y)/Sex	WHO Grade	Histopathologic Diagnosis	Acquisition Method
1/69/M	IV	Glioblastoma multiforme	A, D
2/35/F	IV	Residual glioblastoma multiforme	A, D
3/48/F	IV	Glioblastoma multiforme	A, D
4/21/F	III	Malignant glioneuronal tumor	A, D
5/56/F	III	Recurrent anaplastic mixed glioma (mostly astrocytoma with some oligodendroglioma)	A, D
6/76/F	IV	Glioblastoma multiforme	A, D
7/40/M	III	Anaplastic oligodendroglioma	A, D
8/52/M	III	Recurrent malignant mixed glioma with predominant oligodendroglial component plus anaplastic astrocytoma	A, D
9/44/F	III	Recurrent anaplastic astrocytoma	A, D
10/61/F	IV	Glioblastoma multiforme	A, D
11/53/M	IV	Glioblastoma multiforme	B, D
12/49/M	IV	Recurrent glioblastoma multiforme	B, D
13/75/M	IV	Recurrent glioblastoma multiforme	B, D
14/41/F	III	Anaplastic astrocytoma	B, D
15/38/F	IV	Residual glioblastoma multiforme	B, D
16/58/M	III	Recurrent malignant oligoastrocytoma	B, D
17/45/M	IV	Recurrent glioblastoma multiforme	B, D
18/44/F	IV	Recurrent glioblastoma multiforme	C, D
19/37/M	IV	Recurrent glioblastoma multiforme	C, D
20/46/M	III	Recurrent anaplastic astrocytoma	C, D
21/53/M	IV	Recurrent glioblastoma multiforme with radiation effect	C, D
22/43/M	IV	Recurrent glioblastoma multiforme	C, D

Note.—WHO = World Health Organization.

enhanced MR imaging acquisition, contrast-enhanced T1-weighted spin-echo images were acquired by using the same geometric prescription as was used for the dynamic susceptibility-weighted contrast-enhanced images (repetition time msec/TE msec, 450/11; number of repetitions, two; and matrix, 256×256).

Two acquisition methods were used to collect dynamic susceptibility-weighted contrast-enhanced data from the same patient during the same imaging session (Fig 1). One of three methods (methods A–C) was used to collect data during the primary injection (0.1 mmol per kilogram of body weight) of gadodiamide (Omniscan; Nycomed-Amersham, Princeton, NJ), whereas method D was used to acquire images

during the secondary injection (0.2 mmol/kg) of gadodiamide. With this approach, the primary injection acts as a preload of contrast agent for the subsequent method D study. The preload decreases the initial tissue T1 such that changes in T1, which might occur during the first-pass study, are minimized (9,23,36).

Postprocessing Methods

Six postprocessing methods (Appendix E1 [<http://radiology.rsna.org/cgi/content/full/2492071659/DC1>]) were applied to each of the four types of acquired data, resulting in 24 permutations for determining rCBV values for comparison. Postprocessing was performed off-line by using Analysis of Functional NeuroImages software (37)

and additional programs developed at our institution. The complete dynamic susceptibility-weighted contrast-enhanced MR imaging data analysis procedure consisted of the following steps:

1. The first five times of the raw dynamic susceptibility-weighted contrast-enhanced MR imaging time series were truncated because the MR signal does not reach steady state before this time.

2. Prebolus baseline signal intensity, S_B , was calculated on a voxelwise basis as the mean of the first N_b images of the truncated dynamic susceptibility-weighted contrast-enhanced MR imaging time series, where S_j is the j th image in the time series, with the following equation:

$$S_B = \frac{1}{N_b} \sum_{j=1}^{N_b} S_j. \quad (1)$$

3. The truncated dynamic susceptibility-weighted contrast-enhanced MR imaging time courses were converted into concentration-time (ie, $\Delta R2^*[t]$) curves for single-echo data by using Equation (2):

$$\Delta R2^*(t) = -\frac{1}{TE} \ln \left(\frac{S[t]}{S_B} \right) \quad (2)$$

and for dual-echo data (method C) by using Equation (3):

$$\Delta R2^*(t) = \frac{1}{(TE_2 - TE_1)} \ln \left(\frac{S-TE_1[t]}{S-TE_2[t]} \cdot \frac{S-TE_{1B}}{S-TE_{2B}} \right), \quad (3)$$

where $S-TE_1$ and $S-TE_2$ designate signals from the first-TE and second-TE series, respectively, and $S-TE_{1B}$ and $S-TE_{2B}$ represent the mean of the baseline signals (Equation [1]) for the first TE and second TE, respectively.

