Grading of Astrocytomas

A Simple and Reproducible Method

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This study determines the effectiveness and reproducibility of a previously published method of grading gliomas. The method under study is for use on "ordinary astrocytoma" cell types, *i.e.*, fibrillary, protoplasmic, gemistocytic, anaplastic astrocytomas and glioblastomas, and is based upon the recognition of the presence or absence of four morphologic criteria: nuclear atypia, mitoses, endothelial proliferation, and necrosis. The method results in a summary score which is translated into a grade as follows: 0 criteria = grade 1, 1 criterion = grade 2, 2 criteria = grade 3, 3 or 4 criteria = grade 4. The histologic material and clinical data were derived from a previously reported series of patients with astrocytomas, radiotherapeutically treated at Mayo Clinic between the years 1960 and 1969. From this series, initially graded 1 to 4, according to the Kernohan system, 287 "ordinary astrocytomas" were entered into the study; 51 pilocytic astrocytomas and microcystic cerebellar-type astrocytomas also were included for comparison. Among ordinary astrocytomas, the grading method under study distinguished 0.7% of grade 1, 17% of grade 2, 18% of grade 3, and 65.3% of grade 4. A 15-year period of follow-up was available on all surviving patients. Statistical analysis showed that in ordinary astrocytomas, each of the four histologic criteria, as well as the resultant grade, were strongly correlated to survival ($P < 0.0001$). Median survival was 4 years in grade 2, 1.6 years in grade 3, and 0.7 years in grade 4 tumors. Of the two patients with grade 1 ordinary astrocytomas, 1 had 11 years of survival, and the other was alive at 15 years. Furthermore, based upon the Cox Model, grade was found to be the major prognostic factor, superceding the effects of age, sex, and location. Among ordinary astrocytomas, the grading system under consideration clearly distinguished four distinct grades of malignancy, whereas, the Kernohan grading system accurately distinguished only two major groups of patients. Survival curve of patients with our grade 2 tumors coincided with the grade 1 and 2 Kernohan survival curves. Similarly, our grade 4 survival curve coincided with the Kernohan grade 3 and 4 survival curves. As a result, our proposed grading method generated an individualized curve corresponding to grade 3 tumors. Double-blind grading between two independent observers was concordant in 94% of ordinary astrocytomas; reproducibility was 81% in low-grade (grades 1 and 2) and 96% in high-grade (grades 3 and 4) astrocytomas of ordinary type. Application of this simple and reproducible grading method for the study of ordinary forms of astrocytomas should permit reliable comparison of clinical and therapeutic data emanating from various treatment centers.

Cancer **62:2152–2165, 1988.**

PATHOLOGIC CLASSIFICATION and grading of astrocytic gliomas is a controversial subject. Nevertheless, acceptance of a common system is critical for assessing prognosis and planning therapy as well as for interpreting the results of various therapeutic regimens, often emanating from different centers.

The simultaneous use of different classification systems understandably creates confusion. The Kernohan system, for example, subdivides astrocytic neoplasms by their degree of malignancy into grades 1 through 4.[1] Other approaches often recognize three divisions such as astrocytoma, anaplastic or malignant astrocytoma, and glioblastoma.[2–5]

Considerable skepticism exists concerning the validity of histologic grading as currently applied. A major impediment to effective grading is the lack of firm pathologic criteria for separation of grades. Subjectivity in the appreciation of various morphologic features has resulted

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Supported in part by Grant CA25224 from the National Cancer Institute of the National Institutes of Health and by Grant M73 from the Fraternal Order of Eagles, Rochester, Minnesota.

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Accepted for publication May 5, 1988.

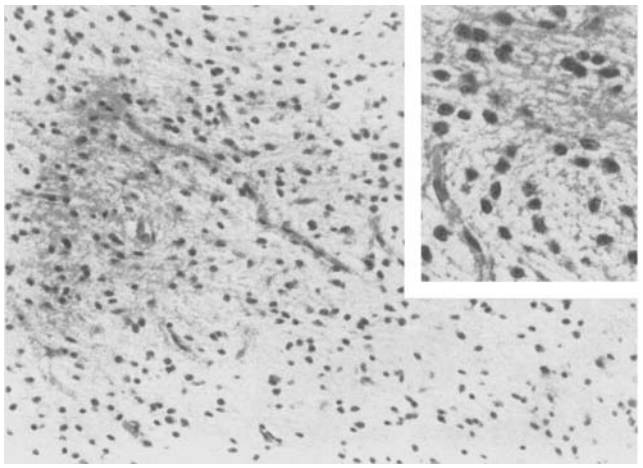


FIG. 1. Grade 1 fibrillary astrocytoma: Notice only minimal variation in shape and size of nuclei. Mitoses, endothelial proliferation and necrosis are absent (H & E, original magnification ×150). *Inset:* Magnification showing tumor nuclei appearance (H & E, original magnification ×450).

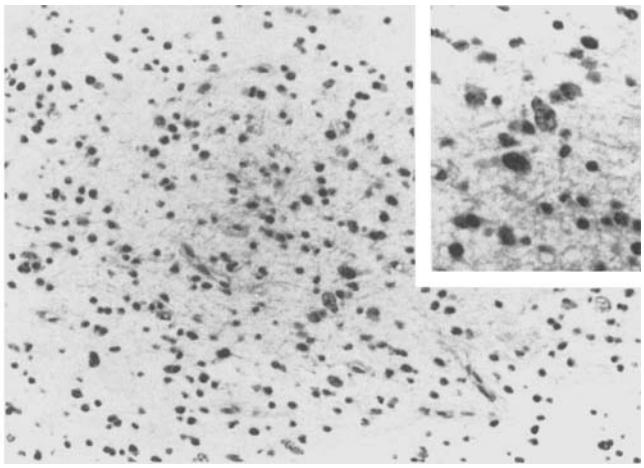


FIG. 2. Grade 2 fibrillary astrocytoma: Nuclear atypia is present but mitoses, endothelial proliferation, and necrosis are not seen (H & E, original magnification ×150). *Inset:* Magnification showing nuclear atypia (H & E, original magnification ×450).

in lack of uniformity and in the proliferation of meaningless subgrades, *e.g.*, grade 2–3, grade 3–4.

We suggest that consideration be given to a simple grading system of gliomas, first described in 1981[6] and 1982[7] as one element of a two-part classification scheme of gliomas. The two features include the following: (1) histologic malignancy and (2) spatial configuration of the tumor. Development of this grading system was prompted by lack of a suitable method applicable to serial stereotactic biopsies. Although originally the four grades of malignancy of the system were designated A, B, C, and D, we now refer to grades 1 through 4 in order to avoid confusion with the alphabetic grades subsequently introduced by Smith *et al.* in their grading scheme of oligodendrocytomas.[8]

This simple grading system for gliomas relies upon recognizing the presence or absence of four parameters: nuclear atypia, mitoses, endothelial proliferation, and necrosis. The method thus is binary and results in a score which is easily translated into a grade as follows: 0 criterion = grade 1, 1 criterion = grade 2, 2 criteria = grade 3, or 3 or 4 criteria = grade 4. Examples of each histologic grade are illustrated in Figures 1 to 4A through 4C. This study assesses the effectiveness and reproducibility of this method in a retrospective study of a large series of uniformly treated astrocytomas of ordinary type with long-term follow-up.

Materials and Methods

The pathologic material and clinical information upon which this study is based were derived from a previously reported series of Scanlon and Taylor[9] of 415 astrocytomas radiotherapeutically treated at Mayo Clinic between the years 1960 and 1969. During that decade, patients undergoing radiotherapy were those with high-grade or subtotally resected low-grade gliomas. Fifteen-year follow-up was available on all surviving patients, as was data regarding age at the time of surgery, sex, and tumor location. The tumors in this series were initially classified according to the Kernohan system[1] into four grades of malignancy, with no distinction having been made between histologic subtypes of astrocytomas.

