Blood-Brain-Barrier Imaging in Brain Tumors: Concepts and Methods

Rajan Jain, MD, Brent Griffith, MD, Jayant Narang, MD, Tom Mikkelsen, MD, Hassan Bagher-Ebadian, PhD, Siamak P. Nejad-Davarani, PhD, James R. Ewing, PhD, and Ali S. Arbab, PhD, MD

ABSTRACT
Malignant gliomas are often very heterogeneous tumors with complex vasculature, frequently exhibiting angiogenesis and increased vascular permeability. In vivo measurement of the tumor vessel permeability can serve as a potential imaging biomarker to assess tumor grade and aggressiveness. It can also be used to study the response of tumors to various therapies, especially antiangiogenic therapy. Central to the concept of permeability is a thorough knowledge of the BBB and its role in brain tumors and angiogenesis. Much work has been done in the past to understand the structural/molecular composition of the BBB and the role it plays in various pathologic processes, including brain tumors. Various imaging techniques have also been used to evaluate BBB leakiness in brain tumors because higher tumor vascular leakiness is known to be associated with higher grade and malignant potential of the tumor and hence poor patient prognosis. These imaging techniques range from routine postcontrast T1-weighted images to measurement of vascular permeability using various quantitative or semiquantitative indices based on multicompartment phamacokinetic models. The purpose of this article is to discuss BBB anatomy; various clinically available imaging techniques to evaluate tumor vascular leakiness (perfusion imaging), including their advantages and limitations; as well as a brief discussion of the clinical utility of measuring vascular permeability in brain tumors. We will also discuss the various permeability-related indices along with the pharmacokinetic models to simplify the “nomenclature soup.”

INTRODUCTION
The BBB consists of a complex of capillary endothelial cells, pericytes, and astroglial and perivascular macrophages, serving as an effective physical barrier to the entry of lipophobic substances into the brain. However, in many brain tumors, as well as other disease processes, the BBB becomes interrupted. It is this disruption and other changes to the BBB in tumors that contribute to contrast enhancement on imaging and serve as a potential surrogate imaging marker.

Malignant gliomas are hypervascular tumors with very heterogeneous and complex vasculature, which is regulated by multiple angiogenic cytokines. Tumors larger than 1–2 mm cannot grow by relying on passive transport of nutrients and oxygen. Further growth is supported by angiogenesis promoted by proangiogenic factors; the most important among those is VEGF. Animal studies indicate that neoangiogenesis and increased vascular permeability are essential for the survival and proliferation of tumor cells. An understanding of this process is essential because it provides the physiologic basis for perfusion imaging in tumors. Tumor angiogenesis involves a multitude of controlled signaling cascades and structural changes that occur in a defined order and continue until
a new vasculature has been formed (Fig 1). The process usually begins when tumor cellular growth outgrows its blood supply leading to hypoxia and low intratumoral pH. These local factors, in turn, lead to expression of HIF-1α, which, in conjunction with genetic pathways (phosphoinositide 3-kinase, isocitrate dehydrogenase-1—they help stabilize HIF-1α by decreasing its degradation), promote the formation of proangiogenic mediators (angiogenic switch) such as VEGF and SDF-1. The effects of VEGF and SDF-1 lead to formation of immature and leaky blood vessels, which results in increased permeability. This increased permeability allows extravasation of plasma and plasma proteins and deposition of proangiogenic matrix proteins, ultimately resulting in microvascular cellular proliferation (MVCP). In addition to VEGF-related pathways, there are various non-VEGF pathways (growth factors, fibroblast growth factor, platelet-derived growth factor, and Notch surface receptors), which also result in endothelial cell upregulation, proliferation, and increased permeability.