4. The rCBV was estimated on a voxelwise basis by using six postprocessing methods, including the following: (a) 120-point trapezoidal integration uncorrected for leakage, hereafter called uncorrected trapezoidal integration (UTI), (b) 120 point trap-

Table 2

Experimental Parameters for MR Imaging Acquisition Methods

Parameter	Method A ($n = 10$)	Method B ($n = 7$)	Method C ($n = 5$)	Method D ($n = 22$)
Pulse sequence	Vendor GRE echo-planar imaging	Vendor GRE echo-planar imaging	Custom dual-echo GRE spiral	Vendor GRE echo-planar imaging
Flip angle (degrees)	90	35	72	90
No. of echoes	1	1	2	1
TE (msec)	30	54	3.3, 30	30
Contrast agent dose (mmol/kg)	0.1	0.1	0.1	0.2
No. of baseline images, or N_b	60	15	60	60
Preload administered	No	No	No	Yes

Note.—Dynamic susceptibility-weighted contrast-enhanced MR imaging was used for data acquisition. The preload refers to the primary injection of contrast agent during acquisition methods A–C, which acts as a loading dose for the subsequent method D. TE = echo time.

Figure 1

Figure 1: Graphic depiction of experimental paradigm. Acquisition method A, B, or C was used to collect data during primary (1°) injection of contrast agent. After clinical contrast-enhanced imaging protocol, acquisition method D was used to collect data during secondary injection (2°) of contrast agent. Primary injection of contrast agent served as preload for acquisition method D. FLAIR = fluid-attenuated inversion-recovery, T1+C = T1-weighted contrast-enhanced image.

ezoidal integration with correction for leakage, hereafter called corrected trapezoidal integration (CTI), (c) gamma-variate fitting, (d) integration after a postbolus baseline correction, hereafter called postbolus baseline subtraction integration (PBSI), (e) maximum signal decrease (MSD), and (f) integration of the negative enhancement portion of the MR signal-time course, hereafter called negative enhancement integration (NEI). These methods and equations are described in Appendix E1 (<http://radiology.rsna.org/cgi/content/full/2492071659/DC1>) and are depicted in Figure 2.

Data Analysis

Data analysis was conducted according to the following steps:

1. Regions of interest (ROIs) were manually constructed by one author (E.S.P., with more than 3 years of experience) for normal-appearing white matter, nonnecrotic enhancing tumor, and contralateral normal-appearing brain regions by using the contrast-enhanced T1-weighted image as a reference. Care was taken to exclude large vessels from the ROIs. The same ROIs for a given patient were applied across all six postprocessing methods.

2. Hot-spot ROIs were obtained by visually choosing the highest 10%–15% of the tumor rCBV values. The rCBV maps used for this selection were those acquired with method D and postprocessed with the CTI method, because these maps were available for all patients who were examined.

3. The rCBV maps (postprocessing step 4) were normalized on a voxelwise basis to the mean rCBV of the normal-appearing white-matter, NAWM, ROI with the following equation:

$$\text{rCBV} = \frac{\text{rCBV}}{\langle \text{rCBV}_{\text{NAWM}} \rangle} \quad (4)$$

Statistical Analysis

All statistical analyses were performed by using software (GraphPad Prism; GraphPad Software, La Jolla, Calif), as illustrated in Figure E1 (<http://radiology.rsna.org/cgi/content/full/2492071659/DC3>). Friedman two-way analysis of variance according to ranks was performed to

determine if differences in median rCBV values exist across postprocessing methods. Kruskal-Wallis one-way analysis of variance according to ranks was performed to determine whether differences exist across acquisition methods. The Wilcoxon matched-pairs test was used to compare values between paired acquisition methods (method A vs D, method B vs D, method C vs D). Finally, Mann-Whitney tests were performed for each of the 24 combinations of data acquisition and postprocessing methods to determine whether there were significant differences between hot-spot or whole-tumor and brain rCBV values.

