

Comparison of Permeability in High-Grade and Low-Grade Brain Tumors Using Dynamic Susceptibility Contrast MR Imaging

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OBJECTIVE. The purpose of this study was to compare permeability measurements in high-grade and low-grade glial neoplasms using a T2*-weighted method. Our hypothesis was that permeability measurements using a T2*-weighted technique would show permeability in high-grade neoplasms to be higher than that in low-grade neoplasms.

MATERIALS AND METHODS. Twelve patients with biopsy-proven high-grade neoplasms and 10 patients with biopsy-proven low-grade neoplasms underwent dynamic susceptibility contrast MR perfusion imaging (TR/TE, 1500/80) after bolus infusion of 0.2 mmol/kg of MR contrast material. Color-coded permeability-weighted maps were created using a model that weights relative contributions to signal intensity from intravascular T2* effects and extravascular T1 effects from blood-brain barrier permeability. Two measures of permeability were performed: mean value of highest permeability found on three images through the tumor (mean regional value) and highest value found at any region of interest in the tumor (single area of maximum permeability). Depending on the normality of the data sets, we used the Wilcoxon's rank sum test or the two-tailed Student's *t* test for statistical analysis.

RESULTS. For low-grade tumors, the range was 0.006–0.041, and the median of the mean regional value for each image was 0.017. For high-grade tumors, the range was 0.005–0.092, and the median of the mean regional value was 0.035 ($p = 0.025$). For low-grade tumors, the range was 0.008–0.045, and the mean of the single area of maximum values was 0.02. For high-grade tumors, the range was 0.007–0.136, and the mean of the single area of maximum values was 0.054 ($p = 0.018$).

CONCLUSION. Permeability values for high-grade tumors obtained using a T2*-weighted method were significantly greater than those for low-grade tumors and are consistent with previous studies reporting results using T1-weighted methods.

Hemodynamic assessment of brain tumors using MR imaging has assumed increased importance given the variety of new therapeutic agents for their treatment. Many novel agents designed to decrease the rate of angiogenesis have been developed and are in clinical trials. For this reason, researchers have placed increased emphasis on development of surrogate markers that could provide information related to angiogenesis in a noninvasive manner [1]. Blood vessel permeability in tumors is one such marker that is possibly thought to reflect the rate of angiogenesis. Previous studies of blood vessel permeability in humans have typically been performed using T1-weighted techniques [2, 3]. In one study using a T1-weighted technique, investigators found a strong correlation between microvascular permeability and tu-

mor grade [3]. We set out to compare permeability measurements in high-grade glial tumors and low-grade tumors using a T2*-weighted dynamic susceptibility technique that allows calculation of a permeability-weighted map [4]. This comparison was performed to determine whether this method would also show a relationship between permeability and tumor grade. Presently, one of the primary uses of the T2*-weighted technique is calculation of a relative cerebral blood volume map. If a relationship between permeability and tumor grade could be shown using a T2*-weighted technique, then this technique could simultaneously be used for derivation of both relative cerebral blood volume maps and permeability maps. Our hypothesis was that permeability measurements using the T2*-weighted technique would also show perme-

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ability in high-grade neoplasms to be higher than that in low-grade neoplasms. To our knowledge, a study attempting to compare permeability measurements obtained using a T2*-weighted technique with tumor grade has not been published.

Materials and Methods

The study population consisted of 10 patients with biopsy-proven low-grade (World Health Organization category I or II) gliomas (age range, 26–58 years; mean, 39 years) and 12 patients with biopsy-proven high-grade (World Health Organization category III or IV) gliomas (age range, 30–75 years; mean, 52 years). Patients were retrospectively chosen from a database of patients with brain tumors who underwent hemodynamic MR imaging between November 1998 and January 2001. For each patient, the initial MR hemodynamic imaging performed at our institution was chosen. Two high-grade tumors and all low-grade tumors were imaged before surgery. Ten high-grade tumors had undergone partial resection, but postoperative imaging showed residual contrast-enhancing tumor. On MR imaging, all 12 high-grade tumors densely contrast-enhanced. Six of the 10 low-grade tumors showed faint contrast enhancement, and four tumors showed no contrast enhancement.

