PRACTICAL NEUROPATHOLOGY OF GLIOMAS

Peter C. Burger, M.D.

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I. INTRODUCTION

As composed of the astrocytic and oligodendroglial neoplasms, infiltrating gliomas make up the vast majority of primary tumors of the cerebral parenchyma of adults. The fibrillary or "diffuse" astrocytomas and the oligodendrogliomas share a number of common features. These include an infiltrating nature which generally precludes total surgical resection, a continuum of "malignancy" that does not spontaneously divide itself into "benign" and "malignant" categories, a propensity for tumor progression so that the histologic grade of a recurrent neoplasm tends to be higher at the time of recurrence than at the time of the initial biopsy, and an association between the histologic grade of the neoplasm and certain clinical variables such age and degree of neurological deficit. Although there are a number of conceptual objections to grading, and there are also practical limitations as is discussed below, the variability in biologic behavior of these gliomas requires some system of grading for the purpose of treatment and prognostication.

II. GRADING OF GLIOMAS

A. Fibrillary or "Diffuse" Astrocytic Neoplasms

The first widely used classification in this country was that of Bailey and Cushing who emphasized the similarity of neoplasms to cells in phases of embryologic development (1). Accordingly, it utilized a three-tiered system composed of an astrocytoma, an astroblastoma, and a spongioblastoma multiforme. Although the overtly malignant cells in the glioblastoma multiforme in a sense recapture the ability of embryonic cells to proliferate and migrate, the classification had serious limitations, largely because of the use of the astroblastoma as an intermediate lesion. This rare

neoplasm has a very distinctive morphology and is not a morphologic bridge between the astrocytoma and the glioblastoma multiforme.

In 1949, Kernohan and his associates presented their classification, and the results when it was applied to astrocytomas at the Mayo Clinic (13,14). It was based on a four-tiered system originally proposed by Broders for squamous cell carcinoma of the lip. Accordingly, the astrocytomas were divided into four grades depending on the presence or extent of certain histologic variables. As in the Broders' classification, an important intrinsic component was the percentage of "normal-appearing" neoplastic astrocytes. The well differentiated lesion had a high percentage of neoplastic astrocytes that simulated the normal cell, whereas only a few of these elements were present in the more malignant lesions. This concept is in direct opposition to the present practice of grading neoplasms on the basis of their most malignant areas without considering the numbers of well differentiated elements. In addition, the pilocytic astrocytomas of childhood including the cerebellar astrocytoma were included in the grade I astrocytoma. Subsequent experience has made it clear that the pilocytic astrocytomas of the cerebellum, optic nerve, and hypothalamus are distinctive entities not to be confused with the infiltrating fibrillary or "diffuse" astrocytomas.

In spite of its limitations, the Kernohan system has been widely embraced by general pathologists familiar with the four-tiered system already in wide use in general pathology. There was, and is, considerable variation in the way this system is applied by individual pathologists, and there is little assurance that a grade II lesion of one pathologist is similar to a grade II lesion of another. The Kernohan grades III and IV represent lesions which today would be diagnosed by most neuropathologists as a glioblastoma multiforme, but there is little agreement what histologic features separate grades III from IV.

With the caveat that pilocytic astrocytomas may have been included and skewed the results, the Kernohan system as applied to material from the Mayo Clinic produced

results which are at the heart of clinical and biological features of the astrocytomas. Thus, with increasing histologic grade, there was an increase in the mean age of the patient, decrease in the duration of preoperative symptoms, and a diminution in the mean postoperative survival (13,14). Extremely close interrelationships between histologic features, clinical presentation, and length of survival have been noted in many subsequent studies of these neoplasms and must have a most fundamental significance about the origin and evolution of these gliomas.

One year after the appearance of the Kernohan system, a three-tiered system was proposed by Ringertz (21) who suggested that these neoplasms were best divided into three, rather than four, grades. The three were the astrocytoma, the malignant or atypical astrocytoma, and the glioblastoma multiforme. Such a system has become increasingly popular and is used presently by three large cooperative study groups, the Radiation Therapy Oncology Group (RTOG), the Brain Tumor Cooperative Group (BTCG), and the Eastern Cooperative Oncology Group (ECOG) (5,9,20). As defined by these groups, the glioblastoma is a histologically anaplastic neoplasm with necrosis, and often vascular proliferation. The anaplastic astrocytoma is usually less undifferentiated than the glioblastoma and, importantly, lacks necrosis. The well differentiated lesion is composed generally of cells with more astrocytic features and lacks necrosis and vascular proliferation. Survival statistics for patients with the glioblastoma as defined by these three Cooperative Groups are exceedingly close, and each Group has demonstrated close correlations between histologic grade, age, and duration of preoperative symptoms.

