Clinicopathologic Study of Forty-Four Histologically Pure Supratentorial Oligodendrogliomas

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Few studies in recent years have specifically focused on pure oligodendroglial neoplasms. We retrospectively reviewed the clinicopathologic features of 44 patients with supratentorial oligodendroglioma diagnosed over a 19-year period (1974 to 1993). The study group consisted of 44 patients (age range, 8 to 69 years; median, 42 years), including 31 males. Thirty-one initially resected tumors (70%) were low grade and 13 (30%) were high grade (anaplastic). Using the St Anne-Mayo criteria for astrocytic tumors, 19 tumors (43%) were grade 2, 17 (39%) were grade 3, and 8 were (18%) grade 4. Histologic features of the tumors at initial resection included prominent nucleoli (N = 18, 41%), vascular proliferation (N = 9, 20%), necrosis (N = 6, 14%), and microcystic degeneration (N = 23, 52%). Nuclear atypia was graded as mild in 22 tumors (50%), moderate in 18 (41%), and marked in four (9%). The highest mitosis counts ranged from 0 to 10 mitotic figures (MF)/10 high-power fields (HPF) (mean, 2.4). Twelve patients (27%) had four or more MF/10 HPF. Initial surgery included gross total resection in 10 patients, subtotal resection in 16 patients, and biopsy in 14 patients. Thirty-one patients received adjuvant radiotherapy and 15 received chemotherapy. MIB-1 labeling indices ranged from 0 to 42.3 (median, 1.2 [low grade tumor median, 0.5; anaplastic tumor median, 6.2]). p53 immunostaining was observed in 18 of 43 stained tumors (41%). Overall, 5- and 10-year survival rates were 71% and 63%, respectively. The entire group had a median follow-up of 5.2 years. Age greater than 45 years (P = .02), mitosis counts of ≥4 MF/10 HPF (P = .0004), and MIB-1 labeling indices < 2 (P = .03) were independent predictors of survival (Kaplan-Meier analysis). MIB-1 labeling indices < 2 (P = .0009) was an independent predictor of disease-free survival. Low cell density (P = .04) and low histologic grade (P = .03) show trends with regard to being associated with longer survival. In conclusion, older patients (>45 years) or patients with tumors with an increased rate of cell proliferation generally have a worse prognosis. Although tumors of high histologic grade generally have a worse survival, the correlation was not statistically significant.

Index Words: Oligodendroglioma, anaplastic oligodendroglioma, MIB-1, p53, glioma
Oligodendrogial neoplasms that demonstrate chromosomal deletions on 1p and 19q are more likely to be chemosensitive compared with tumors that do not demonstrate these mutations.6

Similar to astrocytic neoplasms, grading schemas for oligodendroglial neoplasms are far from perfect. In most cases predicting tumor behavior is still based primarily on the histologic appearance of the neoplasm. This study reviews the clinicopathologic features of 44 histologically pure oligodendrogliomas and examines various histopathologic features and grading schemas with respect to clinical outcome. In addition, the role of MIB-1 labeling indices and p53 immunohistochemistry in assessing these tumors is also evaluated.

Materials and Methods

The surgical pathology files were searched for cases in which a diagnosis of oligodendroglioma, anaplastic oligodendroglioma, or malignant oligodendroglioma was made. Only tumors diagnosed before December 1993 were included for study to ensure an adequate length of follow-up. Seventy-four tumors were histologically reviewed. Only tumors felt represent pure oligodendroglioma (ie, tumors that lacked histologically distinct areas of astrocytic differentiation) were included for study. Forty-four tumors fulfilled this criterion and formed the study group.