All patients underwent MR imaging using dynamic susceptibility contrast imaging with a spin-echo echoplanar technique (TR/TE, 1500/80). Slice thickness was 5 mm with a 2.5-mm interslice gap. This technique typically provided between seven and nine images through the brain per 1.5-sec interval and allowed coverage of the entire tumor in all cases. All examinations were performed using a power injection of 0.2 mmol/kg of gadopentetate dimeglumine at an injection rate of 5 mL/sec through a 20-gauge IV line.

Permeability maps were generated on an Advantage Windows workstation (version 3.1; General Electric Medical Systems, Milwaukee, WI) using a “ K_2 analysis” approach hand-ported into Functool software (General Electric Medical Systems). Permeability images were derived from T2*-weighted data using the method described by Weisskoff et al. [4], which weighs the relative contributions of T1-weighted effects and T2-weighted effects to the signal change observed during rapid passage of MR contrast material. The observed signal, $\Delta R2_{obs}$, is a weighted linear combination of the T2-weighted intravascular effects and the T1-weighted extravascular effects of a gadolinium-based contrast agent, as illustrated in the equation,

$$\Delta R2_{obs}(t) = K_1 \Delta R2_{avg}(t) - K_2 \int_0^t dt' \Delta R2_{avg}(t'),$$

where $\Delta R2$ is assumed to be proportional to local blood volume. K_1 represents the weighting of the intravascular component, and K_2 represents the weighting of the extravascular component. $\Delta R2_{avg}$ is computed by averaging $\Delta R2_{obs}$ for all nonenhancing pixels in the brain, defined by signal enhancement no greater than 1 standard deviation

above baseline. For each pixel in the brain, K_1 and K_2 are determined by a least-squares fit to the equation, which allowed determination of permeability weighting (K_2).

In the original formulation of this model, a preinfusion loading dose of contrast material was used to keep T1-enhancement rates below 30%. This preinfusion loading dose was not performed in our series for practical reasons related to imaging of patients with brain tumor in a clinical environment.

Permeability measurements were obtained by simultaneously viewing axial contrast-enhanced T1-weighted images and corresponding permeability-weighted images on the workstation. The T1-weighted images were used as a guide for the location of the tumor because on some permeability maps showing relatively normal permeability in tumors, the tumor was not obvious against normal background. The T1-weighted images were not used as a specific guide for placement of regions of interest (ROIs) in a tumor after the tumor was identified on a permeability map. Rather, ROIs were moved in the confines of the tumor (whose borders were identified on the T1-weighted image) until regions having highest permeability values for that slice were identified. Three permeability measures in the tumor were obtained for each patient in the following manner. First, an ROI varying between 57 and 63 mm³ was placed on each of three consecutive permeability images that showed the tumor. In a few instances, all three images were not on consecutive slices. On each of these three images, the maximum permeability was sought using manual placement of the ROI (using the T1-weighted image as a guide), and the maximum permeability value was recorded. The average of these three permeability values (termed “mean regional permeability value”) was recorded for each tumor. The distribution of the mean regional permeability values was compared between groups. For each image, the highest permeability value in a normal-appearing thalamus in the contralateral thalamus was recorded as a control value. Second, the highest permeability value at any ROI in the tumor (termed “single area of maximum permeability”) was recorded.

We assumed that permeability effects are due to leakage of contrast material from small vessels (e.g., arterioles and capillaries) that are not visible on MR images. On this basis, we assumed that permeability measurements in ROIs that contained visible vessels would not differ from those that did not contain visible vessels. To test this assumption, smaller ROIs were drawn in regions of highest permeability values. Specifically, small ROIs were drawn that were placed on vessels. Permeability values in these ROIs were then compared with values in ROIs that were placed adjacent to vessels. These measurements showed that permeability values were similar between ROIs placed on vessels and those placed adjacent to vessels.

