Microvascularization of Grade I meningiomas: effect on tumor volume, blood loss, and patient outcome

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OBJECTIVE Quantitative assessment of tumor microvascularity has the potential to improve prognostication, advance understanding of tumor biology, and help narrow potential molecular therapies. While the role of tumor microvascularity has been widely studied in meningiomas, this study examines both the role of automated measurements and the impact on surgical outcome.

METHODS Two hundred seven patients with Grade I meningiomas underwent surgery between 1996 and 2011. Tissue samples from each patient were retrospectively evaluated for histopathological measures of microvascularity, including staining for von Willebrand factor (vWF), CD31, CD105, hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factor, glucose transporter 1, and carbonic anhydrase IX. Manual methods of assessing microvascularity were supplemented by a computational analysis of the microvascular patterns by means of fractal analysis. MIB-1 proliferation staining was also performed on the same tumors. These measures were compared with various patient characteristics, tumor volume, estimated blood loss (EBL) during surgery, progression-free survival (PFS), and overall survival (OS).

RESULTS The mean patient age was 55.4 ± 14.8 years, and 63 (30.4%) patients were male. Patients harboring tumors ≥ 3 cm were significantly older (56.9 ± 15.2 years vs 53.1 ± 13.6 years; p = 0.07), more frequently male (40.8% vs 14.6%; p = 0.0001), and had greater EBL (446.5 ± 532.2 ml vs 185.4 ± 197.2 ml; p = 0.0001), greater tumor volume (33.9 ± 38.1 ml vs 29.4 ± 23.5 ml; p = 0.0001), higher MIB-1 index values (3.0% ± 5.4% vs 1.7% ± 1.7%; p = 0.03), higher vWF levels (85.6% ± 76.9% vs 54.1% ± 52.4%; p = 0.001), lower HIF-1 expression (1.4 ± 1.3 vs 2.2 ± 1.4; p = 0.004), and worse OS (199.9 ± 7.6 months vs 180.8 ± 8.1 months; p = 0.05) than patients with tumors < 3 cm. In the multivariate logistic regression, MIB-1 (OR 1.14; p = 0.05), vWF (OR 1.01; p = 0.01), and HIF-1 (OR 1.54; p = 0.0001) significantly predicted tumor size. Although multiple factors were predictive of EBL in the univariate linear regression, only vWF remained significant in the multivariate analysis (β = 0.39; p = 0.004). Lastly, MIB-1 was useful via Kaplan-Meier survival analysis for predicting patients with disease progression, whereby an MIB-1 cutoff value of ≥ 3% conferred a 36% sensitivity and 82.5% specificity in predicting disease progression; an MIB-1 value ≥ 3% showed significantly shorter mean PFS (140.1 ± 11.7 months vs 179.5 ± 7.0 months; log-rank test, p = 0.05). The Cox proportional hazards model showed a trend for MIB-1 in predicting disease progression in a hazards model (OR 1.08; 95% CI 0.99–1.19; p = 0.08).

CONCLUSIONS These results support the importance of various microvascularity measures in predicting preoperative (e.g., tumor size), intraoperative (e.g., EBL), and postoperative (e.g., PFS and OS) outcomes in patients with Grade I meningiomas. An MIB-1 cutoff value of 3% showed good specificity for predicting tumor progression. The predictive ability of various measures to detect aberrant tumor microvasculature differed, possibly reflecting the heterogeneous underlying biology of meningiomas. It may be necessary to combine assays to understand angiogenesis in meningiomas.

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KEY WORDS meningioma; MIB; microvascularity; angiogenesis; fractal analysis; blood loss; progression-free survival; overall survival; oncology
The quantitative assessment of tumor microvascularity and its impact on aggressiveness was first explored by Brem et al. in the 1970s. Since then, multiple studies have shown the importance of hypoxia and microvasculature in tumor aggressiveness, predicting patient survival, the response to antiangiogenic treatments, the regulation of cancer stem cells, and multiple oncological signaling pathways. The most common benign cerebral tumor, meningiomas are dural-based lesions that arise from the arachnoid cap cells and are organized by histological subtype and categorized as WHO Grade I–III. Previous studies have shown the importance of in vitro vascular density in predicting meningioma recurrence, vascular endothelial growth factor (VEGF) expression, and WHO grade. Further elucidation of the underlying microvascular changes in meningioma may be an avenue for understanding tumor biology and designing future treatments.

