Apoptotic Activity and bcl-2 Immunoreactivity in Meningiomas

Association With Grade and Outcome

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Key Words: Apoptosis; bcl-2 Expression; Meningioma; MIB-1 antibody; Tumor recurrence

Abstract

We retrospectively evaluated 90 meningiomas for bcl-2 expression, apoptosis counts (per 10 high-power fields [HPF]), MIB-1 labeling indices (LI), and mitosis counts (per 10 HPF). Characteristics were as follows: 37 low-grade (benign) meningiomas: mean apoptosis count, 1.2; MIB-1 LI, 1.0; mitosis count, 0.1; and bcl-2 positivity, 40%; 29 atypical meningiomas: apoptosis count, 3.3; MIB-1 LI, 5.5; mitosis count, 2.2; and bcl-2 positivity, 62%; 24 malignant meningiomas: apoptosis count, 6.5; MIB-1 LI, 12.0; mitosis count, 6.0; and bcl-2 positivity, 67%. By univariate analysis, MIB-1 LI, apoptosis and mitosis counts, and tumor grade were associated significantly with death due to tumor; by multivariate analysis, only mitosis count was independently associated with death due to tumor. We compared similar data for 27 patients with nonrecurrent tumors and 32 patients with recurrent meningiomas. Histologic sections from the initially resected tumor and from the most recent recurrence were reviewed. Only the apoptosis count was significantly higher by univariate analysis in the initial resection specimens from tumors that ultimately recurred vs nonrecurrent tumors. Expression of bcl-2, MIB-1 LI, and mitosis count did not correlate with recurrence. By multivariate analysis, only extent of surgical resection was associated significantly with tumor recurrence. Although bcl-2 immunostaining was not associated statistically with outcome, bcl-2 positivity was more common in atypical and malignant meningiomas than in low-grade tumors.

Although most meningiomas behave in a benign fashion, a subset may recur locally, invade brain parenchyma, or, rarely, metastasize. Unfortunately, there are no precise and universally accepted histologic criteria established for grading these neoplasms or for predicting tumor behavior. The most recent revision of the World Health Organization (WHO) classification separates meningiomas into 3 grades: benign (grade I), atypical (grade II), and malignant (grade III). Atypical meningioma is vaguely defined as a tumor with several of the following histologic features: frequent mitoses, hypercellularity, cells with increased nuclear/cytoplasmic ratios, prominent nucleoli, sheet-like growth, and areas of “spontaneous” or geographic necrosis. Malignant/anaplastic meningioma is characterized by features of frank anaplasia that are above and beyond those found in atypical tumors. This classification leaves much room for individual interpretation but reflects the well-known limitations of histologic features for consistently predicting tumor behavior.

Numerous investigators have studied the histopathologic features and the relationship of particular features of meningiomas with tumor recurrence and ultimate clinical outcome. The proliferative activity of the tumor in terms of mitosis count seems to be one of the more important features associated with poor prognosis. Additional studies have examined the potential role of cell proliferation markers, such as MIB-1, in predicting tumor behavior. The MIB-1 labeling index (LI) has been shown to increase generally with increasing histologic tumor grade. Although the MIB-1 LI has been associated significantly with tumor recurrence in some studies, other studies have not supported this finding.

Apoptosis is regulated by several genes, one of which is the bcl-2 proto-oncogene. The bcl-2 gene is responsible...
for the synthesis of bcl-2 protein, a 26-kd protein involved in the inhibition of apoptosis and promotion of cell survival. The bcl-2 protein works in conjunction with p53, bax, and c-myc proteins in the regulation of apoptotic activity. Several studies have examined the expression of bcl-2 in tumors outside the central nervous system in which bcl-2 may have some prognostic significance. The association of bcl-2 expression and prognosis in tumors of the central nervous system and, in particular, meningiomas is not clearly defined.

We retrospectively evaluated apoptotic activity, expression of bcl-2, mitotic activity, and MIB-1 labeling indices in histologically benign, atypical, and malignant meningiomas, as well as between nonrecurrent and recurrent tumors. Correlation with tumor grade and clinical outcome in terms of recurrence and death due to tumor is presented.