Results

Figure 3 displays the normalized mean rCBV values for contralateral brain, whole-tumor, and tumor hot-spot ROIs, as a function of postprocessing method, for methods A–D. The results of the statistical analyses are given in Tables 3–6 and Tables E1 and E2 (<http://radiology.rsna.org/cgi/content/full/2492071659/DC2>).

When the data were collected with a standard GRE echo-planar imaging sequence (method A, Fig 3a), a large variability in tumor rCBV resulted. Results of the Friedman test indicated significant differences across postprocessing methods for both contralateral brain and tumor rCBV values (Table 3). In some cases, tumor rCBV was negative (less than zero). This was true when simple integration of $\Delta R2^*(t)$ was used (UTI) for both whole tumor and tumor hot spots. However, when a leakage correction method (CTI) was applied, significant tumor-brain rCBV contrast emerged for both whole tumor ($P = .0002$) and tumor hot spots ($P < .0001$) (Table 6). Likewise, with gamma-variate fitting of the data, significant tumor-brain rCBV values for both whole tumor ($P = .04$) and tumor hot spots ($P < .0001$) resulted. Conversely, the PBSI method did not work well with this acquisition method, because the tumor rCBV values remained negative. Finally, when the signal postprocessing methods were used, the difference between tu-

mor and contralateral brain was significant for both whole tumor ($P = .002$) and tumor hot spots ($P < .0001$) by using MSD (Table 6) but was significant only for tumor hot spots by using NEI ($P < .0001$).

When the data were collected with a small-flip-angle GRE echo-planar imaging sequence (method B, Fig 3b), a dependence on postprocessing method existed for both brain and tumor hot spots (Table 3). In general, use of this acquisition method improved the tumor-brain rCBV image contrast in that no negative tumor rCBV values resulted. However, for all postprocessing methods, the rCBV image contrast between whole tumor and contralateral brain was poor and only reached significance when mean rCBV values were derived from tumor hot spots (Table 6). For this same group of patients, when acquisition method D was used, significant differences between whole-tumor and brain rCBV values did result for the NEI ($P = .02$) and MSD ($P = .01$) postprocessing methods and for all of the postprocessing methods when hot-spot ROIs were used ($P = .0006$ – $.007$).

Dual-echo acquisition (Fig 3c) gave consistently positive and significant tumor-brain rCBV values for all postprocessing methods and for both whole-tumor and tumor-hot-spot ROIs (Table 6). Only with this method did the Friedman test show no significant difference ($P = .62$) in brain rCBV as a function of postprocessing method (Table 3). However, although T1 leakage effects no longer confounded interpretation, what appeared to remain were residual T2 and/or T2* effects, evident as an elevated postbolus $\Delta R2^*$. Consistent with this explanation, the corrected tumor-brain rCBV contrast (CTI) was less than the uncorrected rCBV contrast (UTI). This finding suggests that an overestimation of tumor rCBV may occur when the full 120-point $\Delta R2^*(t)$ is integrated but that it is at least partially corrected by the postprocessing leakage correction method (CTI) (23). Similarly, by applying the gamma-variate or PBSI fitting to these data, the tumor rCBV values were less than those computed with

Figure 2

Figure 2: Pictorial representations of six data postprocessing methods used to estimate rCBV from the dynamic susceptibility-weighted contrast-enhanced MR imaging data, as follows: (a) UTI, (b) CTI, (c) gamma-variate fit, (d) PBSI, (e) MSD, and (f) NEI. *a.u.* = Arbitrary units.

Figure 3

Figure 3: Study results for acquisition methods (a) A, (b) B, (c) C, and (d) D. Six methods of analysis were used to estimate rCBV from data collected with each acquisition method. Values are mean normalized rCBV values from ROIs. *a.u.* = Arbitrary units, *Gd* = gadolinium, *Neg. Enh.* = negative enhancement, *PostB Sub* = postbolus subtraction, *pt* = point, *reference* = contralateral, *TI* = trapezoidal integration.

trapezoidal integration (UTI), a consequence of these postprocessing methods essentially forcing the postbolus $\Delta R2^*(t)$ back to baseline.