All the pathologic material available in each case was reviewed. Histologic parameters evaluated included necrosis, vascular proliferation, nuclear atypia (evaluated as mild, moderate, or marked), presence of prominent nuleolation, microcystic degeneration, microcalcifications, cell density (evaluated as mild, moderate, or marked), and subpial infiltration or leptomeningeal involvement. Mitosis counts were performed in the most mitotically active area of the tumor at high-power magnification using a Nikon binocular microscope (Melville, NY) with a wide field 3 ocular and 340 objective (high-power field [HPF] area calculated to be 0.17 mm2). The single highest mitosis count per 10 HPF was recorded. All the tissue submitted to pathology was examined in 33 tumors. In the remaining 11 tumors, greater than half of the tissue submitted to pathology was evaluated histologically. One to 54 histologic sections (mean, 12 sections) were examined in each case. Routine sections were generated from formalin-fixed, paraffin-embedded tissues that were sectioned at a thickness of 4 µm.

All tumors were graded using two methodologies. The first involved stratifying tumors into low-grade or anaplastic (malignant) categories based on the most recently revised World Health Organization (WHO) schema.7 The second grading approach involved using the St Anne-Mayo grading schema, originally devised for astrocytomas.8

Immunohistochemical studies using the DO7 monoclonal antibody against p53 protein (dilution, 1:100; DAKO Corp, Carpinteria, CA) and MIB-1 antibody (dilution, 1:10; AMAC, Westbrook, ME) were performed in all but one tumor. Appropriate positive and negative controls were performed with each antibody. Immunostaining was performed using an avidin-biotinylated immunoperoxidase methodology with a microwave processing procedure in the case of the MIB-1 antibody, as previously described.9,10 With MIB-1 immunostaining, only nuclear staining was interpreted as positive. MIB-1 immunostained sections were evaluated at high magnification to identify areas with the highest degree of positive nuclear staining. One thousand tumor cell nuclei were evaluated at high magnification and a labeling index was determined as the percent of positive staining tumor cell nuclei. The degree of p53 positivity was reported as a percent of positive staining tumor cell nuclei.

Age, gender, clinical presentation of tumor, evidence of recurrence, utilization of radiation therapy or chemotherapy, and status of patient at the last known follow-up visit were obtained from review of the medical records. Kaplan-Meier statistical analysis of the data in relation to outcome was performed.

Results

Clinical

Forty-four patients were included for study and consisted of 31 males (70%) and 13 females (30%). Patients ranged in age from 8 to 69 years (median, 42 years) at the time of initial surgery. The most common clinical presentation included seizures, which were experienced by 38 patients (86.4%). Other less commonly encountered initial presentations included headaches in 16 patients (36.4%), focal motor deficits in 12 patients (27.3%), and hemiparesis in 11 patients (25%).

Twenty-six tumors were situated on the right side (59.1%) and 18 tumors (40.9%) on the left. The most commonly involved lobe was the frontal lobe in 17 patients (38.6%). Tissue from multiple lobes was excised in 15 patients (34.1%), most commonly the frontal and parietal lobes. Tumor was felt to arise in the temporal lobe in six patients (13.6%), the parietal lobe in five patients (11.4%), and the occipital lobe in one patient (2.3%).

Initial surgery performed included biopsy alone in 18 patients (40.9%), subtotal resection in 16 patients (36.4%), and gross total resection in 10 patients (22.7%). Nineteen patients (43.2%) required at least one additional surgery for treatment of recurrent or residual tumor. Most of these patients belonged to the group who were initially biopsied. The additional surgery involved either subtotal or gross total resection of these tumors. Thirty-one patients received adjuvant radiation therapy (70.5%). Patients received a median total dose of 58.7 Gy (range, 39–65 Gy). Eight patients receive
whole brain radiation (range, 40–50 Gy; median, 45 Gy) followed by a boost radiation (range, 10–20 Gy; median, 13.3 Gy). Eighteen patients received radiation with a 2- to 3-cm margin around the tumor. Fourteen patients received adjuvant chemotherapy (31.8%). Adjuvant chemotherapy consisted of procarbazine, cis-chloronitrosourea, and vincristine or nitrosourea-based chemotherapy. Follow-up data were available for all but five patients. The follow-up period ranged from .04 to 21 years (median, 5.2 years; mean, 6.3 years). Overall survival of patients is schematically illustrated in Fig 1. At the last known follow-up visit 16 patients had died of tumor (36.4%) (range, 2.7 to 17.8 years; mean, 7.9 years), eight were alive with evidence of residual tumor (18.2%) (range, 2.6 to 17.7 years; mean, 9.4 years), and 20 were alive with no evidence of tumor (45.4%) (range, 0.04 to 12.4 years; mean, 5.3 years). The 5-year survival rate for the entire group was 71% and 10-year survival was 63%.