### Materials and Methods

The pathology files at the Cleveland Clinic Foundation, Cleveland, OH, were searched for meningiomas diagnosed through 1997. All cases of recurrent meningiomas were included if the initial tumor and the most recent (or only) recurrence were resected at the Cleveland Clinic Foundation and slides from both resections were available for review. Recurrence, for purposes of the study, was defined as tumor regrowth requiring a second surgical procedure for removal of tumor. Nonrecurrent tumors were included only as long as there was a long clinical follow-up period, with a minimum follow-up of 88 months. A number of nonrecurrent meningiomas approximately equal to the number of recurrent tumors was desired, so only nonrecurrent tumors resected between January 1986 and December 1988 were included for study. A total of 59 cases met the criteria and included 27 benign, 29 atypical, 24 malignant. None of the worrisome histologic features outlined by the WHO for atypical meningioma (nuclear pleomorphism, prominent nucleoli, mitoses, necrosis, and loss of architectural pattern). Atypical meningiomas were defined by the presence of 2 or more of the listed features. Any tumor that exhibited invasion of brain parenchyma or was associated with clinical metastasis was classified as a malignant meningioma.

Mitosis counts were recorded as the single highest number of mitotic figures (MF) per 10 high-power fields (HPF), evaluated in the most proliferative area of the tumor. Counts were performed using a Nikon (Melville, NY) binocular microscope with a wide field 10× ocular and 40× objective, producing a high-power microscopic field with a calculated area of 0.17 mm² (diameter, 0.47 mm). Apoptosis counts were performed away from areas of geographic tumor cell necrosis, if present, and were recorded as the highest number of apoptotic bodies (AB) per 10 HPF. Apoptosis counts were performed in a fashion similar to that used for mitosis counts. Apoptotic bodies were identified as shrunken cells with pyknotic nuclei and eosinophilic cytoplasm as described previously.

Immunohistochemical staining with MIB-1 antibody (1:10 dilution, AMAC, Westbrook, ME), and staining for bcl-2 (Ventana Medical Systems, Tucson, AZ) was performed using a microwave processing procedure and an avidin-biotinylated immunoperoxidase method. Appropriate positive and negative controls were performed. MIB-1 and bcl-2 immunostaining were performed on 1 representative block of tumor. The MIB-1 LI was recorded as the percentage of positively staining tumor cell nuclei in 1,000 tumor cell nuclei evaluated. The determinations were made on high magnification in the areas with the most immunostaining. Staining for bcl-2 was recorded as positive or negative.

Clinical information for the study patients has been reported previously and was obtained through review of medical records, including radiographic and operative reports and discharge summaries. Specifically noted were the age of the patient at the time of the initial resection, sex, type of surgery performed (gross total vs subtotal), number of recurrences, interval to recurrence, adjuvant therapy, development of metastases, most recent follow-up, and other pertinent medical history.

Comparisons of apoptosis counts and mitosis counts between benign, atypical, and malignant meningiomas were completed by using the Dunn test. MIB-1 LIs were compared between tumor grades by using the Kruskal-Wallis test and the Wilcoxon 2-sample test. Positivity for bcl-2 was compared between tumor grades by using the Cochrane-Mantel-Haenszel test. For testing associations of unfavorable outcome designated as death due to tumor with apoptosis count, mitosis count, and MIB-1 LI, the Wald chi-square test was used. Univariately significant associations then were tested in a...
multivariate model. The Cochrane-Mantel-Haenszel test was used for evaluating association of bcl-2 expression and death due to tumor.

Since the 2 groups of recurrent tumors were from the same patients and, therefore, were paired groups, comparisons of apoptosis count, mitosis count, and MIB-1 LI were made using the Wilcoxon signed-rank test and of bcl-2 expression using the sign test. Similar comparisons of apoptosis count, mitosis count, and MIB-1 LI between the nonrecurrent group and the group of recurrent tumors from the initial resection were performed using the Wilcoxon rank-sum test, and for bcl-2 expression, the Cochrane-Mantel-Haenszel test. To adjust for multiple comparisons, a Bonferroni correction set the significance level at \( P = 0.0167 \). Univariate significant associations then were tested in a multivariate model.