From the series of Scanlon and Taylor[9] of 415 patients, 77 cases were excluded from our review for one of the following reasons: slides not available for review, biopsy specimens were considered insufficient in quantity for proper assessment, tumors containing a major oligodendroglial component, and cases wherein basic diagnostic

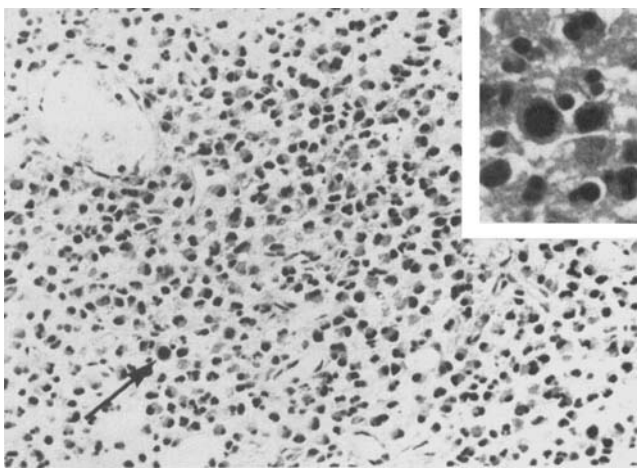
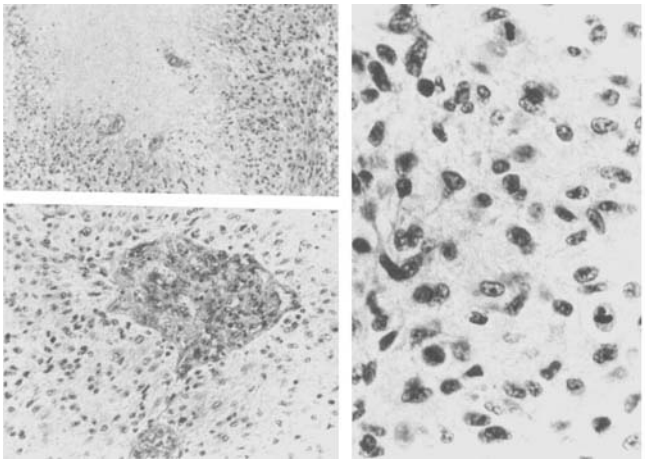


FIG. 3. Grade 3 gemistocytic astrocytoma: Nuclear atypia and mitoses (arrow) are present but endothelial proliferation and necrosis are absent (H & E, original magnification ×150). *Inset:* Magnification of the mitoses (H & E, original magnification ×450).



FIGS. 4A–4C. Grade 4 gemistocytic astrocytoma: Nuclear atypia, mitoses, endothelial proliferation and necrosis are present (A, top left) H & E, original magnification ×80. (B, bottom left) H & E, original magnification ×150. (C, right) H & E, original magnification ×300.

agreement regarding the astrocytic nature of the lesion could not be reached. The remaining 338 cases were accepted into the current study.

In terms of the World Health Organization (WHO) classification,[10] this series included fibrillary, gemistocytic and protoplasmic astrocytomas as well as anaplastic astrocytomas and glioblastomas. This spectrum of tumors, comprising 287 of the 338 astrocytomas, is herein termed "ordinary astrocytomas." In addition to the latter, 51 astrocytomas of either pilocytic or microcystic cerebellar type were encountered and were included in the study solely for the purpose of clinicopathologic comparison.

Histologic Evaluation

Routinely processed sections from all patients were examined independently by two pathologists (C.D.D., B.W.S.) in a double-blind fashion without knowledge of survival data. Each examiner independently recorded the presence or absence of the four morphologic criteria noted above. The summary score and the resultant grade of malignancy were separately recorded.

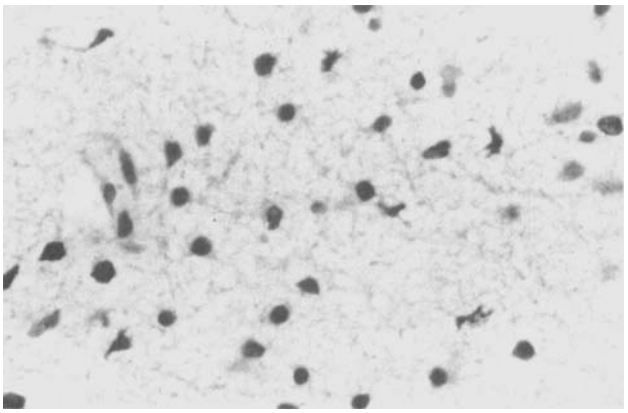


FIG. 5. Artifactual destruction of nuclei mimicking mitoses (×40).

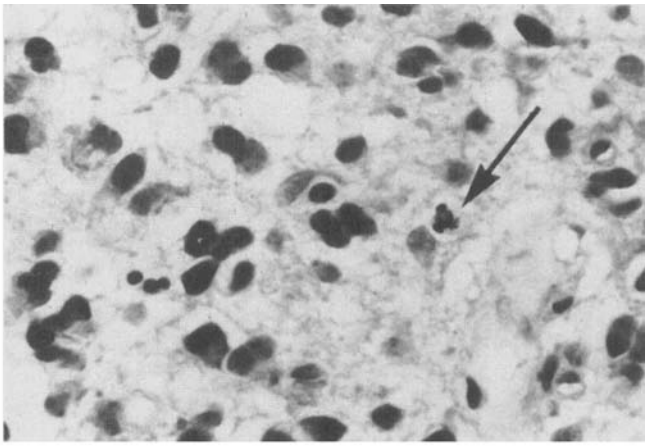


FIG. 6. Picnosis (arrow) mimicking mitoses (H & E, ×300).

The histologic criteria were defined as follows:

1. *Nuclear atypia:* Nuclear atypia was considered as present if nuclei showed hyperchromasia and/or obvious variation in shape and size (Fig. 2), but was considered absent if only minimal variation in nuclear size and shape was observed. Factors such as number and size of the nucleoli as well as chromatin pattern were not considered.

2. *Mitoses:* Mitoses were recorded as present regardless of whether they were normal or abnormal in configuration. A special effort was made to avoid confusing karryorrhexis, artifactual nuclear distortion (Fig. 5), or pyknosis (Fig. 6) for mitotic figures.

3. *Endothelial proliferation:* Endothelial proliferation was recorded as present when vascular lumina were not surrounded by a single layer of endothelial cells but by haphazardly arranged or "piled up" endothelial cells often showing cytologic atypia (Fig. 4). Endothelial proliferation must be distinguished from simple increases in vascularity due to newly formed capillaries. Such neovascularity, seen in both astrocytomas and in nontumoral conditions (Figs. 7A and 7B), may be accompanied by endothelial prominence but in these instances the cells are disposed about the lumina in a single layer. In addition, endothelial proliferation must also be distinguished from tortuous capillaries, which resemble renal glomeruli. Such vessels are typically seen in pilocytic astrocytomas and are lined by a single layered endothelium (Fig. 8A). Smear preparations also clearly show that glomeruloid formations consist of well-differentiated capillaries (Fig. 8B). Endothelial proliferation also must be differentiated from tangentially cut vessels with thick walls.

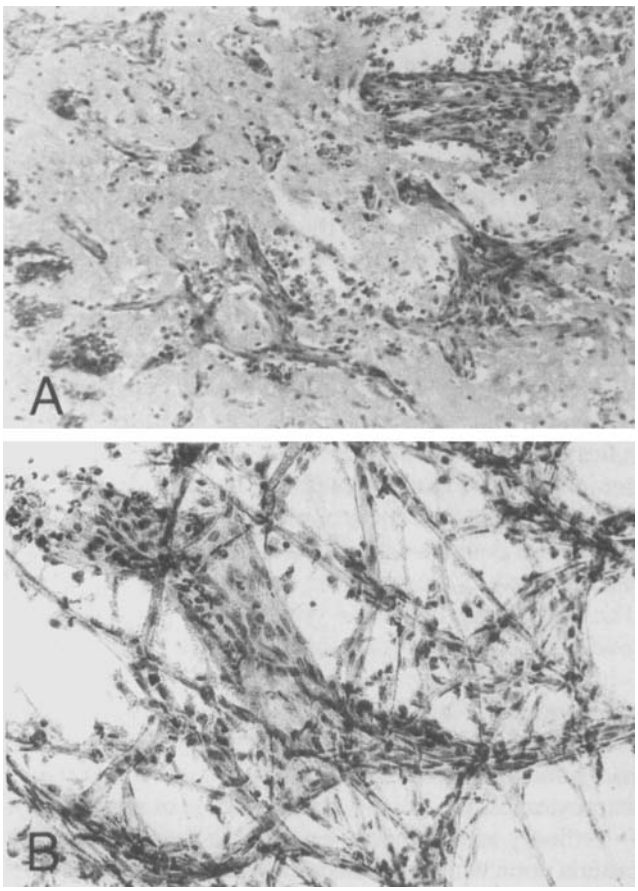
4. *Necrosis:* Necrosis was recorded only when obvious. Simple pseudopalisading of neoplastic nuclei unassociated

with obvious necrosis, the presence of macrophages as well as the finding of individual tumor cell necrosis were not recorded. Necrosis must also be differentiated from processing artifacts, mechanical distortion of cells, and from coagulation artifacts.