For all data sets, the Kolmogorov-Smirnov test of normality was used to determine whether the distribution of values was normal ($p > 0.05$) or not normal ($p < 0.05$) and to indicate whether parametric or nonparametric statistical analysis should

be used to analyze test results. Non-normally distributed data would be analyzed by the Wilcoxon’s rank sum test, and normally distributed data would be analyzed using two-tailed independent samples t test. The mean regional permeability value for high-grade tumors was compared with that for low-grade tumors, and both were compared with the mean value in the thalamus.

Thalamic values were measured as an internal reference point for permeability measurements in normal tissue and to allow comparison between patients. Thalamic values were assessed to determine whether distribution was normal or not normal using the Kolmogorov-Smirnov test of normality. To determine whether thalamic values differed from zero, data that were normally distributed would be analyzed using a paired t test, and data that were not normally distributed would be analyzed using the Wilcoxon’s signed rank test. Thalamic values in patients with high-grade tumors were compared with thalamic values in patients with low-grade tumors using the two-tailed independent samples t test.

Mean regional permeability values were correlated with the mean single area of maximum permeability values using Spearman’s correlation (because data were not normally distributed) to determine whether expression of one of these measurements might prove representative of tumor permeability.

Results

Thalamic values ranged from -0.011 to $+0.017$, resulting in the thalamic permeability values being represented in Figure 1 as a negative value. Thalamic values were normally distributed (Kolmogorov-Smirnov, $p = 0.053$) and were compared with zero using the paired t test. This analysis showed that mean thalamic values were not significantly different from zero ($p = 0.789$), and therefore, we decided not to standardize tumor permeability measurements against thalamic values. Although these values did not differ from zero, both negative and positive values were used to calculate mean thalamic values. Thalamic value distributions were normal in low-grade tumors and in high-grade tumors (Kolmogorov-Smirnov; low-grade, $p = 0.129$; high-grade, $p = 0.2$). The independent samples t test was used to compare mean thalamic values in patients with low-grade and high-grade tumors, which showed that the difference between mean low-grade and high-grade thalamic values was not significant ($p = 0.555$).

The Kolmogorov-Smirnov test of normality showed that the distribution of the mean regional permeability data set was not normal (high-grade, $p = 0.2$; low-grade, $p = 0.007$). For this reason, the Wilcoxon’s rank sum test was chosen for statistical assess-

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ment. For low-grade tumors, the range was 0.006–0.041, and the median of the mean regional permeability value was 0.017. For high-grade tumors, the range was 0.005–0.092, and the median of the mean regional permeability value was 0.03. This difference between the two sample distributions was statistically significant at p equal to 0.025 (Fig. 1).

The Kolmogorov-Smirnov test of normality showed that the single area of maximum permeability data set was normally distributed (high-grade, $p = 0.2$; low-grade, $p = 0.2$). For this reason, the two-tailed independent samples t test was chosen for statistical assessment. For low-grade tumors, the range was 0.008–0.045, and the mean of the single area of maximum values was 0.023. For high-grade tumors, the range was 0.007–0.136, and the mean of the single area of maximum values was 0.054. This difference between the two sample distributions was statistically significant at $p = 0.018$. The Spearman's correlation test comparing mean regional permeability values and single area of maximum permeability values showed that the correlation coefficient was 0.974 with a p value of 0.000.

A side-by-side graphic representation of mean regional permeability values is seen in Figure 2. This plot shows mild overlap between the two populations of tumors. Using an arbitrary threshold of 0.03, we saw that nine high-grade tumors had permeability values above this threshold, and three high-grade tumors had permeability measurements below this threshold. Nine low-grade tumors had permeability values below this threshold, and one low-grade tumor had a permeability measurement above this threshold. Therefore, the positive predictive value for a permeability value of 0.03 was 90%, and the negative predictive value was 75%.