### Results

**Benign, Atypical, and Malignant Meningiomas**

Patients with benign tumors \( (n = 37) \) ranged in age from 30 to 80 years \( (mean, \ 54 \ years) \). Thirty-two patients \( (86\%) \) were women and 5 patients \( (14\%) \) were men. Twenty-three \( (62\%) \) of the benign meningiomas were treated initially by gross total resection, 10 \( (27\%) \) by subtotal resection, and the remainder \( (4\%) \) by biopsy alone or by an unknown extent of resection. Six patients received adjuvant radiation therapy. Thirty-two patients \( (86\%) \) were alive during a mean follow-up interval of 109 months \( \text{range, 37-196 months} \). One patient \( (3\%) \) died with residual tumor at a postoperative interval of 129 months. Four patients \( (11\%) \) died without evidence of residual tumor or had unknown tumor status at postoperative intervals of 101 to 202 months \( \text{median, 127 months} \).

Twenty-nine tumors were diagnosed as atypical meningioma. The patients in this group ranged in age from 35 to 83 years \( (mean, 61 \ years) \) and included 17 women and 12 men. Gross total resection was performed as initial surgery in 22 patients \( (76\%) \). Five patients \( (17\%) \) underwent subtotal resection of the tumor. Information about extent of resection was unavailable for 2 patients. Three patients received postoperative radiation therapy after tumor recurrence. Twenty-four patients \( (83\%) \) were alive at postoperative intervals of 2 to 156 months \( (mean, 54 \ months) \). Two patients \( (7\%) \) were dead of tumor at 36 and 42 months. Three patients \( (10\%) \) died without residual tumor or with unknown tumor status at 0.5 to 84 months after surgery \( \text{median, 18 months} \).

Patients with malignant meningiomas \( (n = 24) \) ranged in age from 18 to 82 years \( (mean, 59 \ years) \) and included 14 women and 10 men. Thirteen patients \( (54\%) \) were treated initially by subtotal resection, and 8 patients \( (33\%) \) by gross total resection. The extent of resection was unknown in 3 cases. Ten patients received postoperative radiation therapy and 2 patients, postoperative chemotherapy. In 5 patients, metastases developed to bone \( (n = 2) \), skin \( (n = 2) \), lung \( (n = 2) \), kidney \( (n = 1) \), and liver \( (n = 1) \). Fifteen patients \( (62\%) \) were alive at postoperative intervals of 3 to 348 months \( (mean, 71 \ months) \). Five patients \( (21\%) \) died of tumor at postoperative intervals of 0.5 to 25 months \( \text{median, 24 months} \). Two patients \( (8\%) \) died without residual tumor or with unknown tumor status at 25 and 412 months after surgery. Two patients were lost to follow-up.

Benign meningiomas demonstrated a generally low level of proliferative activity as measured by mitosis counts and MIB-1 LI. Single highest mitosis counts ranged from 0 to 1 MF/10 HPF \( (mean, 0.1 \ MF/10 \ HPF) \). MIB-1 LIs in benign tumors ranged from 0.0 to 5.5 \( (mean, 1.0) \). In atypical meningiomas, mitosis counts ranged from 0 to 19 MF/10 HPF \( (mean, 2.2 \ MF/10 \ HPF) \) and were significantly higher than counts obtained in benign tumors \( (P < 0.001) \). MIB-1 LIs were 0.1-32.5 \( (mean, 5.5) \) for atypical meningiomas, and also were significantly higher than indices seen in benign tumors \( (P < 0.0001) \). Malignant meningiomas exhibited the highest proliferative activity, with mitosis counts of 0 to 20 MF/10 HPF \( (mean, 6.0 \ MF/10 \ HPF) \) and MIB-1 LIs of 0.3 to 32.5 \( (mean, 12.0) \). Mitosis counts \( (P = 0.01) \) and MIB-1 LIs \( (P = .0012) \) were significantly higher in malignant than in atypical meningiomas.