As these pericyte-poor new vessels (called “mother vessels”) enlarge and give rise to daughter vessels through a complex series of endothelial rearrangements, MVD and total vascular area (TVA) increase; this change leads to further increased permeability (Fig 2). Finally with vessel maturation, the number and area of blood vessels continue to increase to a greater extent than vessel leakiness—evolving into a very heterogeneous tumor with various regions probably demonstrating different mixtures of vessel characteristics and angiogenesis. Given this heterogeneity, not only is an in vivo estimate of the hemodynamics (tumor blood volume) important, but assessment of the physiologic measures (vascular leakiness) is of utmost importance as well. This has become particularly true recently with the increasing use of oncologic imaging, the increasing use of newer antiangiogenic agents, and increasing emphasis on the development of quantitative imaging biomarkers.

Various perfusion imaging techniques have been used in animals and human subjects to estimate tumor vascular permeability, using either quantitative or semiquantitative measures. The remainder of this review will focus on the various clinically available permeability imaging modalities/techniques, their advantages and limitations, and the clinical utility.

**Measuring Tumor Vascular Permeability: Why is it important?**

Tumor blood vessels have defective and leaky endothelium, thereby allowing these abnormal tumor vessels to serve as potential markers for assessing tumor grade and aggressiveness. Thus, in vivo measurement of tumor-vessel permeability may serve a number of purposes, including but not...
limited to grading of tumors, because increased permeability is associated with immature blood vessels, which are seen with angiogenesis, and with assessing response to therapy because changes in permeability may provide a means of assessing treatment response, especially when antiangiogenic therapy has been used. Measuring tumor vessel permeability could also help in better understanding the mechanism of entry of therapeutic agents into the central nervous system, which could be very important for development of methods to selectively alter the BBB to enhance drug delivery.

**Tumor Vascular Permeability: What are we measuring?**

Permeability is related to the diffusion coefficient of contrast agents in the assumed water-filled pores of the capillary endothelium. The diffusion flux of contrast agent across the capillary endothelium is dependent on both the diffusion coefficient and the total surface area of the pores. PS characterizes the diffusion of some of the contrast agent from the blood vessels into the interstitial space due to deficient or leaky BBB and is a product of permeability and capillary surface area. This can be measured using CT perfusion and is commonly used units: mL/100 g/min.

**K<sub>trans</sub>** measures the rate of flux of contrast agent from the intravascular compartment into the extracellular extravascular space and is dependent on both vascular permeability and capillary surface area. This can be measured using DCE MR imaging techniques and is commonly used units: minute<sup>-1</sup>. In states of very high permeability (e.g., very leaky tumors), K<sub>trans</sub> is only limited by blood flow and hence predominantly measures blood flow; whereas in states of very low permeability, contrast agent cannot leak out, so K<sub>trans</sub> measures permeability.

**k<sub>b</sub> or k<sub>epp</sub>** measures the backflow of contrast agent from the extravascular compartment into the intravascular compartment. This can be measured using DCE MR imaging techniques and is commonly used units: minute<sup>-1</sup>. Its measurement is based on a number of factors including the volume of the extravascular compartment and the interstitial pressure.

$$E = 1 - e^{-\frac{PS}{F}}$$

where PS is the permeability surface-area product and F is flow. The PS product has the same dimensions as flow (milliliters/100 gram/minute), and thus the ratio PS/F is dimensionless. In physiologic terms, PS is the rate at which contrast agents flow into the extravascular tissues; it is related to another commonly stated parameter of vascular leakage, K<sub>trans</sub>, by the following equation:

$$K_{trans} = ExF,$$

where K<sub>trans</sub> is the forward transfer constant with, again, the same dimensions as flow (unit commonly used is minute<sup>-1</sup>) (Table 1). It is easily demonstrated that if PS/F \(\ll 1\) (or F \(\gg\) PS, which is usually the case in high-grade leaky brain tumors because blood flow is usually very fast), then K<sub>trans</sub> \(\approx\) PS. In normal cerebral vasculature, PS is negligible for all contrast agents presently in clinical use.

However, in brain tumors, estimates of K<sub>trans</sub> are complicated by the fact that there is backflow of the contrast agent measured by k<sub>epp</sub> or k<sub>b</sub> from the extravascular compartment into the intravascular compartment, which is based on a number of factors including the volume of extravascular extravascular compartment and the interstitial pressure (Fig 3 and Table 1).