High permeability values were evident on color-coded permeability maps as conspicuous regions that were red or yellow (Fig. 3). On permeability maps of patients with low-grade tumors, permeability in tumors did not differ from values seen in normal tissue (Fig. 4). The low-grade tumor with permeability values above the 0.03 threshold is depicted in Figure 5. This tumor had mean regional permeability values of 0.041 and a single area of maximal permeability value of 0.045. Although this lesion was characterized as low-grade (World Health Organization grade II), the neuropathologist noted that the MIB-1 index, which is a monoclonal antibody directed against recombinant parts of the Ki-67

Fig. 1.—Graph shows median of mean regional permeability values in low-grade tumors and high-grade tumors and mean for control regions in normal thalami. Error bars for regional permeability values represent 68% confidence interval calculated on basis of order statistics. Error bar for thalamic mean represents standard error. Note that values for high-grade tumors are substantially higher than those for low-grade tumors and that both differ substantially from thalamic values. Note also that no overlap of error bars is seen.

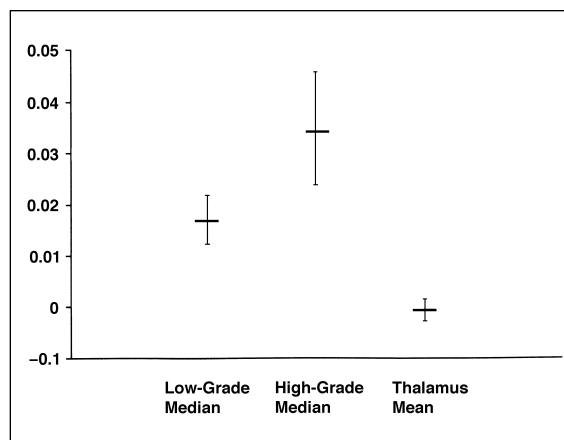
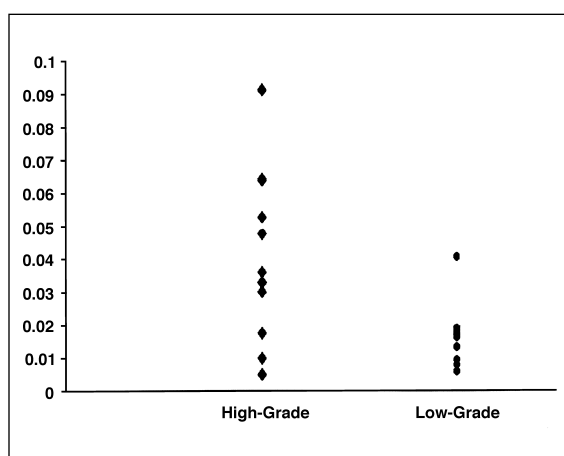


Fig. 2.—Graph shows side-by-side representation of mean regional permeability values in high-grade and low-grade tumors. Note that generally little overlap is seen between two groups although a few high-grade tumors have values that overlap those of low-grade tumors.

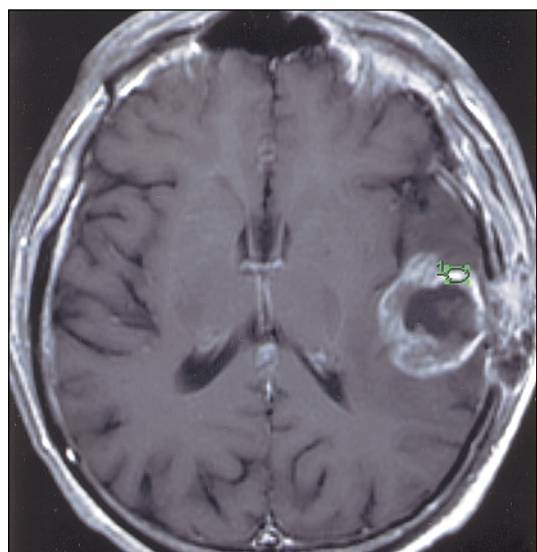


antigen and is a measure of mitotic activity, was particularly high at 5–10% (compared with a typical 1% index in low-grade tumors). On this basis, the neuropathologist suggested that this tumor likely represented a more aggressive tumor than a typical low-grade neoplasm. As this case shows, high permeability values can be seen even in regions that do not densely contrast enhance on conventional T1-weighted images.