Figure 3d demonstrates the effect of using a contrast agent preload before acquiring the rCBV data (method D). In comparison with Figure 3a, for which the same imaging sequence but no preload was used, significant tumor-brain rCBV contrast resulted for all postpro-

Table 3

P Values with Friedman Two-Way Analysis of Variance according to Ranks

Acquisition	Contralateral Brain ROI	Tumor–Hot-Spot ROI	Whole-Tumor ROI
Method A (n = 10)	.03*	<.0001*	<.0001*
Method B (n = 7)	.02*	.006*	.25
Method C (n = 5)	.62	.007*	.02*
Method D (n = 22)	.04*	<.0001*	.0001*

* $P < .05$ indicates a significant difference.

Table 4**P Values with Kruskal-Wallis One-Way Analysis of Variance according to Ranks**

Analysis	Contralateral Brain	Tumor-Hot-Spot ROI	Whole-Tumor ROI
UTI	.006*	.14	.001*
CTI	.003*	.007*	.15
Gamma-variate fitting	.001*	.16	.02*
PBSI	.13	.026*	.0003*
MSD	.027*	.01*	.18
NEI	.023*	.04*	.29

* $P < .05$ indicates a significant difference.**Table 5****Wilcoxon Matched-Pairs P Values**

Structure and Acquisition Method Comparison	UTI Analysis	CTI Analysis	Gamma-Variate Fitting Analysis	PBSI Analysis	MSD Analysis	NEI Analysis
Contralateral brain						
Method A vs D	.38	.56	.23	>.99	.28	.43
Method B vs D	.02*	.047*	.02*	.22	.38	.3
Method C vs D	.44	.31	.31	.44	.31	.63
Tumor hot spots						
Method A vs D	.03*	.19	.28	.006*	.7	.43
Method B vs D	.02*	.02*	.02*	.02*	.02*	.02*
Method C vs D	.44	>.99	.63	.63	.31	.44
Whole tumor						
Method A vs D	.004*	.23	.62	.002*	.38	.11
Method B vs D	.02*	.02*	.02*	.02*	.02*	.02*
Method C vs D	.31	.44	.31	>.99	.31	.31

* Value indicates a significant difference ($P < .05$).**Table 6****Mann-Whitney P Values**

Structure Comparison and Acquisition Method	UTI Analysis	CTI Analysis	Gamma-Variate Fitting Analysis	PBSI Analysis	MSD Analysis	NEI Analysis
Brain vs tumor hot spot						
Method A	.58	<.0001*	<.0001*	.12	<.0001*	<.0001*
Method B	.004*	.03*	.03*	.01*	.21	.073
Method C	.008*	.008*	.008*	.008*	.008*	.008*
Method D	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Brain vs whole tumor						
Method A	.11	.0002*	.04*	<.0001*	.002*	.05
Method B	.07	.901	.13	.8	.21	.1
Method C	.008*	.008*	.008*	.008*	.008*	.008*
Method D	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*

* $P < .05$ indicates a significant difference.

cessing methods (Table 6). Figure 4 demonstrates that when a preload of contrast agent was not used, variability in tumor rCBV was dependent on choice of method of analysis, with negative or zero tumor rCBV results in some cases. Collecting data after preload decreases dependence of tumor rCBV on chosen method of analysis. The effect of the preload is further illustrated by the results from one patient shown in Figure 5. The curves shown in Figure 5b and 5c, obtained from a voxel within the enhancing tumor (Fig 5a), were acquired during the administration of the initial dose or preload of contrast agent (method A). A strong T1 leakage effect is evidenced in that the postbolus $S(t)$ continued to increase above baseline (Fig 5b), corresponding to the decreasing postbolus $\Delta R2^*(t)$ below baseline (Fig 5c). Data from this same voxel, obtained after the preload and during administration of a second dose of contrast agent (method D), no longer demonstrated T1 leakage effects (Fig 5d, 5e). Instead, these curves demonstrated another effect, namely, that of a residual susceptibility and/or dipolar T2 leakage effect, evident in that the postbolus signal remained below baseline (Fig 5d), corresponding to an elevated postbolus $\Delta R2^*(t)$ (Fig 5e). Therefore, under these conditions, contrast agent leakage becomes apparent not as T1 leakage effects but as T2 and/or T2* leakage effects.