A similar three-tiered system is advocated also by the World Health Organization (WHO) classification (25). This system is not widely recognized by neurosurgeons in this country but is in Europe where it is employed almost religiously for therapeutic decisions, i.e. radiotherapy. In the WHO system the astrocytic neoplasms are assigned to one of four grades. Grade I is given to the pilocytic neoplasms, grade II is reserved

for the better differentiated fibrillary or "diffuse" astrocytoma, and grade III for the lesion referred to in the Ringertz system as the malignant astrocytoma. Somewhat confusingly, the glioblastoma multiforme is placed in a category of undifferentiated or embryonal neoplasms. It, along with the medulloblastoma, is not considered an astrocytic tumor and is assigned grade IV.

A four-tiered system in wide use is that of the European Brain Tumor Cooperative Group (EORTC) (4). Although it is stated to be based on the WHO system, it is difficult to compare with this or any of the aforementioned systems since it incorporates the localization of the lesion and the concept of "secondary" glioblastomas as described by Scherer (22). By this system grade I is a focal well differentiated lesion, whereas grade II has similar morphology but is more diffuse. How one can disitnguish these two grades in a needle biopsy specimen is not clear. Grade III lesions are similar histologically to grade II but with foci of anaplastia or "dedifferentiation". These are equivalent to Scherer's "secondary" glioblastomas in which glioblastomas appear to evolve out of a better differentiated precursor astrocytic neoplasm. Grade IV neoplasms, the glioblastoma multiforme, are those in which only overtly anaplastic cells are noted.

Two additional classifications of considerable interest have recently been described. One, as described by Davis et al (personal communications) from the Brain Tumor Research Center in San Francisco, recognizes a four grade system of astrocytic neoplasms: the moderately anaplastic astrocytoma, the highly anaplastic astrocytoma, the gemistocytic astrocytoma, and the glioblastoma multiforme. Although the precise criteria for these four groups remain to be described, it would appear that the highly anaplastic astrocytoma and gemistocytic astrocytomas are approximately the same as both the grade III of the World Health Organization and as the anaplastic astrocytoma of the RTOG and BTCG.

The second recent classification is that formulated by Dumas-Duport and applied recently to material from the Mayo Clinic (11). This system establishes four grades

based on the presence or absence of four variables: nuclear atypia, mitoses, vascular proliferation, and necrosis. Grade I is assigned if any one of these variables is present; grade II if two are present, etc. In concept, this sytem gives equal weight to all histological variables and differs from other grading systems that traditionally assign more weight to certain variables such as necrosis or endothelial proliferation. In this system only a rare neoplasm is classified as grade I, and the grades II, III, and IV are very similar in biological behavior to the astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme of the RTOG, ECOG, and BTCG.

All the above published grading systems show clear-cut separation in overall survival between patients in the different grades. Thus, each classification provides a prognostically usable subclassification. For the purpose of comparisons of therapies between clinical trials run by different organizations, a comparison of these grading systems is obviously important. In this report I have compared these by reference to published survival curves. Since it is sometimes difficult to extrapolate precisely from small published graphs on different scales, the following data should be viewed in that regard and only as preliminary until these survival studies can be compared more rigorously.

Not surprisingly, the survival curves for the grade IV lesions or the glioblastomas of the EORTC, RTOG, BTCG, and Mayo Clinic are exceedingly close since these represent the most malignant lesions. There are, however, considerable difference between the survivals of patients with the grade III lesions, or anaplastic astrocytomas and highly anaplastic astrocytomas. Thus, the two-year survival rate for patients with the UCSF highly anaplastic astrocytoma and the RTOG astrocytoma is approximately 70% whereas the Mayo Clinic grade III and the Brain Tumor Cooperative Group anaplastic astrocytoma is about 50%. The EORTC grade III lesion is only 35%. Some of these differences in survival are understandable in light of the classification system, whereas other differences are not readily explainable. For example, the relatively poor

survival of the EORTC grade III lesions is obviously a consequence of the grading system by which neoplasms that would be considered glioblastoma multiformes by other systems are included. The difference of survival between the Brain Tumor Cooperative Group anaplastic astrocytoma and the RTOG astrocytoma is difficult to explain and may suggest that the RTOG pathologists include lesions that are somewhat less biologically malignant in the category of anaplastic astrocytoma. An extremely important and often unspecified variable is the age of the patient. This has an extremely high prognostic significance in some studies, even more than that of histologic features (10). Thus, without knowledge of some details of the ages of the patients under study, comparisons of survival curves between different groups can be difficult if not impossible.

B. Oligodendrogliomas

Until the last several years there was considerable skepticism that grading of oligodendroglial neoplasms had any prognostic utility. However, there are several recent studies suggesting that the histologic features used to grade the astrocytic neoplasms can also be applied fruitfully to oligodendrogliomas.

In the study by Smith et al. a four-tiered grading system was created, and significant differences were seen between neoplasms grades I and II and those that were grade III or IV. (16,24) Interestingly, only one histologic features, nuclear pleomorphism, was individually significant. In a detailed study from Norway, Mørk et al. studied the relationship between survival and multiple histologic factors, and identified four of the latter that had prognostic utility (15,17). These were necrosis, cellularity, vascular proliferation, and microcystic change. The latter feature was associated with a longer survival prognosis, the former that with a shorter. Our own studies compliment the two previous investigations by suggesting that necrosis, vascular proliferation, mitoses, and cytologic atypia were all significantly related to the duration of postoperative survival (8).