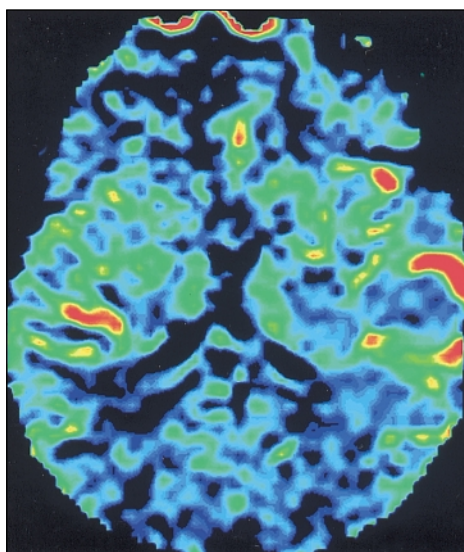
Discussion

Tumor vasculature has a number of features that are almost pathognomonic for malignancy. These features include high permeability to macromolecules, allowed by the presence of large endothelial cell gaps or fenestrae; incomplete basement membrane and relative lack of pericyte or smooth muscle association with endothelial cells; and high vascular tortuosity [5–7]. The high vascular permeability associated with high-grade gliomas is a function of the fact that

these neoplasms have interruption of the blood-brain barrier [1, 3]. Instead of the tight gap junctions found surrounding blood vessels in normal brains, these neoplasms have widened gap junctions rendering the blood-barrier permeable to contrast agents. Vessel permeability appears to be related to the presence of the angiogenic factors that play an important role in growth of human gliomas. One of the major angiogenesis factors associated with growth of tumors, vascular endothelial growth factor, is not only a potent angiogenic factor but also a potent permeability factor (a characteristic for which it has also been called “vascular permeability factor”) [8]. For these reasons, analysis of vascular permeability by dynamic contrast-enhanced MR imaging has been suggested as a method for mapping tumor angiogenesis [1]. Assessment of changes in permeability may provide a surrogate marker of response to antiangiogenesis therapy that would be more valuable than the more standard, but late, changes, such as those in tumor size.