Multiple methods for quantifying microvascularity in meningiomas and other tumors have been suggested. Each method for evaluating the antibody stains of microvessels, such as CD31, CD34, CD105, and von Willebrand factor (vWF), shows specific sensitivity and specificity. The most common method involves the manual evaluation of microvascularity by quantifying the number of vessels per square millimeter in a hot spot or random region. Manual counting and the automated quantitation of vessels on histopathological slides have both been reported. Di Leva et al. described a fractal-based algorithm for quantifying microvascularity by using CD34-stained histopathological slides that aimed to describe the geometrical complexity, not only the density, of the microvessels within the tumor. This method has been used to differentiate tumor histopathological grade as well as to characterize the “microvascular fingerprinting” of distinct tumors. Our previous work demonstrated the correlation of meningioma grade and angiogenic signaling pathway proteins, including VEGF and hypoxia-inducible factor 1 (HIF-1), but we were unable to demonstrate a reliable method for measuring microvascularity that we could correlate with these findings. In this study, we refine our methods for the measurement of tumor microvascularity with additional staining techniques and automated quantification methods that have not yet been previously explored in meningiomas. Using these methods, we test the hypothesis that tumor microvascularity is correlated with tumor size, estimated blood loss (EBL) during surgery, progression-free survival (PFS), and overall survival (OS) of patients with WHO Grade I meningiomas.

**Methods**

**Patients**

Patient demographic data and tumor tissues were collected from 1996 to 2011 in an institutional review board–approved prospective surgical database of patients with diagnosed cerebral meningiomas who underwent surgery performed by the senior author (R.L.J.). Cases were diagnosed based on the 2007 WHO guidelines; diagnoses made prior to 2007 were adjusted based on new criteria when applicable. Analysis was limited to patients with Grade I meningiomas (n = 207). Patient characteristics such as patient outcome and EBL were retrospectively analyzed from the surgical, anesthesia, and electronic medical records. Pathological specimens underwent standard formalin fixation and paraffin embedding followed by histopathological as well as immunohistological analyses. PFS was calculated from the date of diagnosis until the date of radiographic disease progression or the last follow-up evaluation. OS was calculated from the diagnosis date until the date of death or the last follow-up evaluation.

**Tumor Measurement**

Two methods of tumor volume measurement were used. First, volume was calculated using T1-weighted MRI with contrast to determine the maximum length (cm) × width (cm) × depth (cm) × 0.5. Second, volumetric measurements of the regions of interest were summed on each imaging slice using OsiriX software (version 7.5; http://www.osirix-viewer.com/).

**Microvascular Immunohistochemistry**

Immunohistochemical analysis of the MIB-1 proliferation index (reported as the percentage of the field showing staining), microvascularity, and hypoxia-regulated proteins was completed as previously described. Positive controls for MIB-1 were performed on the human thymus, which has > 90% cell staining, and the negative controls involved the replacement of the primary antibody with nonimmune serum. The slides for MIB-1 were stained using the Ki-67 antibody (clone MIB-1, dilution 1:300; Dako). The slides for the microvascularity analysis were treated with factor VIII/vWF (rabbit polyclonal, dilution 1:100; Dako), PECAM-1/CD31 (monoclonal mouse, 1:100; Dako), or endoglin/CD105 (rabbit polyclonal, 1:100; Abcam). Appropriate horseradish peroxidase–linked secondary antibodies were used for detection (Ventana Medical Systems). HIF-1α immunohistochemistry was performed using the Catalyzed Signal Amplification System (Dako) with primary antibody H1α67 (1:1000 dilution; Novus Biologicals). VEGF, glucose transporter 1 (GLUT-1), and carbonic anhydrase IX (CA-IX) were assessed using anti-VEGF Ab-1 polyclonal antibody (1:50 dilution; Calbiochem), anti–CA-IX goat polyclonal antibody (1:200; Santa Cruz Biotechnology), or rabbit anti–GLUT-1 (1:100; Santa Cruz Biotechnology), respectively. Negative controls were prepared by replacing the primary antibody in nonimmune serum, whereas the positive controls were fixed in orthotopic U251 tumor sections that are immunohistochemically positive for HIF-1, VEGF, GLUT-1, and CA-IX. Appropriate horseradish peroxidase–linked secondary antibodies were used for detection.