**Histopathology**

Thirty-one tumors (70%) were histologically categorized as low-grade oligodendrogliomas (WHO grade II) (Fig 2). The remaining 13 neoplasms were felt to represent anaplastic or malignant oligodendrogliomas (WHO grade III) (Fig 3). Using the St Anne-Mayo grading approach, none of the tumors were categorized as grade 1 lesions. Nineteen oligodendrogliomas were felt to represent grade 2 tumors (43.2%). Seventeen grade 3 neoplasms (38.6%) and eight grade 4 tumors (18.2%) were noted.

Nuclear atypia was felt to be mild in 22 cases (50%), moderate in 18 tumors (40.9%), and marked in four neoplasms (9.1%). Nuclear atypia was evaluated in the most atypical-appearing areas of the tumor. Cell densities in the cellular areas of the tumor were rated as mild in 12 tumors (27.3%), moderate in 22 tumors (50%), and marked in 10 tumors (22.7%). Prominent nucleolation was observed in 18 tumors (40.9%). Microcystic degeneration was focally present in 23 neoplasms (52.3%) and microcalcifications in 19 lesions (43.3%). Vascular proliferation consisting of a piling up of cells around vascular lumina was noted in nine neoplasms (20.5%) (Fig 4). Areas of geographic necrosis were noted in six tumors.

![Figure 1. Overall survival in pure oligodendroglioma (n = 44).](image)

![Figure 2. Low-grade oligodendroglioma characterized by a monomorphic proliferation of cells with perinuclear clearing and an arcuate capillary vascular pattern. (Hematoxylin-eosin stain; high-power magnification.)](image)
Subpial infiltration by tumor was identified in six neoplasms (13.6%) (Fig 5). Mitosis counts ranged from 0 to 10 mitotic figures (MF)/10 HPF. The mean mitotic activity was $2.4 \pm 2.9$ MF/10 HPF. Twelve tumors had mitotic counts of $\geq 4$ MF/10 HPF; 11 of these 12 tumors were histologically graded as anaplastic or malignant.

MIB-1 immunohistochemistry yielded labeling indices ranging from 0 to 42.3 (Fig 6). The median labeling index for the entire group of tumors was 1.2. Low-grade oligodendrogliomas had a median labeling index of 0.5. Anaplastic or malignant oligodendrogliomas had a mean labeling index of 6.2. p53 immunoreactivity was identified in 18 of 43 stained tumors (41%). The degree of staining with p53 antibody was graded as follows: less than 5% (13 tumors), 5% to 20% (three tumors), 20% to 50% (one tumor), and more than 50% (one tumor).

Table 1 summarizes the statistical correlation of tumor grade and histologic features with survival. Tumor grade using the WHO schema correlated well ($P = .03$) with overall survival in oligodendrogliomas (Fig 7). Age greater than 45 years ($P = .02$) and mitoses...
counts of $\geq 4/10$ HPF ($P = .0004$) appear to be independent predictors of survival (Fig 8). Low cell density ($P = .04$) and lesions with low WHO histologic grade ($P = .03$) also appeared to be associated with longer survival. MIB-1 labeling indices, particularly labeling indices of less than 2, significantly correlated with disease-free survival ($P = .0009$) and with overall survival ($P = .03$) (Figs 9, 10). p53 immunoreactivity did not appear to correlate with disease-free survival (Fig 11).