Mitosis counts \( (P = .0035) \) and MIB-1 LIs \( (P = .013) \) were significantly higher in meningiomas in patients who died of tumor compared with those in patients who were alive at the time of follow-up. Meningiomas in patients who were alive \( (n = 72) \) showed mitosis counts of 0 to 20 MF/10 HPF \( (mean, 1.6 \ MF/10 \ HPF) \) and MIB-1 LIs of 0.0 to 32.5 \( (mean, 4.5) \). In patients who died of tumor \( (n = 8) \), mitosis counts were 0 to 19 MF/10 HPF \( (median, 5.5 \ MF/10 \ HPF) \), and MIB-1 LIs were 1.0 to 32.5 \( (median, 8.5) \).

Apoptosis counts were associated significantly with tumor grade \( (P < 0.001) \). Benign tumors generally exhibited lower apoptosis counts \( \text{range, 0-8 AB/10 HPF; mean, 1.2 AB/10 HPF} \) than atypical tumors \( (range, 0-14 \ AB/10 \ HPF; mean, 3.3 AB/10 \ HPF) \), and malignant meningiomas generally demonstrated the highest apoptosis counts \( \text{range, 0-16 AB/10 \ HPF; mean, 6.5 AB/10 \ HPF} \). Apoptosis counts also were associated significantly with death due to tumor \( (P = .0039) \). Meningiomas in patients who were alive at follow-up had apoptosis counts of 0 to 14 AB/10 HPF \( (mean, 2.6 \ AB/10 \ HPF) \), while those in patients who died of tumor demonstrated a higher median value \( \text{range, 0-13 AB/10 \ HPF; median, 7.0 AB/10 \ HPF} \). Positivity for bcl-2, however, did not correlate significantly with apoptosis counts, tumor grade, or death due to tumor. A summary of results for proliferative and apoptotic indices for benign,
atypical, and malignant meningiomas is given in Table 1. Table 2 presents proliferative and apoptotic indices for patients who were alive at follow-up and in those who died of tumor.

Variables that were associated significantly with death due to tumor were tested in a multivariate model using a significance level of .05. The extent of surgical resection and tumor grade were included in this analysis. Mitotic activity was the only variable found to have a strong independent association with death from tumor. All other variables became nonsignificant. Finally, determination of a ratio of mitosis count over apoptosis count that might have correlated with death was attempted, but no correlation was found.

**Recurrent vs Nonrecurrent Meningiomas**

Thirty-two patients had recurrent meningiomas with ages at the time of initial surgery ranging from 30 to 83 years (mean, 55 years). Twenty-six patients were women, and 6 were men. Seventeen patients (53%) underwent initial subtotal resection, 10 (31%) gross total resection, and 1 (3%)
biopsy alone. In 4 patients, the extent of initial surgery was unclear from the available information. Two patients received adjuvant chemotherapy, and 15 received adjuvant radiation therapy. All patients developed tumor recurrence requiring additional surgical resection. The number of recurrences, including metastases, ranged from 1 to 5 (mean, 1.4), with the interval to the first or only recurrence ranging from 5 to 183 months (mean, 55.4 months). The interval between the initial resection of tumor to the most recent or only recurrence ranged from 5 to 360 months (mean, 73.2 months). Twenty-one patients (66%) were alive at postoperative intervals of 27 to 348 months (mean, 116 months). Six patients (19%) died of tumor at 19 to 129 months after surgery (mean, 44 months). Five patients (16%) died without residual tumor or with unknown tumor status at 84 to 412 months (mean, 190 months) after surgical resection.

The 27 patients with nonrecurrent tumors ranged in age from 18 to 80 years (mean, 55.4 months) and included 21 women and 6 men. Twenty-five patients (92%) underwent initial gross total resection. One patient (4%) underwent a subtotal resection, and the extent of tumor resection in another patient was not evident from the operative note. None of the patients received adjuvant chemotherapy or radiation therapy. None of the patients developed tumor recurrence during a minimum postoperative interval of 88 months. Twenty-six patients (96%) were alive at postoperative intervals of 88 to 124 months (mean, 109 months). No patients died due to their tumor. One patient (4%) died of unrelated causes at 101 months after surgical resection.