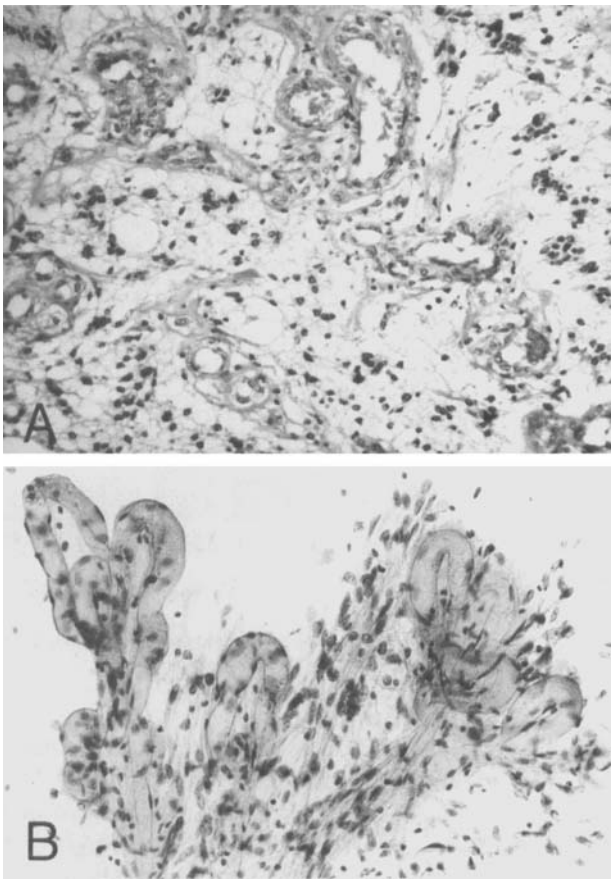
When the presence of any of the above-noted features was doubtful, they were considered to be absent.

Statistical Analysis Methods

Survival was calculated from date of diagnosis to date of death or last follow-up. Survival distributions were estimated by the Kaplan and Meier method[11] and tested for equality with log-rank tests.[12] Cox proportional hazards models[13] were used to assess the strength of association of survival with several analysis variables, individually and jointly, corresponding to the potential prognostic factors: age, sex, site of primary, histologic type, histologic grade, and its determinants, *i.e.*, nuclear atypia, mitoses, necrosis, and endothelial proliferation.



FIGS. 7A AND 7B. New formed capillaries with prominent endothelial cells at the periphery of an organizing hematoma: (A) paraffin section (H & E, ×120); (B) appearance on smear preparation (H & E, ×160).



FIGS. 8A AND 8B. The appearance of glomeruloid vessels in pilocytic astrocytomas: (A) paraffin sections (H & E, ×120); (B) smear preparations (hemalum phloxine, ×120).

Results

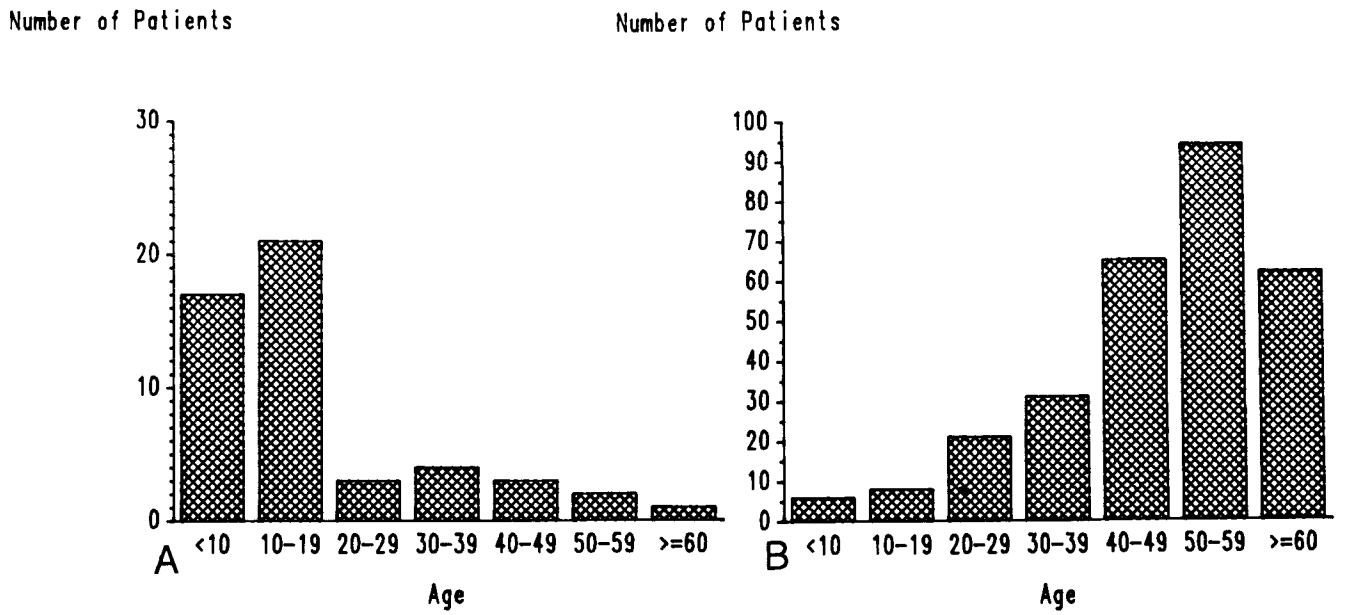
Patient Characteristics

The 205 males and 133 female patients studied (n = 338) ranged in age from 1 to 78 years (median, 49 years). The age distributions of patients with ordinary astrocytomas and with pilocytic or microcystic cerebellar-type astrocytomas are shown in Figures 9A and 9B. The location of the tumors, by histologic subtype, are indicated in Table 1.

Grade

When all astrocytomas, irrespective of histologic subtype, were graded by our method, the following frequencies were observed: 14 grade 1 (4.1%); 79 grade 2 (23.4%); 53 grade 3 (15.7%); and 192 grade 4 (56.8%). When only the ordinary astrocytomas were considered as a group, *i.e.*, exclusive of pilocytic and microcystic cerebellar-type variants, the frequencies were as follows: 2 grade 1 (0.7%); 46 grade 2 (16%); 51 grade 3 (17.8%); 188 grade 4 (65.5%).

Interestingly, in ordinary astrocytomas the four criteria



FIGS. 9A AND 9B. Age distribution for patients with (A) pilocytic and microcystic cerebellar astrocytomas, and (B) with ordinary astrocytomas.

of malignancy tended to appear in a predictable sequence corresponding to increasing degree of malignancy (Table 2). Nuclear atypia, the first criterion, was present in 46 (100%) of grade 2 tumors. The second criterion, mitotic activity, was observed in 47 (92%) of grade 3 tumors but in no grade 2 tumors. Necrosis and endothelial proliferation were almost exclusively present in grade 4 astrocytomas. Only 4 (7.8%) of 51 grade 3 astrocytomas showed either endothelial proliferation or necrosis. Among the 188 cases with grade 4 astrocytoma of ordinary cell types, in addition to nuclear abnormalities and mitoses, 117 tumors (62%) showed both necrosis and endothelial proliferation, 37 (20%) had endothelial proliferation without necrosis, and 34 (18%) had necrosis without endothelial proliferation.

TABLE 1. Locations of 338 Astrocytomas According to Cell Types

Location	Ordinary astrocytomas	Microcystic cerebellar and pilocytic astrocytomas	Total
Temporal	99	8	107
Frontal	93	0	93
Parietal	65	2	67
Occipital	14	0	14
Optic nerve	0	5	5
3rd ventricular/ basal ganglion	6	6	12
Cerebellum	5	16	21
Midline cerebellum	5	14	19
Total	287	51	338

Survival

At termination of the data collection period for the study, 47 patients (14%) were alive. A strong association between cell type and survival was noted (log-rank $P < 0.0001$). Patients with ordinary astrocytomas had a median survival of 0.87 years, less than 5% of patients being alive at 15 years. Of those with pilocytic or microcystic cerebellar-type astrocytomas, 15-year survival was 67% and 91%, respectively.