**Discussion**

A number of attempts have been made to predict tumor behavior in oligodendroglial neoplasms based on the presence or absence of certain histologic parameters. Unfortunately, there continues to be a lack of consensus with regard to precisely what histologic parameters are most relevant and what minimal criteria are required to make a diagnosis of anaplastic or malignant oligodendroglioma. Similar to astrocytic neoplasms, cer-
tain histologic factors have been repeatedly identified in most studies as being associated with more aggressive behavior. In particular, features such as increased cellularity, cytologic atypia, increased mitotic activity, vascular proliferation, and necrosis have been noted to be more common in tumors that have ultimately behaved in a more aggressive fashion.\textsuperscript{11-14} In 1987, Burger et al\textsuperscript{11} demonstrated that mitoses, necrosis, nuclear cytologic atypia, vascular hypertrophy, and vascular proliferation were prognostically significant factors in predicting outcome in oligodendroglial neoplasms. Interestingly, mitotic activity was felt to be the single most important parameter. Of the five most important histologic features identified by Burger et al\textsuperscript{11} in their study, only necrosis was found to be independently a significant variable. Mørk et al\textsuperscript{13} noted necrosis and high cellularity as the two factors most associated with poor prognosis. Smith et al,\textsuperscript{14} attempting to establish a four-tier grading system for oligodendrogliomas based on a variety of parameters including endothelial proliferation, necrosis, nuclear to cytoplasmic ratio, cell density, and pleomorphism, found that only pleomorphism was significant when features were analyzed individually. In the current study, each of the histologic parameters was examined with regard to survival. Of the histologic parameters evaluated, mitosis counts of greater than 4 MF/10 HPF appeared to be an independent predictor of survival, along with overall tumor grade (WHO) and cell density.

### Table 1. Histopathologic Features Correlated With Survival

<table>
<thead>
<tr>
<th>Feature</th>
<th>Number (%)</th>
<th>P Value</th>
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<tr>
<td>WHO Grade</td>
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<tr>
<td>Low grade (grade II)</td>
<td>31 (70.5)</td>
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<tr>
<td>Anaplastic/malignant</td>
<td>13 (29.5)</td>
<td></td>
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<tr>
<td>(grade III/IV)</td>
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<td>St Anne-Mayo</td>
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<tr>
<td>Grade 1</td>
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<td></td>
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<tr>
<td>Grade 2</td>
<td>19 (43.2)</td>
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<tr>
<td>Grade 3</td>
<td>17 (38.6)</td>
<td></td>
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<tr>
<td>Grade 4</td>
<td>8 (18.2)</td>
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<tr>
<td>Prominent nucleoli</td>
<td>18 (40.9)</td>
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<td>Vascular proliferation</td>
<td>9 (20.5)</td>
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<td>Necrosis</td>
<td>6 (13.6)</td>
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<td>Microcystic degeneration</td>
<td>23 (52.3)</td>
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<td>Calcification</td>
<td>19 (43.3)</td>
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<td>Subpial infiltration</td>
<td>6 (13.6)</td>
<td>.616</td>
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<tr>
<td>Nuclear atypia</td>
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<td>.19</td>
</tr>
<tr>
<td>Mild</td>
<td>22 (50)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>18 (40.9)</td>
<td></td>
</tr>
<tr>
<td>Marked</td>
<td>4 (9.1)</td>
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<tr>
<td>Cell density</td>
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<tr>
<td>Mild</td>
<td>12 (27.3)</td>
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</tr>
<tr>
<td>Moderate</td>
<td>22 (50)</td>
<td></td>
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<tr>
<td>Marked</td>
<td>10 (22.7)</td>
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<tr>
<td>Mitosis Counts</td>
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<tr>
<td>Mean</td>
<td>2.4 ± 2.9 MF/10 HPF (≥4 MF/10 HPF; P = 0.00004)</td>
<td></td>
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<tr>
<td>Range</td>
<td>0–10 MF/10 HPF</td>
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<td>Immunohistochemistry</td>
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<tr>
<td>MIB-1 labeling index</td>
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<tr>
<td>Range</td>
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<tr>
<td>MIB-1</td>
<td>&lt;2</td>
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<tr>
<td>p53 positivity</td>
<td>18/43 (41) of stained tumors</td>
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<tr>
<td>p53 negative</td>
<td>0.74</td>
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![Figure 7](image-url) Overall survival in pure oligodendroglioma according to WHO grade.