A

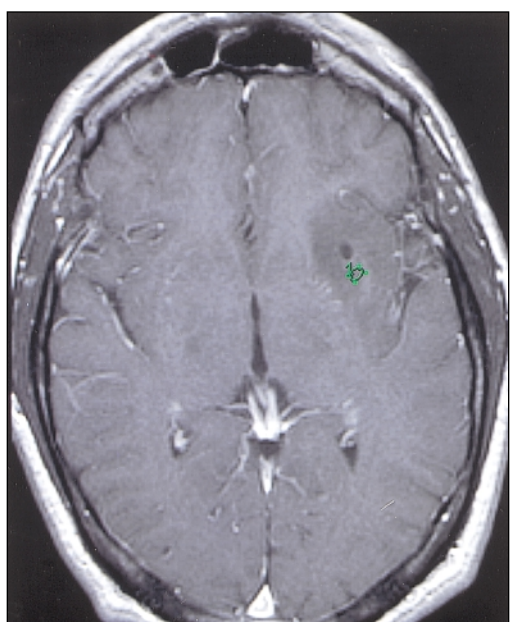


B

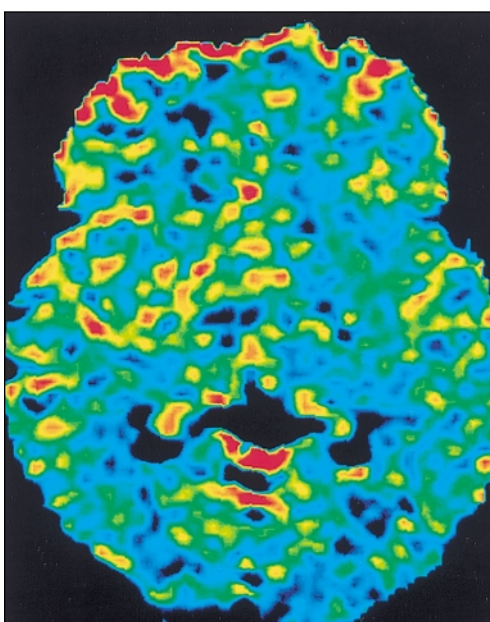
Fig. 3.—70-year-old man who had undergone partial resection of glioblastoma multiforme 4 months before permeability imaging.

A, Contrast-enhanced axial T1-weighted MR image shows rim-enhancing mass in left temporal lobe representing residual tumor. Region of interest (*green circle*) has been placed at site of maximum permeability value measured on permeability map seen in **B**.

B, Permeability map generated from dynamic susceptibility contrast sequence, in which high permeability values are shown in red, reveals crescentic region of high permeability in left temporal lobe. This region corresponds to anterior portion of rim-enhancing mass seen in **A**. In this tumor, mean regional permeability value measured 0.064, and single area of maximum permeability value measured 0.072. Note that other red regions are seen that represent normal vessels rather than regions of elevated permeability.



A



B

Fig. 4.—34-year-old man with recent onset of seizures.

A, Contrast-enhanced axial T1-weighted MR image shows unenhancing left insular mass lesion. Region of interest (*green circle*) that has been placed on site shows area of highest permeability that was found on permeability map seen in **B**.

B, Permeability map shows no areas of elevated permeability in tumor region seen in **A**. Mean regional permeability value in this tumor measured 0.016, and single area of maximum permeability value measured 0.018. At surgery, low-grade glioma was diagnosed.

If analysis of changes in permeability is to play an important role in trials of antiangiogenesis therapies for brain tumors, it is important that MR measurements of permeability be compared with tumor grade and, ultimately, with histologic characteristics of angiogenesis. Dynamic contrast-enhanced MR imaging can provide information on the permeability of the vasculature to the contrast material (given by the product of permeability times and surface area) and the availability of the extracellular ex-

travascular space into which the contrast material can extravasate. Encouraging results have been seen in organs systems other than the central nervous system. For instance, MR derived parameters have been correlated with histologic measures of angiogenesis in cervical cancer [9] and breast cancer [10]. In comparison, experience with MR permeability imaging of human brain tumors is limited, although animal models have correlated permeability*surface area product with histopathologic tumor grade

using a macromolecular contrast medium (albumin-labeled gadopentetate dimeglumine) [11]. In one study of human brain tumors, MR permeability imaging using a dynamic T1-weighted method showed that permeability measurements correlate relatively well with histologic grade of tumor [3].

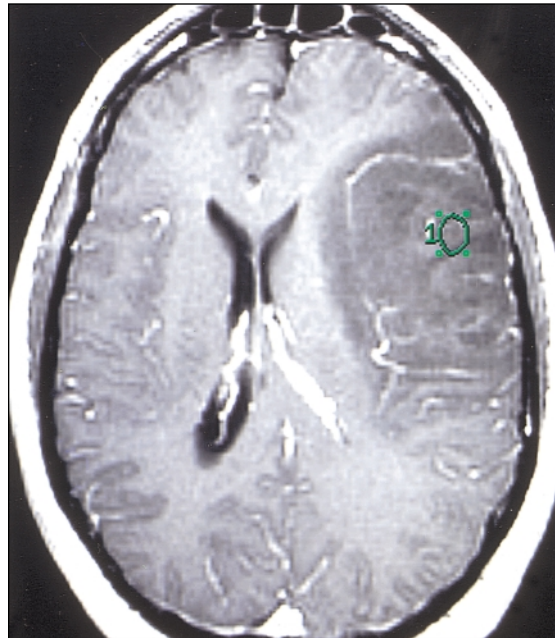
The purpose of our study was to determine whether the positive association between tumor grade and degree of permeability (reported using a T1-weighted method) could be