**Tumor Microvascularity and Proliferation Assessments**

Imaging was performed in a manner similar to that in a previously published method. For the MIB-1 index analysis, 3 pictures of the most vascular area of the slide were taken at ×400 magnification using an Olympus BX41 MicroFire camera (Olympus America Inc.) with Image-Pro Plus 5.0 (Media Cybernetics Inc.) and transferred to Photoshop CS7 (Adobe Systems Inc.). Any positive cell
that was separate from the other stained cells and not contiguous or branching from the other vessels was counted. The results of each slide were averaged to determine the resulting microvascularity and divided by 0.26 mm² to normalize the size of the picture field. The MIB-1 index was calculated as the number of MIB-1–stained cells divided by the total number of cells in the field, and this calculation was repeated 3 times for each picture and averaged. Two observers duplicated the analysis, and the final MIB-1 index represented the mean percentage of cells showing MIB-1 staining. This method was reproducible, as demonstrated by good interrater (p = 0.99; 95% CI 0.99–1.00) and intrarater (p = 0.96; 95% CI 0.92–0.99) reliability compared with prior studies. For CD31, vWF, and CD105, the ratio of positive to negative cells in 3 high-power (×100) fields was calculated and averaged. The immunohistochemical analysis of HIF-1α, VEGF, CA-IX, and GLUT-1 was similarly performed at ×200 and scored from 0 to 4 (0, 0%–25%; 1, 25%–50%; 2, 50%–75%; 3, 75%–100%; and 4, 100%) based on the number of cells stained in a given field.

Fractal-Based Microvascularity Assessment

The methods for the fractal-based analysis of vascular density were used as described by Di Ieva et al. Briefly, the microvascular patterns of the CD31-immunostained histological slides were assessed by the means of a parameter based on the fractal dimension: that is, the space-filling property of an irregular object by a regularly repeating geometrical pattern at every scale. As reported in Di Ieva et al., the local box-counting dimension (Loc bcD) was computed on each slide in a “hot spot” area of the histological slide. Loc bcD is calculated by identifying the minimum number of differently sized boxes needed to cover an object in a defined range of magnifications (defined as the “scaling window.”) For details, see Di Ieva et al. Higher Loc bcD values signify a more complex pattern of the distribution of the microvessels that are embedded within the tumor, not just in terms of quantity but also in terms of size and shape (Fig. 1). The hot spot area was defined as the most vascularized 1-mm² area of the slide (which was automatically selected by the software). The microvascular ratio—defined as the ratio between the immunopositive vessels and the tumor area—served to define the hot spot.

Statistical Analysis

The mean ± SD of each variable was calculated. Statistical analysis of continuous and discrete values was performed using the t-test and chi-square test, respectively. Univariate linear analysis using Spearman’s ρ was calculated, and variables with p < 0.25 were entered into a multivariate, enter-method, linear regression analysis. The correlations of various parameters were determined by bivariate linear correlation with reported correlation (r) and significance (p) values. Kaplan-Meier survival analysis with the Mantel-Cox log-rank statistic was performed, as well as a Cox proportional hazards model with forward stepwise regression using the likelihood ratio. A decision tree analysis was performed for PFS and OS using the chi-square automatic interaction detection method with a maximum tree depth of 3 levels. Statistical significance was defined as p < 0.05, and statistics were calculated using SPSS (version 20.0).

Results

Baseline and Vascularity Characteristics

Evaluation of the 207 included patients with Grade I meningiomas showed a mean age of 55.4 ± 14.8 years, with 63 (30.4%) male patients (Table 1). The mean EBL was 343.1 ± 449.6 ml. The calculated tumor volume (21.63 ± 33.28 ml) and measured volume (27.31 ± 37.18 ml) showed a good correlation (r = 0.997; p = 0.0001). The mean anteroposterior, lateral, and depth dimensions were 3.4 ± 1.7, 2.8 ± 1.6, and 2.7 ± 1.6 cm, respectively. The mean OS was 124.1 ± 64.0 months, and the mean PFS was 115.9 ± 63.9 months; at 6 months only 1 patient (0.5%) demonstrated progression.