In ordinary astrocytomas, survival was strongly associated with grade as computed according to our proposed system (log-rank $P < 0.0001$). In contrast, in patients with pilocytic or microcystic astrocytomas no statistically significant association between grade and survival could be demonstrated. Figure 10 presents the survival curves for each tumor grade in the group of ordinary astrocytomas. Of the two patients with grade 1 ordinary astrocytomas, one survived 11 years and the other was alive at 15 years. The median survival was 4 years for the 46 patients with grade 2 tumors, 1.6 years for the 51 patients with grade 3 tumors, and 0.7 years for the 188 patients with grade 4 astrocytomas. Figure 11 illustrates the survival curves of the entire series of 338 patients according to whether they had pilocytic astrocytomas, microcystic cerebellar-type astrocytomas, or ordinary astrocytomas of grade 1 to 4. In ordinary astrocytomas, each of the four morphologic criteria upon which the summary score or grade was computed was strongly associated with survival (log-rank $P < 0.0001$). Survival curves based upon the presence or absence of nuclear atypia, mitoses, endothelial prolifer-

TABLE 2. Distribution of the Four Criteria of Malignancy According to Grade in 287 Patients With Ordinary Astrocytoma Cell Types

Criteria frequency percent	0 Criterion = grade 1 (2 patients)	1 Criterion = grade 2 (46 patients)	2 Criteria = grade 3 (51 patients)	3 Criteria = grade 4 (71 patients)	4 Criteria = grade 4 (117 patients)
Nuclear atypia	0	46	51	71	117
	0%	100%	100%	100%	100%
Mitoses	0	0	47	71	117
	0%	0%	92%	100%	100%
Necrosis	0	0	1	34	117
	0%	0%	2%	48%	100%
Endothelial proliferation	0	0	3	37	117
	0%	0%	6%	52%	100%

ation and necrosis are illustrated in Figures 12A through 12D. No statistically significant difference in survival was found among patients whose tumors demonstrated the presence of either 3 or 4 criteria. This was consistent with the fact that the survival distributions were almost identical for patients whose tumors showed either endothelial proliferation or necrosis.

Cox proportional hazard modeling was used to assess the strength of association between survival and several potentially prognostic factors taken individually and jointly. In patients with ordinary astrocytomas, six analysis variables were used: (1) age (in years); (2) sex (1 = male, 2 = female); grade (1, 2, 3, 4); and three location variables, (4) frontal (=1 if the primary lesion site was mainly frontal,

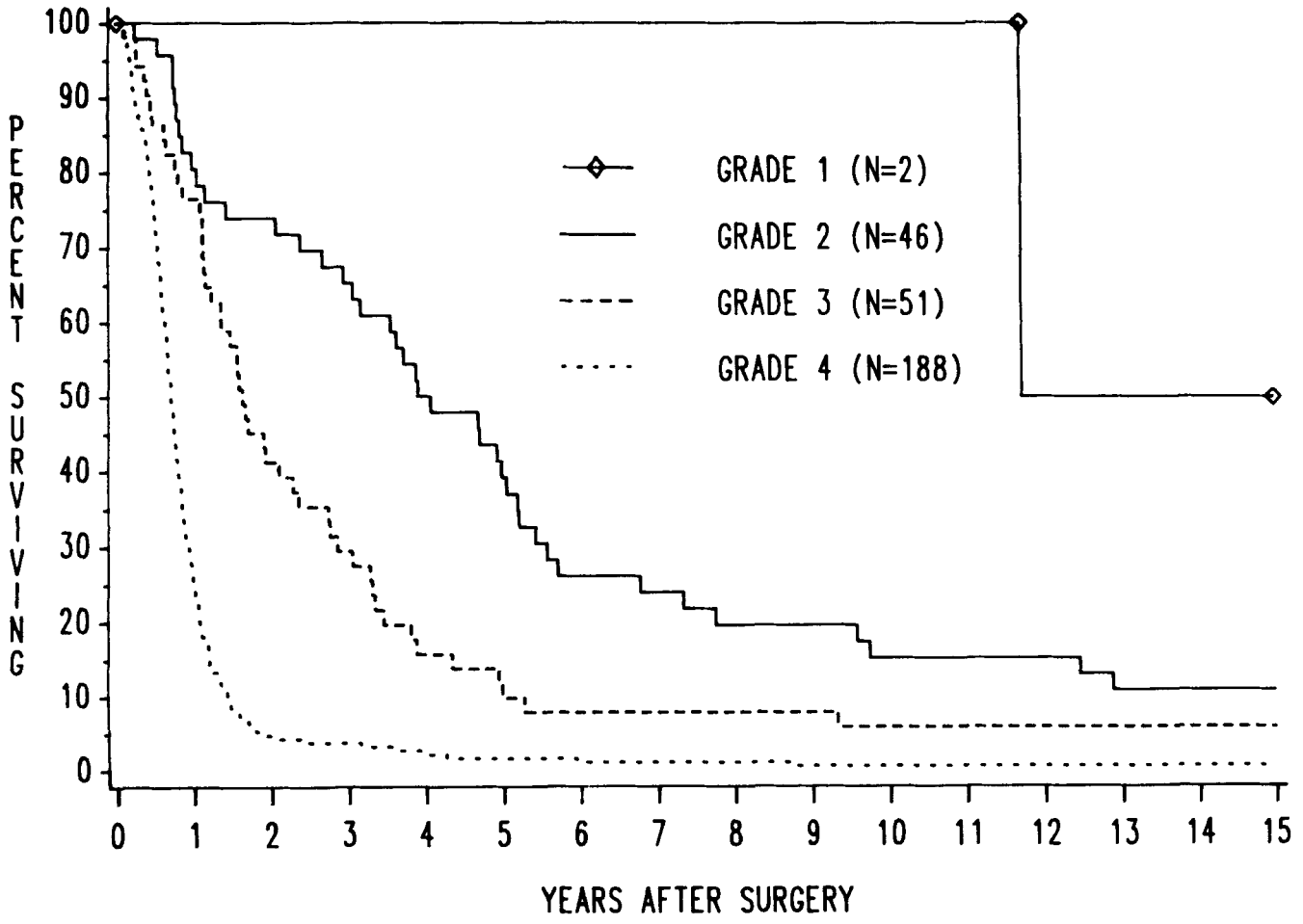


FIG. 10. Survival curves based upon the proposed grading system as applied to 287 patients with ordinary astrocytomas.