---

Table 1—Commonly used permeability indices: definition and important details

<table>
<thead>
<tr>
<th>Permeability Indices</th>
<th>Definition</th>
<th>Important Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>Characterizes the diffusion of contrast agent from the blood vessels into the interstitial space due to deficient or leaky BBB; it is a product of permeability and capillary surface area</td>
<td>• Usually measured using CT perfusion</td>
</tr>
<tr>
<td>K&lt;sub&gt;trans&lt;/sub&gt;</td>
<td>Measures rate of flux of contrast agent from the intravascular compartment into the extracellular extravascular space and is dependent on both vascular permeability and capillary surface area</td>
<td>• Usually measured using DCE MR imaging techniques</td>
</tr>
<tr>
<td>k&lt;sub&gt;b&lt;/sub&gt; or k&lt;sub&gt;epp&lt;/sub&gt;</td>
<td>Measures backflow of contrast agent from the extravascular compartment into the intravascular compartment</td>
<td>• Usually measured using DCE MR imaging techniques</td>
</tr>
</tbody>
</table>

Note: Ve indicates extravascular extravascular compartment.
Understanding Angiogenesis and Its Correlation with Perfusion Parameters

As already mentioned, angiogenesis is an essential aspect of tumor growth because it provides tumors a means of maintaining blood supply. In the initial phase of angiogenesis, vessel leakiness, which is measured by permeability (PS or Ktrans), increases more than the total number and area of blood vessels, which are measured by blood volume. However, as the vessels mature, the total number and area of blood vessels increase more than vessel leakiness, evolving into a very heterogeneous tumor with regions showing different mixtures of vessel characteristics and angiogenesis. This heterogeneity is manifest by differences in tumor blood volume and permeability, which do not necessarily increase or decrease in tandem and probably represent 2 different aspects of tumor vasculature (Fig 4).

Tumor Vascular Permeability: Dynamic Contrast-Enhanced Imaging Techniques

While postgadolinium T1-weighted MR imaging gives a rough estimate of the disruption of BBB by providing a snapshot in time and has been used in the past for quantitative estimation of permeability, dynamic imaging acquisition provides a better estimate of vascular permeability. Various noninvasive dynamic contrast-enhanced imaging techniques including MR imaging\(^{12-14}\) and CT perfusion\(^{15,16,17}\) have been used for in vivo estimation of tumor vascular leakiness in brain tumors (Table 2). DCE MR imaging data acquisition primarily involves 2 techniques; 1) T1-weighted acquisition, which is based on the fact that increase in the rate of T1 relaxation is proportional to the concentration of the contrast agent from which a time-concentration curve can be generated and is tracked during a longer time period (5–10 minutes); and 2) first-pass T2*-based acquisition, which uses susceptibility-weighted imaging to generate a time-concentration curve during the initial 45–60 seconds of circulation. Both DCE MR imaging techniques have advantages and disadvantages; however, the major limitation is a nonlinear relationship of contrast agent concentration with tissue signal intensity.

Dual-echo gradient echo perfusion-weighted imaging\(^{18}\) is based on a simple 2-compartment kinetic model\(^{19}\) and has been used to measure vascular permeability and to correctly estimate blood volume in lesions/tumors with deficient or absent BBB. Several studies have found a correlation between increased vascular permeability and higher tumor grade.\(^{14,20-23}\) However, MR perfusion techniques have certain limitations because of the nonlinear relationship of the signal intensity with the contrast agent, both for dynamic contrast-enhanced imaging with T1-weighting\(^{24-27}\) and for dynamic susceptibility contrast imaging with T2- or T2*-weighting. In the latter case, if the contrast agent remains intravascular, the method is widely accepted as a relative estimate of CBF and CBV, though there is a possibility for artifacts because of difficulties in assessing the shape and timing of the arterial input function.\(^{28-34}\) However, in cases with leaky BBB, there is substantial leakage of contrast agent from the intravascular to extravascu-
lar space and a strong and competing T1 contrast effect is often noticed in areas of pathology. To minimize the competing T1 effect, preloading with contrast agent has been proposed, with some success. However, this approach does not allow an estimate of $K_{\text{trans}}$. An alternative approach has also been proposed to decrease T1 effect, which uses a slower TR, lengthening the TR of the experiment and undermining the estimation of CBF, thus yielding only estimates of CBV and $K_{\text{trans}}$. A further refinement, allowing the estimate of blood volume and producing an index of transfer constant, has been suggested, and a dual-echo gradient echo sequence also shows some potential for an index of blood volume and transfer constant. Despite the partial success of these rapid imaging studies, in contrast to PCT, there does not appear to be an MR imaging technique that will reliably quantify CBF, CBV, and $K_{\text{trans}}$.