Mitosis counts recorded for the nonrecurrent meningiomas were low (range, 0-1 MF/10 HPF; mean, 0.1 MF/10 HPF). Although mitosis counts observed in both groups of recurrent tumors were generally higher than those of the nonrecurrent tumors, the differences were not statistically significant. Likewise, MIB-1 LI was not significantly higher in the initial resections of recurrent tumors compared with those of the nonrecurrent meningiomas and was somewhat lower in recurrent tumors compared with the corresponding initial resection.

Apoptosis counts were significantly higher ($P = .013$) in initial resections of tumors that ultimately recurred (range, 0-13 AB/10 HPF; mean, 2.8 AB/10 HPF) compared with those of nonrecurrent tumors (range, 0-3 AB/10 HPF; mean, 0.9, AB/10 HPF). Apoptosis counts were similar between the 2

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### Table 1

| Apoptotic Activity, bcl-2 Expression, Mitosis Counts, and MIB-1 LI: Comparison With Histologic Grade
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<tr>
<td><strong>Benign (n = 37)</strong></td>
<td><strong>Atypical (n = 29)</strong></td>
<td><strong>Malignant (n = 24)</strong></td>
<td><strong>P</strong></td>
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<tr>
<td>Apoptosis count (AB/10 HPF) Mean (range)</td>
<td>1.2 (0-9)</td>
<td>3.3 (0-14)</td>
<td>6.5 (0-16) (n = 21)</td>
<td>&lt;.001</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>No. (%) bcl-2 positive</td>
<td>14 (40) (n = 35)</td>
<td>18 (62)</td>
<td>14 (67) (n = 21)</td>
<td>.059</td>
<td>.57†</td>
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<tr>
<td>Mitosis count (MF/10 HPF) Mean (range)</td>
<td>0.1 (0-1)</td>
<td>2.2 (0-19)</td>
<td>6.0 (0-20) (n = 23)</td>
<td>&lt;.001</td>
<td>.01*</td>
</tr>
<tr>
<td>MIB-1 labeling index (%) Mean (range)</td>
<td>1.0 (0-5.5)</td>
<td>5.5 (0.1-32.5)</td>
<td>12.0 (0.3-32.5) (n = 22)</td>
<td>&lt;.0001</td>
<td>.0012‡</td>
</tr>
</tbody>
</table>

AB, apoptotic bodies; HPF, high-power field; MF, mitotic figures.
* Comparisons made using the Dunn test. $P$ values of .025 or less are considered statistically significant.
† Cochrane-Mantel-Haenszel test. $P$ values of .0167 or less are considered statistically significant.
‡ Kruskal-Wallis test and Wilcoxon 2-sample test. A Bonferroni correction set the significance level at .025.

### Table 2

| Apoptotic Activity, bcl-2 Expression, Mitosis Counts, and MIB-1 LI: Association With Death Due to Tumor
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<tr>
<td><strong>Alive (n = 72)</strong></td>
<td><strong>Dead of Tumor (n = 8)</strong></td>
<td><strong>P</strong></td>
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<tr>
<td>Apoptosis count (AB/10 HPF) Mean (range)</td>
<td>2.6 (0-14) (n = 70)</td>
<td>7.0† (0-13)</td>
<td>.0039‡</td>
</tr>
<tr>
<td>No. (%) bcl-2 positive</td>
<td>38 (56) (n = 68)</td>
<td>6 (75)</td>
<td>.062§</td>
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<tr>
<td>Mitosis count (MF/10 HPF) Mean (range)</td>
<td>1.6 (0-20) (n = 71)</td>
<td>5.5† (0-19)</td>
<td>.0035‡</td>
</tr>
<tr>
<td>MIB-1 labeling index (%) Mean (range)</td>
<td>4.5 (0.0-32.5) (n = 71)</td>
<td>8.5† (1.0-32.5)</td>
<td>.013‡</td>
</tr>
</tbody>
</table>

AB, apoptotic bodies; HPF, high-power field; MF, mitotic figures.
* $P$ values less than or equal to .05 are considered statistically significant.
† Median.
‡ Wald chi-square test (logistic regression).
§ Cochrane-Mantel-Haenszel test.
groups of recurrent tumors. Meningiomas demonstrating bcl-2 positivity were observed in each study group, and no significant differences were noted between recurrent and nonrecurrent tumors. Table 3 gives the proliferative and apoptotic indices observed for nonrecurrent and recurrent meningiomas.