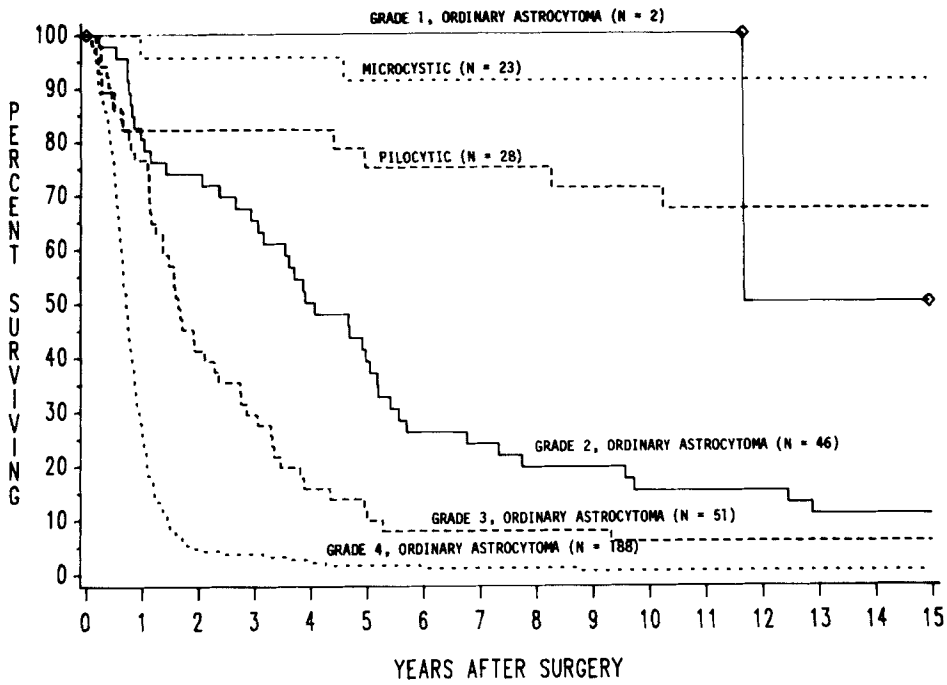
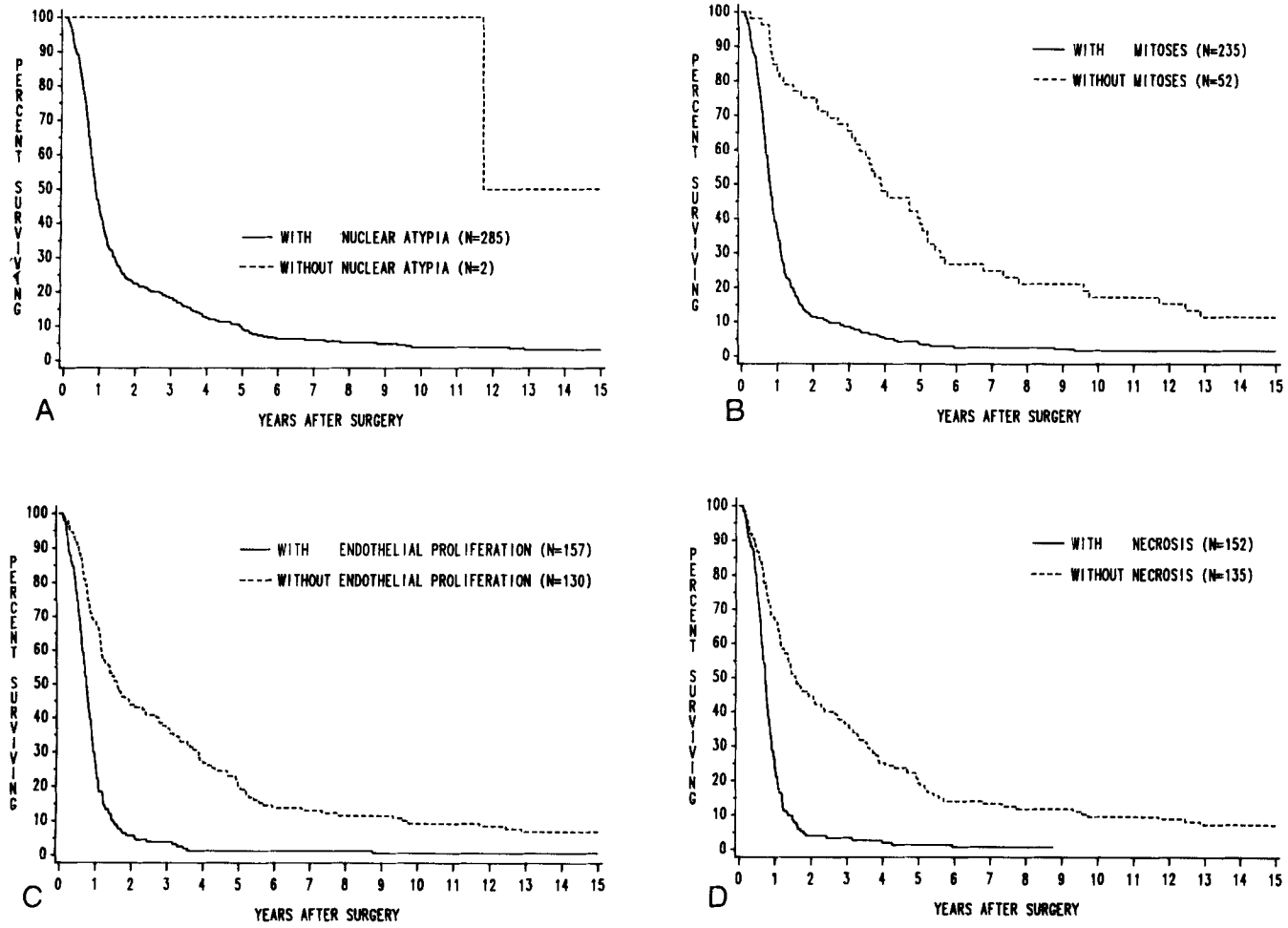


FIG. 11. Superimposition of the survival curves for patients with microcystic cerebellar pilocytic astrocytomas, all types, with the four survival curves generated by the proposed grading in patients with ordinary astrocytomas, all types.



FIGS. 12A–12D. Survival curves for the 287 patients with ordinary astrocytomas based upon the presence or absence of (A) nuclear atypia, (B) mitoses, (C) endothelial proliferation, or (D) necrosis.

TABLE 3. Statistical Analysis: Chi-Square and *P* Value of Different Variables in Cox Proportional Hazards Models in 287 Patients With Ordinary Astrocytomas

Variables	Individual one-variable models		Six-variable model		"Best" five-variable model	
	Chi-square	*P* value	Chi-square	*P* value	Chi-square	*P* value
Grade	97.12	<0.0001	60.69	<0.0001	60.72	<0.0001
Age	36.34	<0.0001	12.57	0.0004	12.63	0.0004
Sex	4.77	0.0290	5.71	0.0168	5.71	0.0169
Location						
Temporal	0.95	0.3286	2.15	0.1423	4.05	0.0441
Parietal	2.21	0.1374	3.02	0.0825	5.23	0.0222
Frontal	3.54	0.0600	0.03	0.8564	—	—

=0 otherwise), (5) temporal (=1 if the primary lesion site was mainly temporal, =0 otherwise), and (6) parietal (=1 if the primary lesion site was mainly parietal, =0 otherwise). Table 3 shows the chi-square and *P* values for all analysis variables in the individual one-variable, full six-variable, and "best" five-variable Cox models. Of these six variables, "grade" was the single factor most strongly associated with survival; "age" and "sex" were also significantly associated with survival when taken individually, but the three location variables were not. In the full model containing all six variables, "grade" remained the most strongly associated with survival ($P < 0.0001$) even after adjustment for the effects of the other five variables. "Age" and "sex" were also significantly associated with survival (*P* values = 0.004 and 0.0168, respectively) and two of the three location variables were nearly significant. When one of the location variables, *i.e.*, "frontal," was removed from the full model, all five of the remaining variables were significantly associated with survival even after adjustment for the effects of the other variables in the model.

From the coefficients (not shown) of the various factors in the "best" model, we can conclude that survival is significantly diminished for males, older persons, temporal or parietal lesions, and higher values of grade. The *P* values convey the information that survival is most strongly associated with grade in patients with ordinary astrocytomas.

Reproducibility

Double-blind grading between two independent observers was concordant in 94% of the 287 ordinary astrocytomas. When considered separately, the percentage of agreement was 81% in 48 low-grade (grades 1 and 2) and 96% in 249 high-grade (grades 3 and 4) ordinary astrocytomas (Table 4). Disagreement between the observers was found in 17 cases. In 15 cases the under or overestimation was of one grade; in two cases the difference was of two grades. The percentage of concordance according to presence or absence of each of the histologic criteria of malignancy were the following: nuclear atypia = 99.9%; mitoses = 97%; endothelial proliferation = 91.6%; and necrosis = 95%.

Comparison With Kernohan Grading

The 338 astrocytomas in this study were initially graded according to the Kernohan classification.[1] There were 24 grade 1 (7.1%); 71 grade 2 (21%); 163 grade 3 (48.2%); and 80 grade 4 (23.7%) tumors. When the 287 ordinary astrocytomas were considered apart from pilocytic and microcystic astrocytomas, their frequency according to the Kernohan system was as follows: eight grade 1 (2.8%); 40 grade 2 (14%); 159 grade 3 (55.4%); and 80 grade 4 (28%).