Tumor Vascular Permeability Imaging: Limitations and Controversies

Low spatial resolution has been one of the major limitations of the available clinical imaging tools, which limits a detailed assessment of the complex and heterogeneous vascular microenvironment. Another major limitation has been the use of the currently FDA approved low-molecular-weight CT and MR imaging contrast agents ($\sim$0.5–0.9 kDa) for human subjects, which might limit the accurate differentiation of vascular permeability and blood volume. Permeability measurements depend on the leakage of particles from the blood to the interstitial space; it is hypothesized that low-molecular-weight contrast agents leak relatively easily from the blood into the interstitium. This might also lead to overestimation of the permeability, which will be influenced by and will approximate tumor blood flow.

On the other hand, high-molecular-weight contrast agents leak from the vessels and move through the interstitium with relative difficulty and are hence flow-independent. Therefore, macromolecular permeability and vascular volumes may be best measured by high-molecular-weight contrast agents. Even though CT and MR imaging contrast agents do not differ much in their molecular weight, different charges of nonionic CT contrast compared with ionic MR imaging contrast may also be responsible for the differences in permeability measured with these 2 modalities. Blood pool contrast agents, particularly albumin-binding agents (such as gadofosveset, ABLAVAR; Lantheus Medical Imaging, North Billerica, Massachusetts; which was recently FDA-approved for MR angiography of peripheral vascular disease) may solve some of the issues associated with easy leakage of extracellular contrast agents into the interstitium.

Another controversial aspect of measuring permeability with various perfusion imaging techniques has been the scanning time. It is presumed that delayed permeability due to slow leakage of contrast from a leaky blood vessel may not be accurately measured with the first pass of the contrast agent by using a 45- or 60-second scanning time and can be measured only with longer acquisition times. However, there is no consensus or criterion standard for the optimal acquisition time. Gliomas, particularly high-grade gliomas, can have extremely variable and heterogeneous blood flow due to the complex tumor vasculature, which can influence permeability. Various other intratumoral factors that can also influence permeability include luminal surface area and interstitial, hydrostatic, and osmotic pressure across the endothelium. Slow blood flow and/or low osmotic gradients, which can occur in high-grade
tumors with a lot of vasogenic edema and also in the central necrotic or hypoperfused parts of large tumors, can lead to a larger component of delayed permeability, which may need longer acquisition times for accurate estimation.17

Another major limitation of permeability imaging is complex multicompartment modeling needed for postprocessing calculations. Quantification of vessel permeability with various perfusion techniques requires a 2 or more compartment pharmacokinetic model with an arterial input function, making these studies more complex than CBV estimation,11 and quantification is dependent on the imaging technique and the mathematic model43 that is used.

Steroid use is another confounder that can affect blood volume and tumor-permeability measurements, affecting permeability measurements more than blood volume,44,45 and, hence, should be known before comparing results across different patients and different time points in the same patient.