Mitosis and apoptosis counts, along with extent of surgical resection and overall tumor grade, were tested in a multivariate model with recurrence as the outcome. Extent of surgical resection was the only significant variable independently associated with tumor recurrence with an odds ratio of 44.2 (95% confidence interval, 4.6-427.7). This wide confidence interval may be due to the relatively small sample but, regardless, reflects the highly variable risk of recurrence associated with incomplete surgical resection.

Discussion

It is well known that the clinical behavior of meningiomas cannot be predicted consistently using histopathologic features alone. A number of studies have examined the expression of proliferative markers in meningiomas in the hope of identifying the tumors at increased risk of recurrence. Fewer studies have investigated the role of apoptosis and expression of bcl-2 in determining the biologic behavior of meningiomas.

The MIB-1 antibody recognizes the Ki-67 antigen, which is a nuclear protein expressed only during the active phases of the cell cycle. Although a few studies have failed to demonstrate a correlation of tumor grade and MIB-1 LI, most studies have, including the present study. This is consistent with the apparent importance of mitotic activity in determining tumor grade and the assertion by Perry et al10 that mitosis counts are probably the most important histologic feature predicting biologic tumor behavior. In the study by Perry et al10 of 581 patients with meningiomas, one of the most significant parameters associated with tumor recurrence was a mitotic rate of at least 4 MF/10 HPF. That study was followed by another demonstrating MIB-1 LI to be significantly associated with decreased recurrence-free survival.22

However, there are a few limitations in using MIB-1 LI to predict clinical behavior in a given tumor. One problem is that there is considerable overlap with regard to MIB-1 LI ranges between tumor grades. In general, a high MIB-1 LI probably indicates a tumor that will behave in a more aggressive fashion, but the issue of how high is too high is problematic. Another potential drawback is that the amount of MIB-1 immunostaining can be variable in different regions of a tumor, so sampling becomes an important issue. Finally, laboratories may generate different labeling indices related to differences in technique, making comparison of MIB-1 LIs from institution to institution difficult.

In the present study, mitotic activity was the only variable independently associated with death due to tumor. Mitoses were not, however, independently associated with tumor recurrence. Extent of surgical resection was the only independent predictor of tumor recurrence. The tumors in the nonrecurrent and recurrent study groups were not all resected to the same extent, and this may explain the lack of a significant association in this study between mitotic activity and recurrence that was found in the study by Perry et al.10

The process of apoptosis, or programmed cell death, has been studied only recently in tumors of the central nervous system. Patsouris and colleagues37 studied 26 brain tumors, including a varied number meningiomas, astrocytomas, and oligodendrogliomas, with respect to apoptotic indices as determined by an in situ end-labeling method. They found that the meningiomas (n = 7) exhibited a higher mean apoptosis index (1.129%) than did the WHO grade II (n = 4) and

**Table 3**

<table>
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<tr>
<th>Apoptotic Activity, bcl-2 Expression, and Proliferative Indices in Recurrent and Nonrecurrent Meningiomas</th>
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<tr>
<td>Apoptosis count (AB/10 HPF)</td>
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<tr>
<td>Mean (range)</td>
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<tr>
<td>No. (%) bcl-2 positive</td>
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<tr>
<td>Mitoses count (MF/10 HPF) Mean (range)</td>
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<tr>
<td>MIB-1 labeling index (%) Mean (range)</td>
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</table>

AB, apoptotic bodies; HPF, high-power fields; MF, mitotic figures.

*P* values less than or equal to 0.0167 were considered statistically significant.