Survival curves for the four Kernohan grades in patients with tumors of all cell types are shown in Figure 13. Survival by Kernohan grade in ordinary astrocytomas is shown in Figure 14. Comparison of these survival curves showed a marked decrease in survival in astrocytomas of Kernohan grades 1 and 2 when pilocytic and microcystic cerebellar astrocytomas were excluded from consideration. In addition, the Kernohan grades 1 and 2 survival curves were nearly superimposable. Survival curves in patients with Kernohan grades 3 and 4 also were very

TABLE 4. Interobserver Reproducibility of the Proposed Grading System in 287 Patients With Ordinary Astrocytomas

Frequency / Row percent	Grade observer 2				
	1	2	3	4	Total
Grade observer 1					
1	2	0	0	0	2
	100.0	0.0	0.0	0.0	0.7
2	1	37	6	2	46
	2.2	80.4	13.0	4.4	16.0
3	0	1	48	2	51
	0.0	2.0	94.1	3.9	17.8
4	0	0	5	183	188
	0.0	0.0	2.7	97.3	65.5
Total	3	38	59	187	287
	1.05	13.2	20.6	65.2	100.0

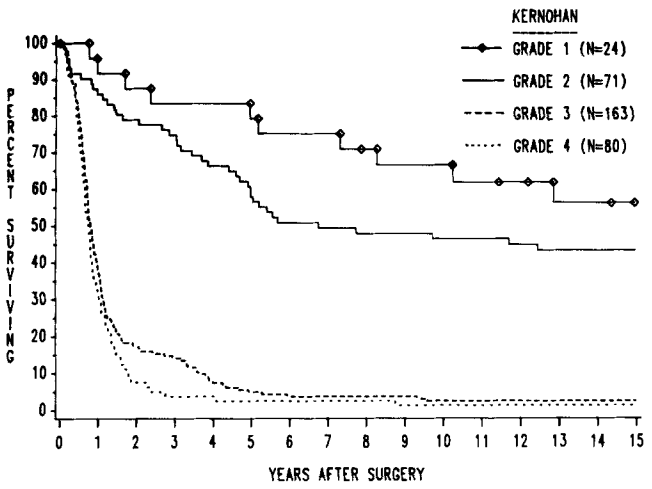


FIG. 13. Survival curves for the 338 patients, based upon Kernohan grade as applied to all astrocytomas irrespective of cell type.

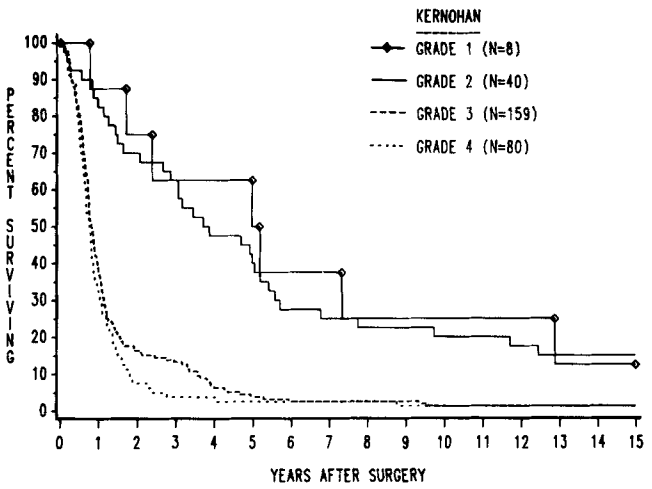


FIG. 14. Survival curves based upon Kernohan grading as applied to 287 patients with ordinary astrocytoma cell types.

similar. Indeed, the Kernohan grading system accurately distinguished only two major groups of patients, *i.e.*, those with low-grade ordinary astrocytomas (grades 1 and 2) and those with high-grade neoplasms (grades 3 and 4).

Figure 15 illustrates the survival curves, generated on the basis of our grading system, as applied to all histologic subtypes. Comparison with the survival curves in Figure 9 shows that the exclusion of pilocytic and cerebellar-type astrocytomas results in a marked decrease in survival time in grade 2 astrocytomas. Among ordinary astrocytomas, the grading system under consideration clearly distinguishes four distinct grades of malignancy. As illustrated in Figure 16, survival curve of patients with grade 2 tumors coincided with the grade 1 and 2 Kernohan survival curves. Similarly, our grade 4 survival curve coincided with the Kernohan grade 3 and 4 survival curves. As a result, our proposed grading method generates an individualized curve corresponding to grade 3 tumors.

A comparison of the distribution of grades as determined by the Kernohan and by our grading system is as follows: the highest percentage (91.3%) of agreement was observed in astrocytomas designated grade 4 by the Ker-

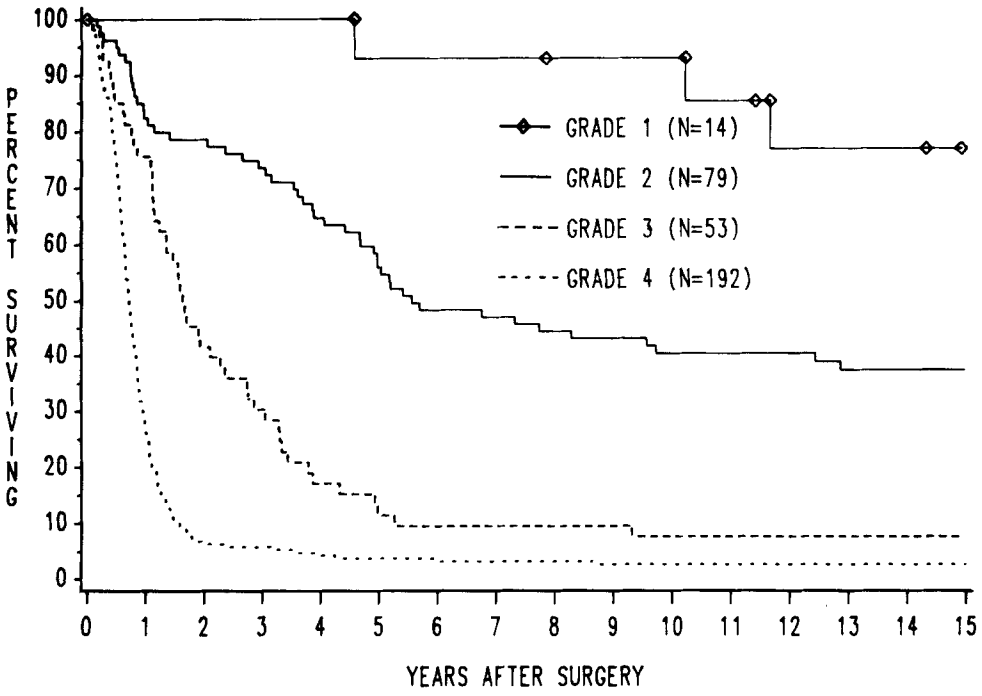


FIG. 15. Survival curves based upon the proposed grading system as applied to 338 patients with astrocytomas of all cell types.

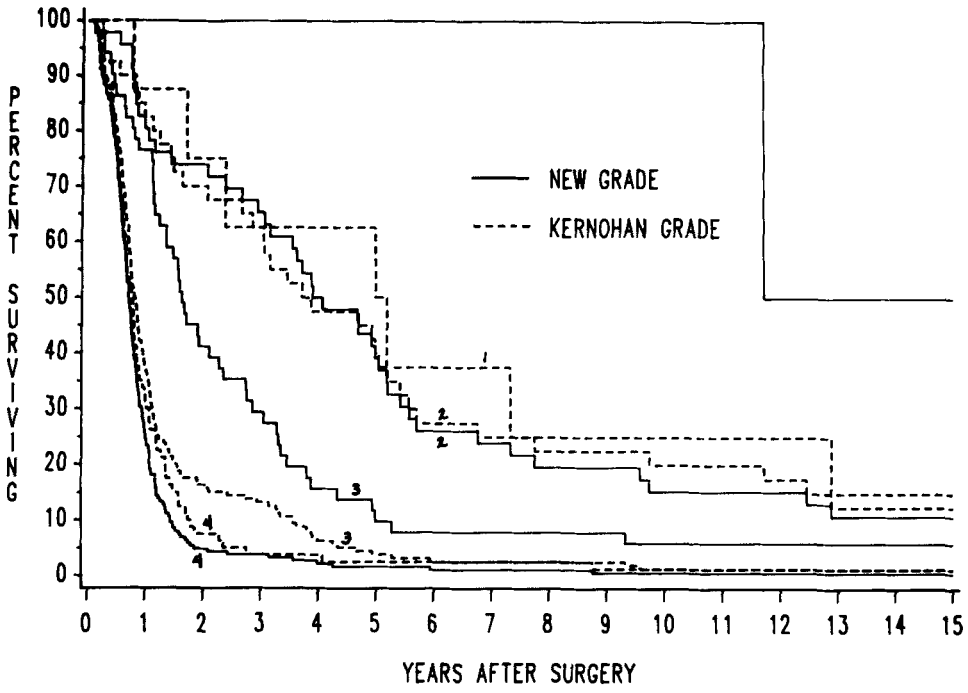


FIG. 16. Superimposition of the survival curves generated by the Kernohan and the proposed grading system in 287 patients with astrocytomas of ordinary cell types.

nohan system and by ours. However, there was no agreement in grade 1 lesions; our two examples of grade 1 astrocytoma were designated grade 2 by the Kernohan system. Seven of the eight ordinary astrocytomas having been classified as grade 1 by the Kernohan system were designated as grade 2 by our system. Only 20.2% of agreement was found among ordinary astrocytomas of grade 3; 114 of 159 cases (71.5%) originally graded 3 with the Kernohan system were graded 4 by our method. Of 40 tumors originally considered grade 2, 12 (30%) were upgraded to 3 by our system.