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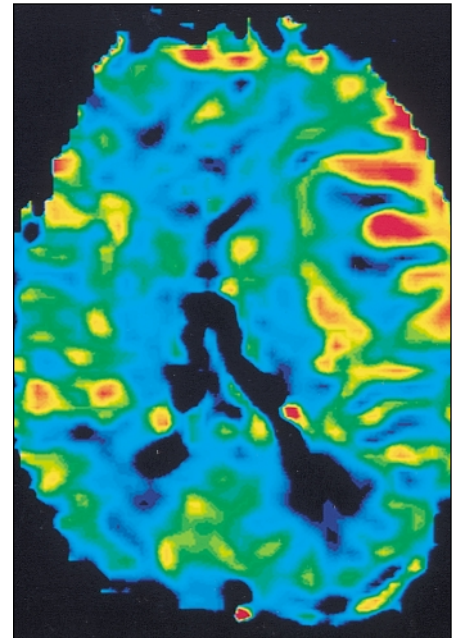
Fig. 5.—34-year-old man with new onset of generalized seizures. Permeability maps show that areas of high permeability can be seen in regions that do not densely enhance on T1-weighted images.

A, Contrast-enhanced axial T1-weighted MR image shows large mildly enhancing left frontal and temporal lobe mass. Region of interest (*green circle*) corresponds to site having highest permeability on this image as seen on permeability maps (**B**).

B, Permeability map shows multiple cortical and subcortical areas of elevated permeability in tumor region seen in **A**. Mean regional permeability value in tumor measured 0.041, and single area of maximum permeability value measured 0.045. Surgical biopsy showed World Health Organization grade II well-differentiated astrocytoma, but pathology report noted that MIB-1 labeling index (a measure of mitotic activity) measured 5–10%, which is much more indicative of aggressive high-grade neoplasm than of typical low-grade neoplasm.



A



B

reproduced using measurements obtained from dynamic susceptibility contrast T2*-weighted images. Permeability measurements can be obtained from T2*-weighted techniques using a calculation that allows separate analysis of T1-weighted effects [4]. This method has the advantage of derivation of both a cerebral blood volume-weighted image and a permeability-weighted image, thereby providing two distinct types of information from one data set. The model used by Weisskoff et al. [4] used a preimaging infusion of a small loading dose of MR contrast material (0.05 mmol/kg). This infusion was performed to preload tissues in an attempt to decrease the likelihood of large (i.e., <30%) degrees of T1-enhancement. For practical reasons related to patient care, we did not use a preinfusion loading dose of contrast material.

Our study showed that mean permeability values measured using the T2*-weighted method were significantly higher in high-grade tumors than those in low-grade tumors. This result was found whether analysis was performed by using the mean of highest values on three separate images or by comparing highest values in the entire tumor between the two populations. Overall, only a small amount of overlap was seen between high-grade and low-grade tumors on mean regional permeability measurements (Fig. 1). The permeability values used in our study are relative; the approach used here measures the

permeability–surface area product, which has the same units as flow. In comparison, one previous study of tumor permeability used a dynamic T1-weighted method that allowed derivation of microvascular permeability measured as the transendothelial transfer constant KPS [3]. In that study, mean microvascular permeability was measured as 6.32 mL/100 cm³ per minute for high-grade (World Health Organization grade III or IV) tumors compared with 1.63 mL/100 cm³ per minute for low-grade tumors (i.e., permeability values in high-grade tumors were approximately four times those seen in low-grade tumors). The permeability values found in our high-grade tumors using a T2*-weighted technique were also substantially greater than those in low-grade tumors, being approximately twice those seen in low-grade tumors. The differences between the permeability values in tumor types in the two studies may reflect differences in pulse sequences used to obtain permeability data, differences between patient populations, or other (as yet) undetermined factors. A direct comparison between T1-weighted and T2*-weighted techniques would be the most appropriate method to determine whether the differences between our study and previously published T1-weighted permeability results were solely due to differences in patient populations.