A summary of the vascularity assessments is provided in Table 2. The mean percentage of the total field for the MIB-1 index was 2.5% ± 4.3%, and the mean microvascularity percentages as measured by vWF, CD31, and CD105 were 73.1% ± 69.8%, 18.2% ± 12.1%, and 13.4% ± 8.0%, respectively. The fractal-based microvascularity measure Loc bcD was 1.1 ± 0.1. The mean expression of VEGF was 2.4 ± 1.2, HIF-1 was 2.0 ± 1.4, GLUT-1 was 2.0 ± 1.3, and CA-IX was 2.5 ± 1.3. The bivariate linear correlation showed a significant correlation among the microvascularity measures, automated fractal-based quantitation, and gene expression patterns (Supplemental Table S1).

Evaluation of Tumor Size

The relationships among the various measures of microvascularity, tumor size, and biology were evaluated using the t-test or chi-square test (Table 3). Patients with tumors < 3 cm tended to be slightly younger than patients with tumors ≥ 3 cm (53.1 ± 13.6 years vs 56.9 ± 15.2 years; p = 0.07). Patients with smaller tumors were less likely to be male (14.6% vs 40.8%; p = 0.0001), had significantly lower EBL (185.4 ± 197.2 ml vs 446.5 ± 532.2 ml; p = 0.0001) and smaller tumor volume (29.4 ± 23.5 ml vs 33.9 ± 38.1 ml; p = 0.0001), and had a lower MIB-1 index (1.7% ± 1.7% vs 3.0% ± 5.4%; p = 0.03), vWF (54.1% ± 52.4% vs 85.6% ± 76.9%; p = 0.001), and HIF-1 expression (2.2 ± 1.4 vs 1.4 ± 1.3; p = 0.004). In addition, significantly lower mortality (17.1% vs 30.4%; p = 0.03) was also observed in patients with smaller tumors.

Kaplan-Meier survival analysis with the log-rank test was performed with a tumor size cutoff of 3 cm (Fig. 2A and B). There was no difference in PFS related to tumor size (180.9 ± 90 months for patients with tumors < 3 cm vs 166.0 ± 83 months for patients with tumors ≥ 3 cm; p = 0.16); however, a significantly worse OS was seen in patients with tumors ≥ 3 cm (199.9 ± 7.6 months vs 180.8 ± 8.1 months; p = 0.05).

Multivariate logistic regression was used to evaluate the contributors to larger tumor size (Table 4). Using a variable enter-method regression model for variables with p < 0.1 on the univariate linear regression analysis, sex (OR 3.75, 95% CI 1.75–8.00; p = 0.001), MIB-1 (OR 1.14,
95% CI 1.00–1.31; \( p = 0.05 \), vWF (OR 1.01, 95% CI 1.00–1.01; \( p = 0.01 \)), and HIF-1 (OR 1.54, 95% CI 1.22–1.94; \( p = 0.0001 \)) were significant predictors of greater tumor size.

### Linear Regression Analysis of EBL

Univariate and multivariate linear regression analyses were performed to identify predictors of EBL (Table 5). Univariate linear regression analysis showed that EBL could be predicted by age (\( \rho = 0.16, p = 0.021 \)), sex (\( \rho = 0.198, p = 0.004 \)), calculated tumor volume (\( \rho = 0.415, p = 0.0001 \)), vWF (\( \rho = 0.229, p = 0.006 \)), and CD31 (\( \rho = 0.321, p = 0.006 \)); however, multivariate linear regression analysis showed that only vWF remained significant (\( \beta = 0.363, p = 0.01 \)).

### Analysis of Survival

We evaluated which factors might be used to predict disease progression (PFS) or death (OS) using the t-test.
and chi-square analysis (Supplemental Table S2). Younger patients had a significantly longer time to disease progression (p = 0.0001) and longer survival (p = 0.0001). Similarly, those with lower EBL had longer PFS (p = 0.0033) and OS (p = 0.003). Univariate and multivariate linear regression analyses showed that the MIB-1 index had a good correlation with PFS (p = 0.002) and OS (p = 0.003). Univariate and multivariate linear regression analysis, and thus MIB-1 index was further explored as a prognostic marker. No other factor was predictive of improved PFS and OS except for the correlation of CD105 with OS on the multivariate analysis (β = 0.4, p = 0.009) (Supplemental Table S3).