Discussion

The aim of histologic grading is less an effort to predict survival in individual patients than to define, in conjunction with other parameters, a homogeneous group of patients. The development of a simple and reproducible grading method is of paramount importance for planning therapy as well as for the interpretation of results of various therapeutic regimens. The current study demonstrates that grading can establish categories which are statistically related to survival. Applying rigorous statistical methods, we found that grade was the major prognostic factor in ordinary astrocytoma cell types, superceding the effects of age, sex, and location.

Historical Considerations

In 1948, following the introduction by Broders of his grading system for epithelial tumors,[14] Kernohan introduced a system of grading for gliomas.[1] Adhering to the concept that gliomas may arise from preexisting adult cell types still capable of proliferation by a process of anaplastic transformation or "dedifferentiation," Kernohan simplified the histogenetic classification of Bailey and Cushing.[15] He rejected the designations astroblastoma, glioblastoma multiforme and polar spongioblastoma (the latter being subsequently referred to as the pilocytic and microcystic cerebellar forms of astrocytoma), and grouped these variants, together with fibrillary, gemistocytic and protoplasmic astrocytomas into the single category of astrocytoma of grades one through four.

The validity of the Kernohan grading system for astrocytomas has been questioned ever since its introduction.[16] In addition to the criticisms leveled against its "lumping" of histologically distinct astrocytic tumor types, several authors, as well as the current study, have failed to demonstrate a significant difference in survival between astrocytomas of Kernohan grades 1 and 2,[9,16-19] and of Kernohan grades 3 and 4.[9,20,21]

The grading scheme of Kernohan also has been found difficult to apply and its reproducibility has been low.[22] In fact, it is likely that the Kernohan method of grading is rarely implemented as originally intended, wherein grade is determined according to the degree of presence of multiple features, *i.e.*, anaplasia, cellular and nuclear pleomorphism, hyperchromasia, vascularity, cellularity, necrosis, endothelial proliferation, and mitotic rate and abnormalities, as well as tumor delimitation.

Alternative grading systems and classifications: A three-step grading system was proposed by Ringertz (1950),[16] which divided the astrocytic neoplasms into the astrocy-

TABLE 5. Histologic Criteria of Three-Tier Grading Schemes or Classification of Astrocytic Tumors*

	Astrocytoma	Anaplastic astrocytoma	Glioblastoma multiforme
WHO classification (modified from Zulch[10])	Tumor composed primarily of astrocytes (fibrillary, protoplasmic, gemistocytic, giant cell and combinations thereof)	Astrocytoma showing areas of anaplastic transformation; the latter may be "difficult to distinguish from glioblastoma"	Anaplastic glial tumor with high cellularity and necrosis with pseudopalisading; little astrocytoma may be still recognizable; less commonly, oligodendroglioma or ependymona may be the underlying lesion
Ringertz' classification (modified from Ringertz[16])	Tumor showing infiltrative growth pattern and moderate hypercellularity; cytologic features resembling normal astrocytes with only mild nuclear abnormalities	Cellular infiltrative astrocytic tumor containing astrocytes with moderate pleomorphism; mitoses and moderate vascular proliferation may be seen but necrosis is absent	Markedly pleomorphic astrocytic tumor with high cellularity, frequent mitoses, increased vascularity, and necrosis; often show little infiltration
Rubinstein classification (modified from Rubenstein[3])	Astrocytic tumor with mild increase in cellular density, mild nuclear enlargement, mild hyperchromasia and scant mitotic figures; no endothelial proliferation or necrosis	Also termed "malignant astrocytoma," this tumor shows increased cellularity, nuclear irregularities, hyperchromasia, and occasional giant cell formation; mitotic figures and vascular endothelial proliferation are seen	Highly cellular tumor with nuclear pleomorphism and hyperchromasia, many mitotic figures (often atypical), endothelial proliferation, and necrosis; cellular polymorphism is usual with anaplastic foci and differentiated astrocytic components both being evident
Nelson's classification (modified from Nelson *et al.*[5])	Uniform cells closely resembling mature, resting or reactive, nonneoplastic astrocytes; moderate cell density; mitoses absent or very rare	Multifocal or diffuse tumor with cellular and/or nuclear pleomorphism; increased cell density and frequency of mitotic figures; vascular prominence but no tumor necrosis	Features of anaplastic astrocytomas plus one or more foci of coagulation necrosis involving neoplastic astrocytes
Burger's classification (modified from Burger[21])	Mildly hypercellular astrocytic tumor with pleomorphism but no vascular proliferation or necrosis	Also termed "astrocytoma with atypical or anaplastic features," this tumor shows moderate hypercellularity and pleomorphism; vascular proliferation is permitted but no necrosis is seen	Moderate to marked hypercellularity and pleomorphism; necrosis with or without pseudopalisading is required but vascular proliferation is optional

WHO: World Health Organization.

* In the WHO classification, glioblastoma multiforme is not included in the astrocytic tumor group.

toma, intermediate tumor and glioblastoma multiforme.[16] Recently, Nelson *et al.*[5] and Burger and Vogel[4] have also proposed three-tier grading schemes for astrocytomas; the various criteria of these methods are detailed in Table 5.

Skepticism has been expressed concerning the efficacy of grading astrocytomas. The objections are most often based on the following: (1) available sampling size, *e.g.*, tumors may display different degrees of histologic malignancy in different areas[2,3,10,23,24]; (2) progressive evolution, over a period of months or years, of an originally benign neoplasm to a more malignant one[2,3]; and (3) grading alone fails to take into account the topography of a tumor, a factor which has been found to affect clinical behavior rather consistently.[3]

On the basis of these criticisms, Russell and Rubinstein[2,3] as well as the WHO[10] have rejected the principle of assigning a numerical grade of malignancy to a given cytologic type of glioma. Instead the WHO classification attributes a grade to each of the histologic subtypes of glioma. Thus, astrocytomas are considered as grade 2, anaplastic astrocytomas as grade 3, and glioblastomas as grade 4. Interestingly, when all is said and done, this classification as well as any which distinguishes these three categories, *de facto,* utilizes a scheme based upon "increasing grade of malignancy."

Special Astrocytoma Variants

Since the validity and reproducibility of any grading system depends on homogeneity of the lesions under con-

sideration, it is of no surprise that a strict definition of histologic subtypes of astrocytomas is a major element determining the efficiency of our proposed method.

The behavioral characteristics of pilocytic and cerebellar-type astrocytomas prompted their consideration as separate entities.[2,3,10] Criteria of malignancy in ordinary astrocytomas, *e.g.*, nuclear atypia and endothelial proliferation, have not been found to have a sinister implication in these lesions.[2,3] The application of our grading system to these tumors showed no correlation between grade and survival. The number of cases in these two categories, however, may have been too small to permit the detection of meaningful differences in survival. In addition to their distinctive natural history as compared to ordinary astrocytomas, pilocytic and cerebellar-type astrocytomas occur primarily in younger patients and arise at characteristic sites. Therefore, as demonstrated in this study, their inclusion with ordinary astrocytoma cell types introduces a bias which not only dramatically modifies the expected survival in low-grade gliomas but also influences other variables related to survival, such as age and tumor location. Indeed, in studies of low-grade astrocytomas which include pilocytic and microcystic cerebellar-type variants,[18,19,25] patient age at diagnosis has been found to have an overwhelming association with outcome, eclipsing all other variables, including the effects of all forms of management.