![Figure 8](image-url) Overall survival in pure oligodendroglioma according to mitosis counts. High mitosis group, ≥4 MF/10 HPF; low mitosis group, <4 MF/10.
density. Similar to the findings of Mørk et al., tumors with lower cellularity appeared to be more likely associated with longer survival.

As is the case with other glioma types, there appears to be a variety of factors other than histology that also play a role in determining outcome. Age of patient appears to be a potentially important factor in predicting outcome in oligodendrogliomas. Burger et al. intimated that older age may be associated with a more aggressive behavior. Kros et al. noted that age was only "faintly" correlated with survival, with younger patients tending to survive longer. Wilkinson et al. in a study of 63 oligodendrogliomas, found that age proved to be the single most important prognostic factor, with patients younger than 45 years having a significantly longer survival than older patients. Other investigators have similarly shown correlation between good prognosis and younger age. Our study reconfirmed this general impression by noting that age greater than 45 years was an independent predictor of poor survival. A number of studies have also demonstrated the effect of other clinical parameters on prognosis in oligodendrogliomas, variously citing extent of resection, location (better prognosis in frontal lobe based tumors), and increased survival with postoperatively radiated neoplasms. Similar to astrocytic neoplasms, oligodendrogliomas, particularly higher-grade tumors, tend to be somewhat heterogeneous. Limited biopsies may not necessarily sample the highest-grade area of the lesion and may potentially result in undergrading. The extent of sampling becomes important in the evaluation of these tumors and arrival at an accurate histologic assessment. Further compounding the problems of tumor grading due to tumor heterogeneity is interobserver variability with regard to reproducibility of grading parameters. Many of the histologic parameters that are often used in assessing histologic grade, such as degree of cellularity and nuclear atypia, are especially subject to interobserver variability. Even parameters that one would think are more objective, such as identifying mitotic figures and vascular proliferation, are also subject to considerable interobserver variability with regard to interpretation. Identification of mitotic figures has a particular impact on the St Anne-Mayo system which suggests that the identification of a single mitotic figure is enough to warrant advancement of the tumor a grade.

Figure 9. Overall disease-free survival in pure oligodendroglioma according to MIB-1 labeling index. Low MIB-1 group, <2%; high MIB-1 group, ≥2%.

Figure 10. Overall survival in pure oligodendroglioma according to MIB-1 labeling index. Low MIB-1 group, <2%; high MIB-1 group, ≥2%.

Figure 11. Overall disease-free survival in pure oligodendroglioma according to p53 features.
In 1997, Coons et al.\textsuperscript{23} examined the issue of improving diagnostic accuracy in interobserver concordance in the classification and grading of gliomas. As somewhat expected, diagnostic concordance increased over time with increased interactions and refinements of histologic criteria among the group of neuropathologists involved in grading. Interestingly, most of the initial disagreements related to actual classification of tumor type, including distinction of astrocytoma from oligodendroglioma, mixed glioma, and pilocytic astrocytoma.\textsuperscript{23}

Part of the problem of glioma tumor classification lies in the lack of a precise definition of what constitutes a mixed glioma (oligoastrocytoma). Many studies have presented precise definitions with regard to percent of a minor component that needs to be identified before designating the lesion as mixed; figures of 20\% and 25\% have been variously suggested.\textsuperscript{11,13,24,25} Such definitions, albeit imperfect, provide general guidelines for purposes of diagnosis and as an attempt to standardize studies that have tried to evaluate the clinical significance of making such a diagnosis. The issue is further complicated, as previously mentioned, by issues of tumor heterogeneity, tumor sampling, and observer variability with regard to exactly what represents astrocytoma and what represents oligodendroglioma. Recognition of the association of loss of heterozygosity for 1p and 19q in a substantial subset of oligodendrogliomas may be a step in the direction of obtaining more diagnostic homogeneity, particularly if such analysis becomes more widespread. The attempt of the current study was to confine it to only those cases felt to be “pure” oligodendroglioma. The identification of an astrocytoma component to the tumor was a sufficient reason to exclude the case from current study.