Going Backwards with Tumor Vascular Permeability: Semiquantitative Indices

Accurate and absolute quantitative estimates of permeability may not be easily possible due to complex multicompartment physiologic models needed to derive these metrics by using DCE MR imaging techniques as described above. On the contrary, various model-free “semiquantitative” indices derived from DCE-MR imaging have been successfully used in the past in the evaluation of prostate, breast, cervical, and pancreatic cancers.11,41-48 These parameters include onset time of contrast agent arrival, initial and mean gradient of the upslope of the enhancement curve, maximum signal intensity, and washout gradient.46 Narang et al17 used MSIVP, normalized MSIVP, normalized slope of the delayed equilibrium phase, and nIAUC at 60 and 120 seconds (nIAUC60 and nIAUC120) to differentiate recurrent tumors from treatment-induced necrosis (Fig 5). The major disadvantage with these is that these parameters lack a physiologic basis limiting their growth as absolute quantitative measures of tumor vascular leakiness. Despite that, these model-free metrics could have a more practical role to play in the routine clinical setting because they do not depend on the technical expertise needed for the more complex model-based analysis. However, their direct comparison across multiple centers or even individual examinations could be limited due to the fact that these do not accurately reflect contrast agent concentration in the tissue and hence can be influenced by scanner type and settings.

Previous authors have also successfully used similar indices such as relative PSR or signal-intensity enhancement-time curves as an indirect measure of vascular leakiness.48,49 Barajas et al48 showed lower relative PSR in
recurrent GBM compared with radiation necrosis by using DSC MR perfusion imaging, suggesting a disrupted BBB that was more permeable to macromolecular contrast agents; however, their measurements were not a direct estimate of lesion leakiness. They also noted a large degree of overlap between the 2 groups, making rPSR a less robust predictor of recurrent tumor. The same group also published the same methodology with stronger results by using rPSR to differentiate metastatic tumors from radiation necrosis, suggesting that rPSR may be a better prognostic indicator of tumor recurrence than rCBV.50

Tumor Vascular Permeability: Molecular Basis and Correlation with Immunohistologic Markers

Tumor vascular permeability has been shown to correlate with VEGF, VEGFR-2 expression, and tumor growth in breast cancer tumor models in rats.51 However, direct in vivo correlation of PS estimates with molecular angiogenic markers has not been performed in the past in the human subjects, to our knowledge. Jain et al52 showed MVCP to be significantly correlated with PS and not with CBV. This finding suggests that MVCP is associated with leakier tumor vessels. Therefore, regions of increased PS within a heterogeneous tumor might indicate more immature vasculature, whereas higher CBV regions might indicate hyperperfusion and more mature vasculature. In that study, even though correlation of PS with VEGFR-2 expression did not reach statistical significance, it did show a positive trend, suggesting that regions with higher VEGFR-2 expression and higher MVCP could be localized by using PS parametric maps.16,52 These regions of hyperpermeability have been shown to be associated with insufficient blood flow and oxygen transport, resulting in tumor hypoxia53; hence, identifying these in vivo could potentially help target and monitor treatment.

Given the increasing focus on the use of quantitative imaging biomarkers for patient survival and treatment response, it is critical to understand the molecular basis of these imaging features. Recent literature has tried to correlate morphologic imaging features with gene expression in GBMs54-57; however, there has been little emphasis on correlating metabolic or physiologic imaging biomarkers with gene expression. Perfusion parameters such as CBV and PS estimates in GBMs have been shown to correlate positively with proangiogenic genes and inversely with antiangiogenic genes. This correlation can help establish a genomic/molecular basis for these commonly used imaging biomarkers58 and potentially add to our existing knowledge of their immunohistologic bases.36,52