The statistical comparisons across acquisition methods for a given postprocessing method (Table 4) showed significant differences for each method for at least one of the tissue ROIs analyzed. For all tissue ROIs, methods C and D showed consistently similar results for all postprocessing methods, with no significant differences in the median of the rCBV values (Table 5).

Discussion

In February 2004, for the first time ever, a biologic agent that targets angiogenesis (38) was approved by the U.S. Food and Drug Administration for clinical use. Since then, additional antiangiogenic agents have received U.S. Food

Figure 4

Figure 4: Parametric maps for rCBV demonstrate utility of contrast agent preload. One section of rCBV maps generated for 21-year-old patient diagnosed with malignant glioblastoma. Top (A–F) and bottom (G–L) rCBV maps were created from data collected with acquisition methods A and D, respectively. Although GRE echo-planar imaging sequence with 90° flip angle was used for both methods, method A was collected during primary injection of standard dose of contrast agent, and method D was collected during secondary injection of double dose of contrast agent after administration of preload. Methods of analysis were as follows: UTI (A, G), CTI (B, H), gamma-variate fitting (C, I), PBSI (D, J), MSD (E, K), and NEI (F, L). Note that when preload of contrast agent was not used (top), variability in tumor rCBV was dependent on choice of method of analysis, with negative or zero tumor rCBV results in some cases. Collecting data after preload (bottom) decreases dependence of tumor rCBV on chosen method of analysis.

and Drug Administration approval, and a large number of clinical trials with these agents are under way (39). Promising findings from two such trials in patients with brain tumors (40,41) are already affecting the way almost every glioblastoma patient is treated. At the same time, it is becoming increasingly clear that, for this class of drugs, the standard way of evaluating treatment response, by monitoring enhancing tumor volumes, is no longer routinely reliable (42,43). It is therefore timely that the study described here directly addresses the capability of the dynamic susceptibility-weighted contrast-enhanced MR imaging method to provide reliable measures of brain tumor vascularity appropriate for this task.

A disparity in rCBV estimates was found among and between acquisition

and postprocessing methods, especially in regions of tumor where contrast agent extravasation occurs. Because high-grade tumors are known to exhibit increased vascularity, it is expected that tumor rCBV should exceed normal brain rCBV. However, this is not the case for several results, which we attribute to the variable sensitivities of the different approaches to leakage effects. In the past, although most attention was paid to T1 leakage effects, it became apparent in this study that when leakage effects are no longer apparent as T1 effects, they can become apparent as T2 or T2* leakage effects.

Extravasation of contrast agent results in simultaneous changes in T1, T2, and T2* within the extravascular-extracellular space. These changes can confound the vascular-extravascular sus-

ceptibility-induced signal changes that result from the contrast agent passing through the vasculature. In the normal brain, it is expected that the signal will transiently decrease and return to baseline after the bolus is administered. If leakage occurs, and T1 effects dominate, the signal will increase above baseline ($\Delta R2^*[t]$ will decrease below baseline), and an underestimation of rCBV may result if methods are not used to diminish or correct for this effect. Conversely, T2* leakage effects are evident as a decrease in the postbolus baseline signal (or elevated postbolus $\Delta R2^*[t]$) beyond that caused by recirculation, which can result in an overestimation of rCBV. The degree of overestimation will depend on the extent to which the postbaseline $\Delta R2^*(t)$ is used in the calculation of rCBV and whether

leakage effects that occur during the signal transient are taken into account. These fundamental concepts, together with the experimental results of this study, have elucidated several important points with regard to obtaining rCBV maps with dynamic susceptibility-weighted contrast-enhanced MR imaging. Several of these points are discussed in the next paragraphs.

Use of GRE echo-planar imaging with a flip angle of 90° does not consistently provide the expected positive tumor-brain rCBV contrast except in the case when a postprocessing correction

algorithm is applied. In fact, in some cases, the tumor rCBV is negative, demonstrating the high sensitivity of this acquisition approach to T1 leakage effects. Although, in some cases, measurements determined directly from signal-time courses resulted in significant tumor-brain contrast, this approach is problematic, as addressed later.