Because of the potential for a lead time bias in identifying covariates, a Cox proportional hazards model was used to evaluate key factors predictive of PFS and OS (Table 6). Factors were selected for this model based on their significance (p < 0.05) in the univariate and/or multivariate linear regression analysis. Age was a significant factor that predicted a modest increase in PFS (OR 1.05, 95% CI 1.01–1.09; p = 0.007) and OS (OR 1.12, 95% CI 1.05–1.18; p = 0.0001). Sex, CD105 expression, and HIF-1 expression did not show a significant impact on the risk of PFS or OS. In addition, MIB-1 was not associated with an increase in the risk of disease progression (OR 1.05, 95% CI 0.99–1.19; p = 0.08).

Threshold Values for Survival Analysis

A receiver operating characteristic curve analysis was used to evaluate the correlations of various microvascularity measurements and PFS and OS (Supplemental Figure S1). Age demonstrated good discriminative ability for delineating PFS, while MIB-1, CD105, and HIF-1 showed intermediate discriminative ability. Sex did not discriminate PFS well. Age also showed a strong discriminative ability for OS, while CD105 and HIF-1 showed an intermediate ability. MIB-1 and sex did not show good discrimination of OS. An MIB-1 cutoff value was further evaluated for predicting PFS and OS (Supplemental Table S4; Fig. 2C and D). An MIB-1 cutoff value of ≥3% yielded a 36% sensitivity and 82.5% specificity for PFS and 11.8% sensitivity and 72.9% specificity for OS. Use of an MIB-1 cutoff value of ≥3% showed a significant difference in PFS (p = 0.05) but not OS (p = 0.56) on the Kaplan-Meier survival analysis with the log-rank test (Fig. 2C and D). For an MIB-1 cutoff value of ≥3% compared with <3%, there was a significant difference in PFS (140.1 ± 11.7 months vs 179.5 ± 7.0 months; p = 0.05) but not OS (172.7 ± 11.8 months vs 195.4 ± 11.6 months; p = 0.05).
months vs 192.5 ± 6.6 months; \( p = 0.56 \)). Decision tree analysis of all microvascularity-related variables for predicting PFS and OS yielded significant results only for the MIB-1 index (results not shown).

Discussion

Improved understanding of tumor microvascul arity may aid in the understanding of tumor biology, patient prognosis, and treatment development. Our study demonstrated that various clinical and microvascul arity measures in meningioma patients could be predictive of tumor size, EBL, PFS, and OS. Tumors ≥ 3 cm showed significantly greater staining for MIB-1 and vWF as well as lower HIF-1 expression compared with tumors < 3 cm. Moreover, a significantly worse OS was seen in patients with larger tumors, whose survival time was on average 19 months shorter. The vWF microvascul arity measure predicted EBL, showing greater expression in tumors with greater EBL despite tumor size. These results suggest that assessing vWF may be able to detect an alteration in tumor biology with larger meningiomas. Multivariate analysis showed that CD105 and possibly MIB-1 predicted OS, but only MIB-1 showed a trend on a hazard model and was thus further evaluated. Survival analysis showed that an MIB-1 index ≥ 3% demonstrated high specificity (82.5%) but not sensitivity (36%) for predicting PFS, suggesting that elevated MIB could be useful in predicting tumor recurrence regardless of size or other factors. However, tumors with a low MIB-1 index could not be ruled out from possibly demonstrating future progression. The ease of calculating MIB-1 adds to its potential value as a practical clinical tool. These results suggest a potential benefit from the inclusion of microvascul arity measures in the prediction of morbidity and mortality from Grade I meningioma and provide a greater understanding of the underlying tumor biology.

Microvascul arity Measures in Meningiomas

Microvascul arity measures were useful for predicting tumor size, EBL, and PFS. Of the various factors evalu-
ated, MIB-1, vWF, and HIF-1 were the most predictive of tumor size, vWF was the most predictive of EBL, and MIB-1 was the most predictive of PFS. Various microvascularity measurements have been described in the literature as having diverse effects depending on the tumor type and grade. CD105 has been reported to be a more specific marker of neovascularization in meningiomas by staining newly formed vessels, but not preexisting ones.5 The expression of CD105 has been shown to correlate with shorter PFS in patients with Simpson Grade I resections.6 CD34 was reported as a more sensitive marker of meningiomas compared with CD31.38