Previous studies using dynamic MR imaging to examine rate of leakage of MR contrast

material across the blood–brain barrier in humans have predominantly involved T1-weighted methods [2, 3]. In one previous study, a dynamic T1-weighted fast spin-echo technique was used to study rates of contrast enhancement as a means to distinguish treatment-related changes from primary brain tumors [2]. However, correlation between rates of contrast enhancement and tumor grade was not a part of the study design. In another study, a T1-weighted permeability technique was successfully used to show that permeability surface area significantly correlates with tumor grade [3]. Our study using a T2*-weighted technique verifies these results and provides an alternative means of measuring permeability in tumors. The technique used in our study has been previously used to study permeability in a small sample of patients with brain tumor [12]. In that study, substantial decreases in permeability in brain tumors were seen after dexamethasone administration, presumed to be the result of stabilization of the blood–brain barrier by corticosteroid treatment.

We recognize that a selection bias was present in our study population because most high-grade tumors were residual tumors after surgery, and all low-grade tumors were preoperative lesions. Therefore, if factors related to surgery increased tumor permeability, differences between high-grade and low-grade tumors could be related to surgery rather than strictly to tumor grade. However, we are presently per-

forming a study comparing mean regional permeability in unresected high-grade tumors and resected high-grade tumors. Preliminary data from this comparison show that permeability values are similar between the two groups. Therefore, we believe that it is unlikely that surgical status substantially influences permeability, and it is likely that tumor grade is the predominant factor influencing tumor permeability. Another potential limitation of our study was the fact that a preloading dose of contrast material, such as was used in the initial formulation of the T2*-weighted permeability model [4], was not used because it would limit the practical utility of the method. The effect of deletion of the preloading dose is difficult to assess because the conditions under which tumors have an enhancement rate greater than 30% is not known. Future studies to assess the effect of a preloading dose on tumor permeability imaging are indicated. Finally, an important limitation of our study is the assumption we have made about the link between permeability, angiogenesis, and malignancy. Although our data suggest that permeability is correlated with malignancy, this suggestion does not establish a link between angiogenesis and permeability or angiogenesis and malignancy. Animal models showing angiogenesis independent of permeability changes [13] have highlighted this potential limitation. Whether permeability and angiogenesis and malignancy are dependent in humans will require further study.

The exact relative merits of T2*-weighted dynamic susceptibility contrast techniques and T1-weighted techniques for evaluation of hemodynamic alterations in tumors are still a matter of debate. The T1-weighted pulse sequences used to derive permeability data in previous human studies vary in duration from approximately 3 to 5 min and use a dose of 0.1 mmol/kg of MR

contrast material via either a hand bolus or power injection [2, 3]. T1-weighted techniques provide permeability measurements but not relative cerebral blood volume. The T2*-weighted method optimally uses a power injection of 0.2 mmol/kg of MR contrast material with rapid imaging performed over the period of 1 min. Both permeability and relative cerebral blood volume can be obtained. However, in clinical studies, an additional contrast-enhanced T1-weighted sequence should be obtained for anatomic images; this additional sequence essentially offsets part of the time savings provided by the T2*-weighted sequence.

In summary, our findings indicate that permeability measurements in high-grade tumors obtained using a T2* technique are significantly higher than those in low-grade tumors. Future studies directly comparing permeability measures obtained using T2*-weighted techniques and T1-weighted techniques are warranted. At present, to our knowledge, no published data exist that assess change in permeability values in response to chemotherapy or antiangiogenesis agents. Future studies should address the use of this method to follow tumor response. Furthermore, because it is impossible to tell whether lack of a preloading dose of contrast material affected our results, future studies to address the effect of a preloading dose on permeability measurements are warranted.

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