Administration of a preload of contrast agent improves the results for all postprocessing methods. In this study, administration of the standard dose of gadolinium-based contrast agent served

as the preload for acquisition method D. The obvious improvement in the tumor-brain rCBV contrast that resulted, for all postprocessing techniques, supports the contention that the preload plays an important role in diminishing the T1 leakage effects that confound dynamic susceptibility-weighted contrast-enhanced data.

The postprocessing correction algorithm (CTI) performs well with both T1 and T2* leakage conditions. When one uses the 90° flip angle and GRE echo-planar imaging sequence without a preload, the T1 leakage effect can be severe

Figure 5

Figure 5: (a) Contrast-enhanced anatomic image from patient 4. (b–e) MR signal- and concentration-time curves demonstrating confounding T1 and T2 leakage and/or residual susceptibility effects. Data in b and c were collected with acquisition method A (GRE echo-planar imaging with 90° flip angle during primary injection of standard dose of contrast agent). Data acquired during primary injection demonstrate strong T1 leakage effect, as evidenced by the fact that postbolus signal continues rising above its prebolus baseline on b, which corresponds to postbolus portion of $\Delta R2^*(t)$ decreasing below its prebolus baseline level on c. Data on d and e are same types of curves, taken from same voxel, but were acquired with acquisition method D (GRE echo-planar imaging with 90° flip angle during secondary injection of double dose of contrast agent after preload administration). Postbolus signal remains below its prebolus baseline level on d, which corresponds to elevated postbolus portion of $\Delta R2^*(t)$ on e. This is consistent with a dipolar T2 leakage effect or a residual susceptibility leakage effect. a.u. = Arbitrary units.

and can result in negative values for tumor rCBV. However, when leakage correction is applied (24), positive tumor-brain rCBV values result. This result is consistent with data in previous studies in which a loss of rCBV correlation with grade was observed when leakage correction was not applied (9,24). A new finding is that this correction algorithm appears to also correct T2* leakage effects. This finding was demonstrated with the result that the tumor rCBV is lower after correction for the case where the rCBV data were collected by using method D. Although the preload diminishes sensitivity to the T1 leakage effect, the subsequent administration of a double dose of contrast agent, with a cumulative triple dose present, often results in a residual baseline level, that is, a residual T2* (leakage) effect. Therefore, it makes sense that the corrected rCBV is less than the uncorrected rCBV.

A secondary single rather than double dose of contrast agent should be used. A double dose appears to exacerbate residual T2* effects. If the contrast-to-noise ratio of $\Delta R2^*(t)$ is sufficient, as it typically is with GRE methods, a second double dose is neither necessary nor recommended. If spin-echo methods are used, this recommendation needs to be reevaluated.

Small-flip-angle methods reduce sensitivity to T1 leakage effects but result in poor tumor-brain rCBV contrast. These methods were successful in reducing sensitivity to T1 leakage effects in that no negative tumor rCBV values resulted, as was the case with the large-flip-angle T1-sensitive methods. However, the tumor-brain rCBV contrast was poor and only became significant for tumor-hot-spot ROIs. This result is consistent with findings in previous reports in which this acquisition method was used (44,45).

The reduced sensitivity of this method (method B) may be attributed to several factors. First, even though this technique minimizes sensitivity to T1 leakage effects, they may still be present, as previously demonstrated (36). Second, when using this acquisition method, the data are usually fit with

either a gamma-variate function (20,44) or a PBSI performed (46), both in an effort to remove any elevated postbolus baseline. Although this elevated end line is often attributed entirely to recirculation, it may also result from leakage effects, as shown in this study. Any leakage effects that occur during the first-pass transient are ignored with gamma-variate fitting. Finally, although use of a small flip angle reduces T1 sensitivity, it also reduces SNR. For maximum SNR at 1.5 T with repetition time of 1 second, the flip angle should be approximately 72°, as determined from the Ernst equation. The sensitivity of gamma-variate fitting to SNR has been well described (47,48). In fact, in an effort to resolve poor fitting with gamma-variate functions, some have resorted to performing the fits on an average time course from an ROI rather than on a per-voxel basis (20,46).