Having a better understanding of the tumor microvasculature in meningiomas may help us improve targeted therapies as well as understanding regarding resistance to antiangiogenic molecules. Tumor vessels in meningiomas, as well as other tumors, demonstrate multiple distinct abnormalities in comparison with normal vessels, including abnormal capillary division, glomeruloid bodies, and abnormal molecular signaling such as those within VEGF and HIF-1;1,3,25,39 however, not all studies have shown a correlation between VEGF expression, microvascularity, and meningioma WHO grade.37 Although anti-VEGF inhibitors, namely bevacizumab, have been evaluated in meningioma, results demonstrating a sustained tumor response remain limited.29 It is likely that multiple signaling pathways are involved in regulating angiogenesis in meningiomas and that single markers may not be sufficient to categorize these findings. The strong correlation among the various microvascularity measurements in our study suggests that identifying one robust marker could be sufficient for predicting other changes in angiogenesis, but further study of the key angiogenic pathways is warranted.

Our results are also the first to explore the use of fractal-based microvascular assessment of Grade I meningiomas, and we were able to quantify the complexity of the microvascular patterns in an automated fashion. Automated microvascular assessment has been a useful tool in evaluating a variety of tumors, including malignant tumors and pituitary tumors.17 Our results failed to show a significant correlation between fractal-based microvascular assessment and either PFS or OS. It is likely that markers vary in their sensitivity and specificity for assessing tumor vascularity.2,3,11,17 While these fractal-based markers have been more successful for aggressive tumors, they may have been more limited for Grade I meningiomas in our series because differences in microvascularity may have been subtler. Further study is required to evaluate fractal-based microvascular measurements in meningiomas and other tumors.

### Table 4: Logistic regression of predicting tumor size in Grade I meningiomas

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
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<tr>
<td>Sex</td>
<td>3.75</td>
<td>1.75–8.00</td>
<td>0.001</td>
</tr>
<tr>
<td>MIB-1, % of field</td>
<td>1.14</td>
<td>1.00–1.31</td>
<td>0.05</td>
</tr>
<tr>
<td>vWF, % of field</td>
<td>1.01</td>
<td>1.00–1.01</td>
<td>0.01</td>
</tr>
<tr>
<td>HIF-1 expression</td>
<td>1.54</td>
<td>1.22–1.94</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

### Table 5: Factors predictive of EBL during meningioma resection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>β</td>
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<tr>
<td>Age, yrs</td>
<td>0.16</td>
<td>0.021</td>
</tr>
<tr>
<td>Male</td>
<td>0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Tumor vol, ml</td>
<td>0.42</td>
<td>0.0001</td>
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<tr>
<td>MIB-1, % of field</td>
<td>0.014</td>
<td>0.84</td>
</tr>
<tr>
<td>vWF, % of field</td>
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<td>0.001</td>
</tr>
<tr>
<td>CD31, % of field</td>
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<tr>
<td>CD105, % of field</td>
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<td>0.18</td>
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<tr>
<td>Loc bcD</td>
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<td>0.22</td>
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<tr>
<td>VEGF expression</td>
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<tr>
<td>HIF-1 expression</td>
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</tr>
<tr>
<td>GLUT-1 expression</td>
<td>-0.03</td>
<td>0.67</td>
</tr>
<tr>
<td>CA-IX expression</td>
<td>0.09</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Boldface type indicates statistical significance.

**Practical MIB-1 Threshold for Clinical Use**

The MIB-1 index can be a useful tool for evaluating meningioma aggressiveness. While MIB-1 has been correlated with outcomes in some studies, this correlation has not been seen in other studies.1,21,28,40,51 Various cutoff values for MIB-1 in predicting disease grade and progression have been suggested, including 15%, 25%, 3%, 2.6%, and 2%.41 Our results suggest that a value of 3% shows 82.5% specificity in predicting disease progression but only 36% sensitivity. In other words, patients with MIB-1 indices ≥ 3% showed a high likelihood of tumor progression, but patients with MIB-1 indices < 3% could not necessarily be ruled out from future disease progression. In a meta-analysis of 53 studies, the average cutoff values for Grade I, II, and III meningiomas were 3%, 8%, and 17%, respectively.2 A recent study of 240 patients with Grade I meningioma from a single center showed that among patients who had Simpson Grade II and III resections, an MIB-1 index of ≥ 3% would be sufficient for predicting other changes in angiogenesis, but further study of the key angiogenic pathways is warranted.