Quantitative evaluation of tumor proliferative activity by a variety of methodologies has become potentially useful as an additional means of evaluating behavior, with the recognition that tumors that generally have higher proliferative labeling indices tend to behave in a more aggressive fashion. A number of studies have examined the role of a variety of cell proliferation markers, including Ki-67, proliferative cell nuclear antigen, and MIB-1 antibodies, and have been evaluated in the literature in oligodendrogliomas either independently, as a group, or as part of larger surveys of central nervous system neoplasms.\textsuperscript{26-32} In 1996, Kros et al.\textsuperscript{28} examined MIB-1 labeling indices in 108 tumors classified as oligodendroglioma. Fifty of the tumors had labeling indices of less than 0.1, 26 tumors had labeling indices between 0.1 and 0.2, and the remaining 32 tumors had labeling indices $\geq$0.2. Multivariate analysis of their data indicated that MIB-1 labeling indices had independent prognostic significance. Coons et al.\textsuperscript{29} examined 81 astrocytomas with MIB-1 antibody and noted a statistically significant difference between tumors that have labeling indices of $\leq$5 and those that had indices greater than 5.\textsuperscript{29} Dehghani et al.\textsuperscript{30} also demonstrated a correlation between MIB-1 labeling indices and 5-year survival. Others have noted a less-convincing correlation with survival. Wharton et al.\textsuperscript{31} concluded, in their study of 32 oligodendrogliomas, that stratification of cases by MIB-1 labeling indices alone did not predict a significantly different survival. The current study seems to confirm the impression of most of these previous works, however, that MIB-1 labeling indices do yield potentially prognostic information in the evaluation of oligodendrogial neoplasms. The current study indicated that MIB-1 indices of less than 2 were an independent predictor of recurrence-free survival. Although the overwhelming evidence suggests that there may be some utility in the evaluation of these tumors with proliferation markers, caution needs to be taken in the relative weight such information is afforded. Again, issues of tumor heterogeneity and sampling are important. It has been well documented that regional heterogeneity in proliferative activity of gliomas does occur and certainly may influence a proliferation index that is determined in a given neoplasm.\textsuperscript{33} Consequently, something as simple as which paraffin block one selects to perform immunohistochemical analysis may have an impact on the labeling index and may not necessarily be reflective of the most proliferative area of the tumor. Further clouding the issue are factors of stain interpretation, ie, defining what exactly is positive staining. Differences in methodology are significant enough that care needs to be taken in extrapolating other laboratories’ numerical results to one’s own experience.

Evaluation of p53 abnormalities in oligodendrogliomas has resulted in conflicting reports. p53 is a well-recognized tumor suppressor gene located on the short arm of chromosome 17. A subset of oligodendrogliomas do appear to express p53 by immunohistochemistry or demonstrate p53 gene abnormalities.\textsuperscript{34-40} Most studies have demonstrated a somewhat low rate of p53 immunoreactivity or p53 mutation in oligodendrogial neoplasms.\textsuperscript{35-40} Using polymerase chain reaction and single-strand polymorphism analysis, Ohgaki et al.\textsuperscript{36} noted p53 gene mutations in only two of 17 oligodendrogliomas (12\%). In contrast to these studies, which admittedly comprise relatively small numbers of oligodendroglial neoplasms, Kros et al.\textsuperscript{34} noted evidence of p53 immunoreactivity in 75\% of the 84 oligodendrogliomas they
evaluated. In addition, these investigators observed that tumors that had more immunostaining (>75% of tumor cells) behaved in a more aggressive fashion. In our experience, only 41% of oligodendrogliomas demonstrated any p53 immunoreactivity; in the vast majority of cases it involved <5% of tumor cell nuclei. Correlation of low p53 with recurrent-free survival was not statistically determined. Most but not all of the tumors that did demonstrate greater than 5% nuclear immunoreactivity; in the vast majority of cases it involved low p53 with recurrent-free survival was not statistically determined. Most but not all of the tumors that did demonstrate greater than 5% nuclear immunoreactivity with p53 histologically were higher-grade tumors.1-6 It does not appear that p53 analysis adds much significant information to the evaluation of these tumors.5,6

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References