The extent to which indiscriminant "lumping" of histologic subtypes complicates the evaluation of therapeutic modalities in low-grade astrocytoma series is also illustrated by publications showing adjuvant radiotherapy to be either of benefit[18,19] or to be ineffective.[22] Similar observations have been reported regarding the efficacy of radical surgical resection, some series showing prolonged survival[17,18,25] and others demonstrating no effect.[9]

Issue of Sampling

It has been stated that grading must not be based on a limited biopsy, but rather on a thorough evaluation of tissue obtained at autopsy.[3] Without doubt, the effectiveness of histologic grading of astrocytomas is dependent upon obtaining an adequate sample. We agree that pathologists should be cautious in grading a small biopsy, except in cases wherein the criteria of malignancy present readily permit a grade 4 designation. However, nowadays computerized tomography (CT) scan images resemble anatomical brain sections[26] and provide the surgeon with a better conceptualization of a tumor. As a result, adequate sampling can more readily be obtained. Improved biopsy techniques, such as CT-based serial stereotactic biopsies[27,28] can provide samples from differing CT attenuation areas which are representative and lend themselves readily to grading.[29–31]

Proposed Grading Method

Criteria

The four criteria of malignancy, nuclear atypia, mitoses, necrosis and endothelial proliferation, were selected because they can, with little subjectivity, be easily recognized and stated to be either present or absent. Other morphologic criteria often associated with malignancy were rejected because their assessment required subjectivity and since they appear to be covariable with other more objective parameters. For example, in ordinary astrocytomas, it has been our experience that increased cellular density is usually associated with the presence of mitoses. Structuring a grading scheme according to the presence or absence of specific histologic features seemed to us to be justified in that, in our experience, increasing degree of atypicality of a given parameter often coincides with the appearance of additional parameters, *e.g.*, marked nuclear atypia usually coincides with the presence of mitoses and numerous mitoses are usually associated with the presence of endothelial proliferation or necrosis.

We chose to determine grade based upon a cumulative score rather than to ascribe specific criteria to each grade, since a score precludes the making of arbitrary choices in instances wherein a tumor fails to readily enter a predefined category. It is of interest that, in ordinary astrocytomas, the four criteria of malignancy tend to make their appearance in a predictable order corresponding to increasing degree of malignancy. The observation is of practical significance, *e.g.*, astrocytomas with endothelial proliferation or necrosis but without mitosis should be reexamined in order to exclude confusion with pilocytic or microcystic cerebellar astrocytomas which often show complex vascularity.

Prognostic Relevance of Criteria

Several recent studies[5,21,32,33] have been aimed at testing the association of specific histologic features with tumor behavior. Unfortunately, the approach was, in most instances, impaired by the fact that the significance of the factors under consideration was tested within predetermined categories of malignancy, *i.e.*, either in glioblastoma multiforme,[32] in glioblastoma multiforme and anaplastic astrocytoma,[21] or in anaplastic astrocytomas.[5–33] This approach introduces an obvious bias since the presence, absence or degree of a given histologic feature is related to case selection and does not reflect its effect upon the biologic features of the neoplasms. The results are contradictory observations. For example, in some studies, necrosis was found to be highly correlated to survival,[5] the most significant criteria distinguishing glioblastomas from anaplastic astrocytoma,[5–21] whereas in others, necrosis was not correlated with survival in either malignant

astrocytomas[21] or glioblastoma multiforme.[32] Similarly, the presence of endothelial proliferation has been reported to correlate with a shorter survival in anaplastic astrocytomas[21,33] but to be of no prognostic significance in glioblastoma multiforme.[32] Contradictory results also have been reported with respect to mitotic activity. By some, the presence of mitoses was not found to correlate with survival in glioblastoma multiforme[32] whereas others found this to be either correlated[33] or unassociated[21] with shorter survival in anaplastic astrocytomas.

Comparison of Available Grading Schemes

Ordinary astrocytomas that we considered grade 1 are no doubt considered as astrocytomas in the three-step classifications. When all histologic subtypes were considered together, most grade 1 tumors in this study were pilocytic or microcystic cerebellar-type astrocytomas. Nonetheless, grade 1 ordinary astrocytomas do occur (Fig. 1). In view of their rarity, such tumors must be carefully examined in order to avoid a misdiagnosis of gliosis or to exclude pilocytic or microcystic cerebellar-type astrocytoma, *i.e.*, tumors with little or no tendency to "dedifferentiate" with time.

Our ordinary astrocytomas of grade 2 which roughly correspond to grade 1 and 2 tumors of Kernohan (Fig. 16) are similar to the "astrocytomas" of Nelson *et al.*[5] who defined them as having no or very rare mitoses. Tumors graded 2 according to our system should be considered "astrocytomas" in other three-tier classifications. However, our grade 2 tumors are likely to be associated with a better survival than are "astrocytomas" as defined in the WHO[10] and Rubinstein[3] classifications or by the Burger grading method,[4,21] because these schemes include, among "astrocytomas," tumors with mitotic activity.

One of the major advantages of our grading system is that it clearly distinguishes grade 3 astrocytomas from grade 4 tumors. Currently, great confusion exists regarding the significance of such terms as "malignant astrocytoma," "anaplastic astrocytoma," and "glioblastoma," which according to various classifications are either distinct[10] or synonymous[1] lesions.

Our grade 3 designation, which in almost all cases selects tumors with only nuclear abnormalities and mitoses, is neither similar to the WHO's anaplastic astrocytomas nor to malignant astrocytomas according to Rubinstein,[3] both of which include tumors showing necrosis and endothelial proliferation. Our grade 3 astrocytoma is, however, roughly similar to the "anaplastic astrocytoma" of Nelson *et al.*[5] and Burger *et al.*[4,21] in that both use necrosis as the key criterion distinguishing glioblastoma from anaplastic astrocytoma.

Our grade 4 tumors are similar to the grade 3 and 4 astrocytomas of Kernohan (Fig. 16) and also to glioblastoma as described in the WHO[10] and Rubinstein[3] classifications. The mean survival in our grade 4 group (8.4 months) was similar to that of the patients with glioblastoma studied by Burger *et al.*[4] (10 months, as deduced from survival curves) and by Nelson *et al.*[5] (8 months).

Interobserver Reproducibility

Overall reproducibility, as tested by two pathologists (B.W.S., C.D.D.) independently evaluating each ordinary astrocytoma in a double-blind fashion, was shown to be 92%. This high degree of concordance resulted in part from the high proportion (86%) of grade 3 and 4 tumors in this study. Reproducibility in low-grade tumors (81%) was somewhat lower. The higher degree of reproducibility, found in grade 4 tumors (97%), was attributed to (1) the marked degree with which atypicality was present in these tumors thus rendering morphologic features easy to detect, and (2) the definition of grade 4 astrocytomas as lesions having either 3 or 4 criteria. The latter served to minimize the influence of interobserver discrepancies in recognition of necrosis or of endothelial proliferation, factors associated with 6% and 3.5% frequency discrepancy respectively.

The lowest percentage of interobserver agreement was noted in grade 2 tumors (80.4%) (Table 4) and was mainly due to discordance in identifying mitoses in instances wherein they were rare. No discordance was found in identifying mitoses in higher grades wherein they were numerous.

The percentage of agreement in identification of nuclear atypia was surprisingly high since this criterion might be considered to be the most subjective of the four criteria studied. In ordinary astrocytomas, the relative abundance of cells showing nuclear abnormalities makes it unlikely that the presence of nuclear atypia would go unrecognized.

Reproducibility of the proposed grading system cannot be compared with other systems in that, to our knowledge, reproducibility studies of other grading systems of astrocytomas have not been published. Our satisfactory reproducibility rates are attributed to the selection of easily identified morphologic criteria and to their simple recognition as being either present or absent. The use of such a binary system minimizes subjectivity in grading. It should be pointed out, however, that accurate application of our grading scheme rests upon adherence to strict histologic definitions of the four histologic features as defined above in "Materials and Methods."

In conclusion, we think that the simple objective and reproducible method of tumor grading described, designed for application to the ordinarily occurring forms of astrocytoma, will permit reliable comparison of clinical and therapeutic data emanating from various centers.

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