### Table 6: Time-to-event–dependent Cox proportional hazards model

<table>
<thead>
<tr>
<th>Variable</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, yrs</td>
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<td>1.01–1.09</td>
</tr>
<tr>
<td>Sex, male</td>
<td>1.08</td>
<td>0.43–2.72</td>
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<tr>
<td>MIB-1, % of field</td>
<td>1.08</td>
<td>0.99–1.19</td>
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<tr>
<td>CD105, % of field</td>
<td>1.00</td>
<td>0.96–1.04</td>
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<tr>
<td>HIF-1 expression</td>
<td>1.18</td>
<td>0.89–1.58</td>
</tr>
</tbody>
</table>

*Boldface type indicates statistical significance.
was predictive of a shorter time to recurrence.\textsuperscript{44} The effect of MIB-1 in this study was also seen in the Cox proportional hazards model (OR 4.65, 95% CI 1.59–14.0; \( p = 0.006 \)). These results are reflective of our findings showing MIB-1 to be a significant predictor of progression, although a lower OR of 1.14 was found in our series. Our previous results support good intra- and interobserver reliability between MIB-1 measurements.\textsuperscript{30} Nevertheless, not all areas of a tumor are typically evaluated during pathological evaluation, which may account for the variation in MIB-1 thresholds among different studies.

**Noninvasive Methods for Microvasculature Assessment**

Recent studies have supported using various imaging modalities to quantify tumor vascularity in order to better target treatment and predict prognosis. Perfusion MRI is one modality that allows the quantitation of vascularity parameters by compartmental modeling and has been shown to predict outcome in gliomas\textsuperscript{33,37} and meningiomas.\textsuperscript{35} Imaging markers of vascularity have been shown to correlate with and predict the MIB-1 index\textsuperscript{36} and VEGF expression\textsuperscript{9} during immunohistological analysis of meningiomas in some studies, although other studies have not shown a correlation between imaging and pathological markers.\textsuperscript{19} Assessment of vascularity has also been suggested to aid in distinguishing tumor progression from pseudoprogression or radiation necrosis.\textsuperscript{26} These imaging modalities may serve as useful adjuvant tools for clinicians to assess the aggressiveness of a tumor prior to resection and guide surgical treatment and also as a method for the noninvasive evaluation of tumors on follow-up.

Preoperative noninvasive measures of microvascularity can also be helpful in improving surgical strategies to reduce patient morbidity. In addition, the limited response of CNS tumors to antiangiogenic treatments for meningiomas and other diseases can be evaluated by examining the tumor vasculature on dynamic MRI.\textsuperscript{32} One hypothesis has been that antiangiogenic therapies may result in the transient normalization of tumor vasculature as opposed to the destruction of tumor vessels.\textsuperscript{24} Tumor responsiveness to radiotherapy also greatly depends on vascularity, and evaluating microvascularity may be a useful strategy for selecting patients for adjuvant therapies.

**Limitations**

Our results did not include Simpson grade or evaluate the use of postoperative radiotherapy. Although most patients with Grade I meningiomas in our series underwent a Simpson Grade I resection, intraoperative and postoperative imaging were not evaluated in a blinded manner. Several recent studies have suggested limits to the usefulness of Simpson grading for predicting recurrence,\textsuperscript{44,50} although the short follow-up period has been one limitation of these data. Our results suggested that MIB-1 could help explain meningioma recurrence after subtotal resection and be a useful adjuvant to Simpson grading. In addition, the impact of adjuvant radiotherapy on tumor recurrence was not delineated. Our study also omitted Grade II and III meningiomas, which may have been helpful in evaluating the various markers of microvascularity that were evaluated in this study.

**Conclusions**

Our results support the assertion that evaluation of microvascularity has a role in the differentiation of tumor volume, EBL, PFS, and OS in patients with Grade I meningioma. Specific measures of microvasculature were predictive of preoperative (e.g., MIB-1, vWF, and HIF-1 for tumor size), intraoperative (e.g., vWF for EBL), and postoperative (e.g., MIB-1 for PFS) outcomes in patients with Grade I meningiomas. No specific factor was robust in determining OS; however, an MIB-1 index cutoff value of 3% showed a specificity of 86.5% and sensitivity of 36% for predicting PFS, suggesting that it could be used to identify patients likely to progress only when positive. Microvascularity likely plays an important role in the disease progression of Grade I meningiomas and has the potential to aid in the prediction of patient morbidity and mortality.

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Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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