Tumor Vascular Permeability: Clinical Utility

Glioma Grading and Prognosis. Glioma grading historically has been based on the histology of the resected specimen, which is dependent on tumor cellularity, cellular pleomorphism, mitosis, necrosis, and endothelial hyperplasia and does not include microvascular density or total vascular area. Histologic grading is thus limited by sampling error, wide ranges of classification and grading systems, inter- and intrapathologist variability, and, most important, by the evolving nature of central nervous system tumors. In contrast, an in vivo technique to assess tumors may provide similar information about the whole tumor and can be repeated noninvasively and hence could assess the evolution of tumors and help monitor treatment response. Tumor blood volume and permeability are the two important vascular parameters that have been shown to correlate with glioma grade (Fig 6),14,17,20,59,60 treatment response, and prognosis. The information provided by CBV and PS is probably complimentary, with CBV revealing information about the amount of total vessels in that part of the tumor, whereas PS provides information about the degree of abnormality of the BBB,36,52 which, in turn, could potentially correlate with proangiogenic cytokines activity61 within the tumor.
Most perfusion imaging studies have focused on blood volume estimates and correlation with survival prediction in mixed populations of gliomas, whereas only a few studies have explored tumor leakiness or permeability estimates as prognostic factors. Mills et al used both CBV and Ktrans in a single experiment, though they found the relationship of Ktrans with survival to be unexpected and counterintuitive in high-grade gliomas because patients with higher Ktrans showed better survival. Dhermain et al recently showed that low-grade gliomas with microvascular leakage and contrast enhancement show poor progression-free survival compared with those without it. In a recently presented study and our unpublished data, we have evaluated both rCBV and leakiness (PS) estimates as prognostic factors in high-grade gliomas and found that both of these parameters, which can be estimated with a single experiment by using PCT, correlated significantly with overall patient survival. However, after adjusting for World Health Organization grade, PS estimates were not statistically significant. However, within the grade III glioma group, patients with grade III gliomas and lower PS (<1.7) had a significantly better overall survival (P = .011) compared with grade III gliomas with higher PS estimates, suggesting that measuring tumor leakiness could provide additional prognostic information in high-grade gliomas.

**Assessing Treatment Response and Treatment Planning.**

Most of the imaging follow-up for brain neoplasms is based on measurement of contrast-enhancing tumor. However, the increasing use of antiangiogenic agents and multitechnique treatment regimens for treatment of recurrent high-grade neoplasms has raised doubts about just using contrast-enhancing tumor as a marker of treatment response. While antiangiogenic agents inhibit angiogenesis and seem to control tumor enhancement initially, infiltrative nonenhancing tumor may continue to grow. Thus, measuring only the enhancing lesion might overestimate the response. As such, other imaging criteria and methods have become more important in accurately assessing treatment response. Permeability measurements allow more accurate assessment of treatment response following antiangiogenic treat-
ment as measured by a decrease of vessel permeability and $K^{\text{trans}}$ on serial imaging.\textsuperscript{74,75} Measuring tumor permeability could also be important to determine the choice and timing of therapeutic agents for optimal combination therapy because it is important to understand how various agents influence BBB and the optimal timing of maximal opening of the BBB, whether during radiation therapy\textsuperscript{76} or administration of antiangiogenic agents.\textsuperscript{77} Measuring permeability could influence decisions regarding treatment planning by differentiating recurrent/progressive tumor from treatment effects (Fig 8).

**Differentiating Non-Neoplastic Lesions from Neoplasms.**

Occasionally multiple sclerosis or vasculitis/angiitis manifests as a single large tumefactive lesion with atypical morphologic imaging features, thus necessitating biopsy for an accurate diagnosis. Enhancement in tumefactive demyelinating lesions (TDLs) is thought to occur due to the BBB breakdown and macrophage infiltration,\textsuperscript{78} whereas enhancement in tumors and especially high-grade tumors, which TDLs mimic, occurs due to neoangiogenesis and a break in the BBB. Hence, perfusion imaging can help to differentiate these 2 entities because TDLs usually show low blood volume and also low permeability (Fig 9) due to lack of neoangiogenesis or immature leaky vessels, whereas tumors, especially high-grade gliomas, will show high blood volume and higher permeability due to tumor neoangiogenesis.\textsuperscript{79-81}

**CONCLUSIONS**

Despite the limitations and lack of standardization of the clinically available imaging techniques to measure tumor vascular permeability, additional information obtained from permeability imaging may be used in a number of important ways, including glioma grading and patient prognosis, as well as treatment guidance and assessing treatment response. With improvements of image acquisition and postprocessing software in the future, permeability
imaging could be an important clinical tool in the diagnosis and management of brain tumors.

REFERENCES

34. Calamante F, Morup M, Hansen LK. Defining a local arterial