1. *P* values for comparisons between nonrecurrent and initially (i) resected recurrent tumors were determined by using a Wilcoxon rank-sum test except for comparison of bcl-2 positivity, which was determined by using a Cochran-Mantel-Haenszel test.

2. *P* values for comparisons between the 2 groups of recurrent tumors (initially resected [i] and recurrent [r]) were determined by using a Wilcoxon signed-rank test except for comparison of bcl-2 positivity, which was determined by using a sign test.
III (n = 8) gliomas (1.097% and 0.699%, respectively). Glioblastoma multiforme (n = 5), however, demonstrated a higher apoptosis index (1.649%) than either meningiomas or the lower-grade gliomas. Ellison et al\(^3\) compared apoptotic indices among 16 low-grade fibrillary astrocytomas, 19 anaplastic astrocytomas, and 46 glioblastomas and found that apoptotic indices increased with increasing histologic grade, with median apoptotic indices of 0.0%, 0.072%, and 0.120%, respectively.

Few studies have focused on apoptotic indices specifically in meningiomas. Ng and Chen\(^2\) found that the apoptotic index as measured in the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate–biotin nick-end labeling (TUNEL) assay was significantly higher in 12 atypical/malignant meningiomas (apoptosis index, 0.12%) compared with 39 benign meningiomas (apoptosis index, 0.023%). Although Maier and colleagues\(^2\) found that apoptotic indices (determined by in situ tailing) increased with histologic grade of meningioma, the results were not statistically significant.

In our study, apoptosis counts increased significantly with increasing histologic grade ($P < .001$) and were significantly associated with death due to tumor ($P = .0039$) in univariate analysis but not in multivariate analysis. They also were significantly higher in primary resections of tumors that later recurred than in nonrecurrent tumors ($P = .013$) in univariate analysis. This association became nonsignificant in multivariate analysis in which extent of surgical resection became the only independent variable associated with tumor recurrence. In the present study, similar to MIB-1 LI and mitosis counts, there was a great deal of overlap with respect to apoptosis counts observed between tumor grades and between patients who were alive and those who died of tumor. Less overlap was seen between recurrent and nonrecurrent tumors, with none of the nonrecurrent meningiomas having apoptosis counts higher than 3 AB/10 HPF.

It has been shown that the detection of apoptosis by counting apoptotic bodies on H&E-stained slides is as accurate as the TUNEL assay in evaluating apoptotic activity.\(^3\) The choice of method often is influenced by economic resources. However, the detection of apoptotic bodies and the interpretation of apoptosis assays, such as the TUNEL assay, may be affected by delays in tissue fixation and by lengthy strong fixation. In formalin-fixed autopsy tissue, a higher false-positive rate of apoptotic body detection has been reported owing to intense staining of postmortem material with H&E.\(^4\)

Tateyama and colleagues\(^5\) noted that a 2-hour delay in tissue fixation was the maximum period allowed for accurate assessment of apoptotic activity by the TUNEL assay. Longer delays in fixation resulted in false-positive cells, while increased length of fixation had no apparent effect on apoptotic activity as measured by the TUNEL assay.\(^6\) Others, however, have reported long fixation time to be associated with false-negative results with the in situ end-labeling method.\(^7\)

The correlation of bcl-2 expression with prognosis in tumors of the central nervous system and, in particular, meningiomas, is unclear. Hara and associates\(^8\) examined bcl-2 and bax expression in a variety of brain neoplasms and found no correlation between expression of either of these 2 regulatory proteins and tumor grade. In the study of astrocytomas by Ellison et al,\(^9\) bcl-2 expression was found in 44% of fibrillary astrocytomas, 42% of anaplastic astrocytomas, and 28% of glioblastomas. There was no definite correlation of bcl-2 positivity and apoptosis index in this set of tumors. Reactive astrocytes also were reported to stain positively for bcl-2, thus complicating the matter. Nakasu and colleagues\(^10\) examined bcl-2 staining in 140 varied brain tumors and reported more frequent immunostaining in low-grade astrocytomas than in high-grade tumors, and almost half of the recurrent gliomas and medulloblastomas showed increased protein expression compared with tissue from initial resections.