Analysis of rCBV data by using hot-spot ROIs may improve tumor-brain rCBV contrast but may be suboptimal in practice. Because the rCBV contrast of tumor hot-spot to brain was significant in more cases than it was for whole tumor, one may conclude that it does not matter which acquisition or postprocessing method is chosen if hot-spot analysis is performed. However, choosing the hot spot is typically subjective and, thus, error prone. Also, the location of the hot spots can change with the choice of acquisition and postprocessing methods. This is problematic if rCBV is to be used as a guide for selecting a site for biopsy. Also, an emerging and possibly primary role for rCBV mapping is to monitor therapies. For this purpose, it is going to be critically important that an approach is used that gives the most accurate tumor-brain rCBV contrast throughout the entire tumor.

Obtaining estimates of rCBV directly from the MR imaging signal is not a recommended approach. Because it is easy to implement and readily available with many MR imaging systems, estimates of tissue "perfusion" are sometimes determined directly from $S(t)$ rather than from $\Delta R2^*(t)$. Either the signal transient is integrated or the maximum signal decrease is used for

this estimate (NEI, MSD). However, signal is directly proportional to contrast agent concentration with limited conditions of repetition time and relaxation rate changes and may be influenced by changes in tissue T1 and T2 that are not related to vascular contrast agent concentration. Consequently, it is not surprising that a positive rCBV contrast of whole tumor to brain is not consistently observed when GRE echo-planar imaging is used with flip angles of either 35° or 90° with these methods of analysis. Only when a preload of contrast agent is administered, or the dual-echo sequence is used, does consistent and significant tumor-brain rCBV contrast result.

The dual-echo acquisition method results in positive tumor-brain rCBV contrast for all postprocessing methods with only a single dose of contrast agent. This method has the capability to help detect differences between tumor and brain rCBV, even for a small sample size; such an observation suggests that the dual-echo acquisition method may be one of the best to robustly evaluate tumor rCBV. With this method, T1 effects are completely removed because $R2^*$ is calculated at each time point. What may remain are T2 and/or T2* leakage effects, which can result in overestimations of the rCBV. Therefore, an approach with a dual-echo acquisition and subsequent correction for residual T2 and/or T2* effects may prove to be the most accurate and robust approach for the determination of tumor rCBV (49,50).

With an intact blood-brain barrier, the rCBV results are less dependent on the choice of methods of acquisition and analysis. However, some variability in the rCBV estimates still remains. This variability can be attributed to sensitivity to recirculation effects and remaining issues in regard to acquisition and postprocessing methods that influence or are influenced by SNR. These include the number of baseline points collected, choice of TE, and choice of fitting algorithm (48,51).

Limitations of this study include the fact that tumor and contralateral brain tissue blood volumes were not indepen-

dently measured. This would require careful stereotactic colocalization of the imaging and tissue sampling sites. It is for this reason that the study was confined to high-grade tumors, because it is reasonable to expect greater vascularity in these tumors compared with the reference brain. However, the degree to which tumor rCBV is expected to exceed reference brain rCBV is probably less for this study, which includes recurrent tumors, than it would be for a study that included only de novo brain tumors, because recurrent tumor is likely to be a mixture of tumor and treated tissue, such as radiation necrosis.

An additional confounding factor that underlies most perfusion studies performed in patients with brain tumors involves the administration of steroids, which decrease the permeability of the blood-brain barrier. Steroids can result in less contrast agent extravasation at MR imaging studies and, thus, less of a confounding leakage effect for rCBV calculations. The fact that most of the patients enrolled in this study were being treated with steroids, at the time of the rCBV measurement, should result in less, and not more, of a difference between the techniques studied. Therefore, the result that important differences in tumor rCBV were observed further supports the conclusions of this study that the rCBV measurement does depend on the choice of the data acquisition and postprocessing methods.

In conclusion, with the success of new antiangiogenic therapies for solid tumors, including primary brain tumors, there will be a new demand for brain perfusion imaging. Therefore, a robust approach to measure rCBV, which works well even in the presence of contrast agent extravasation, is needed. This study addresses that need with a comparison of the most commonly published approaches for determining rCBV in patients with high-grade primary brain tumors. In this context, we conclude that the preload-postprocessing correction and dual-echo approaches are the most accurate and robust for the determination of tumor rCBV.

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