It remains to be seen how the above histological factors will be formulated grading systems, and how the survival curves generated will appear when neoplasms are so classified. In the interim, it is our own feeling that the oligodendrogliomas should be divided into the well differentiated oligodendroglioma and the anaplastic oligodendroglioma. The latter diagnosis is appropriate when high cellularity, mitoses, and vascular proliferation are noted. The glioblastoma multiforme would complete a three-tiered system.

It is of interest that as oligodendroglial neoplasms become more malignant they develop cells which have strongly GFAP-positive cytoplasm (8). These cells usually have nuclear cytologic features of oligodendroglial rather than astrocytes, however, it has been suggested that as the oligodendroglioma become more malignant it approaches the glioblastoma multiforme in histologic appearance because of an overgrowth of cells with astrocytic features. It is not clear what percentage of oligodendroglial neoplasms develop into an unequivocal glioblastoma multiforme. In our experience, oligodendroglial neoplasms retain a distinctive oligodendroglial character and generally do not merge in morphology with the classic glioblastoma which we consider an astrocytic neoplasm.

III. NEWER METHODS FOR GRADING OF GLIOMAS

Given the subjectivity of the classic histologic grading, there have been attempts to develop more objective means for assessing a neoplasm's proliferative potential. Two recent methods hold considerable promise, more are undoubtedly to be developed. The first is the immunohistochemical detection of the compound bromodeoxyuridine (BdUr) incorporated into dividing cells in a fashion similar to that of triated thymidine as used in classic autoradiographic studies (12). In such preparations for BdUr, the nuclei of cells in the DNA synthesis, or S, phase show brown reaction product. The number of these cells as well as the unstained cells can be determined to provide a labeling index.

Traditionally, this method has required the intravenous administration of the BdUr at or shortly before surgery, but it is also possible on small pieces of tissue removed from the tumor and immersed in the BdUr solution prior to fixation. The limited penetration of this pyrimidine into the tissue may be a limiting factor for this in vito method. Another method, which we favor, also uses immunohistochemistry but detects a nuclear antigen in cells in all phases of the cell cycle except Go. This antibody, Ki-67 cannot be applied to paraffin-embedded tissue and is, unfortunately, not applicable to archival material. With this method, fresh frozen neoplasms are stained with the immunoperoxidase method and produce a preparation in which the nuclei of cycling cells are stained.

There has been considerable more experience with BdUr than Ki-67 in the study of tumors of the brain and meninges, and this has generally shown a good correlation between the labeling index, the biological behavior, and the classic histological features. Studies for Ki-67 are more limited, but the labeling indices of neoplasms are in accord with their histologic grades (7). There seems little reason to doubt that this rapid, relatively simple method will become widely used. Detailed comparisons of BdUr and Ki-67 are in progress. The utility of these two methods, and the additional antibodies undoubtedly to be developed, would appear to be most suited for lesions of grades lower than that of the glioblastoma multiforme. They theoretically provide a way to predict which low grade astrocytomas are truly low grade and which have increased proliferative potential and will, or already have, become anaplastic.

It seems likely that help in the classification and grading of gliomas will also come from techniques of molecular biology. A recent study of malignant gliomas in regard to the amplification of the c-erb B oncogene which codes for the epidermal growth factor receptor suggests that approximately half of glioblastomas are so amplified (2,3). Interestingly, most of the better differentiated lesions were unamplified except for several tumors classified as an anaplastic astrocytoma. These latter lesions may have been poorly sampled glioblastomas, and that studies for oncogene amplification could

have prognostic utility in such situations, particularly in needle biopsy specimens where the amount of tissue is small and potentially not representative of the area that is histologically most malignant. In addition, it was of interest that the malignant oligodendroglial neoplasms were negative for amplification suggesting biological differences between the oligodendroglial and astrocytic neoplasms.

IV. LIMITATIONS OF GRADING

Although the grading of neoplasms is necessary for purposes of prognosis and therapy, it has a number of limitations. In the first place, as is obvious from the variety of survival curves produced by the different grading systems, that are no absolute criteria to define grades. That is, grading is entirely artificial. In addition, grading cannot account for the frequent transition of lesions to higher grades that have been best studied for both the astrocytic and the oligodendroglial neoplasms by Müller et al (18,19). In the case of the astrocytomas, comparison of the tumors at first and at second operations showed increase of grade in at least half of the astrocytic tumors. One should also be hesitant to take grading at face value is the powerful effect of clinical variables on the course of the patient. These factors include the age of the patient, the extent of neurological deficit at the time of presentation, and the length of preoperative symptoms (6,9,10,23). Although not as clearly related to the survival is the location of the lesion and extent of resection. It is the age of the patient that has the strongest effect of the clinical variables and in at least one study was shown to be more predictive of outcome than the histological grade. Thus, a neoplasm's histological grade is just one of a number of factors which must be used to predict the course of the patient's disease or determine the most appropriate therapy.

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