Regarding bcl-2 expression in meningiomas, Karamitopoulou and colleagues,\(^11\) in a study of 60 meningiomas, compared bcl-2 expression between histologic tumor grades and found bcl-2 positivity (defined as positive staining in 10% or more of the tumor cells) in 22% of the benign, 20% of the atypical, and 46% of the malignant meningiomas. When they compared bcl-2 expression between benign meningiomas that did not recur and benign tumors that did, they found that the benign meningiomas that ultimately recurred were significantly more often positive for bcl-2 expression than were tumors that did not recur.

In our study, bcl-2 expression increased slightly with increasing tumor grade, but the difference was not statistically significant. Positivity for bcl-2 was not associated with tumor recurrence or death due to tumor. These results are similar to those from other studies. Konstantinidou et al\(^12\) reported no association between bcl-2 expression and either tumor grade or recurrence in their study of 52 totally resected meningiomas. Most of the atypical/malignant meningiomas (64%) included in their study were negative for bcl-2 immunostaining altogether, and the benign tumors demonstrated a slightly higher mean bcl-2 labeling index relative to the higher-grade tumors.

It is interesting that benign meningiomas express bcl-2 to any degree since bcl-2 inhibits apoptosis and intuitively would lead to increased cell survival and tumor progression. Perhaps the bcl-2 that is detected by immunostaining represents a dysfunctional protein, or there is accumulation of protein that is produced constitutively at low levels that accumulates as a result of decreased degradation. Apoptosis is a
complex regulated process in which many other proteins are involved, and bcl-2 expression alone is probably insufficient for predicting tumor behavior.

Mosnier and coworkers\(^46\) studied 39 meningiomas, including 32 benign, 6 atypical, and 1 malignant tumor, and reported bcl-2 positivity primarily in the spindle cell component of transitional and fibroblastic meningiomas. This result is in contrast to that of Hara et al.,\(^43\) who found bcl-2 immunoreactivity only in meningothelialomatous meningiomas and not in fibroblastic tumors. Mosnier and colleagues\(^46\) also found no correlation between bcl-2 expression and either Ki-67 LI or sex hormone receptor status. They compared bcl-2 expression in meningiomas evaluated by immunohistochemical staining with those evaluated by the Western blot technique and found that Western blot detected bcl-2 positivity in 32 (82%) of the 39 meningiomas, while positive immunostaining was present in only 17 (44%) of the tumors.\(^46\)

Expression of bcl-2 in our study was detected by immunohistochemistry, and the staining in most cases was focal, involving fewer than 10% of the tumor cells. It is possible that our study underestimated the true number of bcl-2-positive cells in meningiomas and that Western blot is a more sensitive technique, but one that requires fresh tissue.

One might expect apoptotic activity as determined by apoptosis counts or TUNEL assay to be inversely related to bcl-2 expression, given that bcl-2 is a protein involved in the inhibition of apoptosis. We found no correlation between apoptosis counts and bcl-2 positivity, similar to other investigators. Ng and Chen\(^26\) studied 51 meningiomas with regard to apoptotic activity as measured by the TUNEL assay and compared apoptotic indices with p53, c-myc, and bcl-2 expression determined by immunohistochemical staining. They found no relationship between the apoptosis index and expression of any of these 3 apoptosis-related regulatory proteins. Our study also failed to demonstrate a statistically significant association between proliferative indices (mitosis counts and MIB-1 LI) with either apoptotic body count or bcl-2 positivity. This result is similar to that of Mosnier and colleagues,\(^46\) who reported no correlation of Ki-67 index with bcl-2 expression. Karamitopoulou and coworkers\(^13\) reported higher MIB-1 LIs in meningiomas that also expressed bcl-2, although this was not statistically significant.

Apoptosis is a complex process of cell death regulated by a number of genes, only one of which is bcl-2. Poor clinical outcome, defined by death or tumor recurrence, is associated with meningiomas characterized by increased mitosis counts and incomplete surgical resection. Apoptosis counts, MIB-1 LI, and bcl-2 positivity were not independently associated with adverse outcome in the present study.

References