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Pathology of malignant gliomas: Challenges of everyday practice and the WHO 2007

Malign gliomların patolojik incelenmesi: Tanıda pratik sorunlar ve Dünya Sağlık Örgütü 2007 sınıflaması

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ABSTRACT

The recent revision of the "*2007 World Health Organization Classification of Tumours of the Central Nervous System*" introduces a series of new entities and variants in addition to bringing more clarity to existing ones. It is critical for the practicing surgical pathologist to be aware of these changes, especially those relating to common primary tumors such as malignant gliomas. This study presents a critical review of the changes and attempts to provide practical insights for the surgical pathologist.

The morphological spectrum of malignant gliomas is quite diverse, and with definition of newer variants and patterns, there is an increasing need to be more specific on the type and the grade of these aggressive neoplasms. The added value of special stains, immunohistochemistry and molecular/genetic analysis is expected to gradually increase in everyday practice. Thus, the surgical pathologist must be in tune with the progress in these fields and must be in a position to apply and interpret these techniques.

Appropriate management of the patients and the correct interpretation of the disease always depend on effective, direct communication of the neuropathologist, neuroradiologist and the neurosurgeon, coupled with the application of carefully chosen ancillary techniques. The combination of collaborative efforts and special techniques is certain to be more critical in the future, and effective utilization of all these elements will better characterize these neoplasms and improve patient management.

Key words: Malignant glioma, glioblastoma, oligodendroglioma, oligoastrocytoma, ependymoma

ÖZET

"*2007 Dünya Sağlık Örgütü Merkezi Sinir Sistemi Tümörleri Sınıflaması*" (WHO 2007) uğraşıları daha önceki sınıflamalarda bulunmayan histolojik tür *(entity)* ve alt türlerin *(varyant)* tanımlanmasını ve var olan histolojik türlerin özelliklerinin açıklık kazanmasını sağlamıştır. Cerrahi patoloji uzmanlarının günlük çalışmalarını doğrudan etkileyeceğine inandığımız bu değişikliklerin bilinmesi bizce büyük önem taşımaktadır. Bu nedenle, malign gliomlar ile ilgili olan WHO 2007 değişikliklerinin bir özetini bu yazıda derlemeye çalıştık.

Malign gliomların histopatolojik özellikleri çok çeşitlilik gösterir. Malign gliomların tiplendirme ve derecelendirmesinde yeni histolojik tür, alt tür ve biçimlerin *(pattern)* göz önüne alınması, ve yeni sınıflamaya uygun daha özgün bir tanıya ulaşılması gereklidir. Özel boyalar, immünohistokimya ve moleküler/genetik incelemeler günlük uygulamada giderek daha fazla değer kazanmaktadır. Bu durum, cerrahi patologların teknik gelişmeleri takip etmelerini ve bu gelişmeleri çalışma ortamlarına uyarlamalarını gerekli kılmaktadır.

Bir olgunun uygun biçimde ele alınması ve doğru yorumlanması her zaman patolog, radyolog ve cerrahın etkin ve dolaysız iletişimini gerektirir. Bunun yanısıra dikkatle seçilmiş olan yardımcı tanı yöntemlerinin doğru değerlendirilmesi de büyük önem taşır. Uzmanlar arası iletişimin ve özel tanı yöntemlerinin etkin bir biçimde kullanımı gittikçe daha fazla önem kazanacak ve tedavinin başarısını artıracaktır.

Anahtar sözcükler: Malign gliom, glioblastom, oligodendrogliom, oligoastrositom, ependimom

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GENERAL CONSIDERATIONS

The new "2007 World Health Organizati-

on Classification Tumors of the Central Nervous System" (WHO 2007) clarified and revised some of the highly diverse and diagnostically challenging tumor categories (1). Two clarifications effect the organization of the classification scheme. First, WHO 2007 provides a better definition of types and subtypes of brain tumors. All brain tumors have now been categorized as either *Entity, Variant, or Histological Pattern*. An *Entity* represents a unique form of a neoplastic disease with a defined clinicopathological spectrum and is given its own chapter in WHO 2007. *Variant* represents a significant subtype of an entity with sufficiently distinctive biological properties and/or clinical behavior. *Histological Pattern* represents a particular differentiation pattern or phenotype that does not have a different biological behavior or prognosis within a specific entity.

The second important clarification is an attempt to better define "Grading" philosophy of WHO 2007. The grading, occasionally detached from histological typing, reports a "stage of malignancy" or "biological behavior". The revised scheme defines grades as follows:

GRADE I=typically well-circumscribed, slowly progressing and may be cured by resection; GRADE II=partly or mostly infiltrative with low proliferation rates, but have a higher likelihood of recurrence compared to grade I tumors; GRADE III=histologically malignant and require aggressive adjuvant therapy; GRADE IV=highly aggressive and usually rapidly fatal with all the histological features of malignancy.

While the improvements in the current classification scheme are not revolutionary, they allow better definition and description of the primary central nervous system (CNS) tumors, and will significantly affect everyday surgical neuropathology practice.

THE SPECTRUM OF MALIGNANT GLIOMAS

The following review focuses on the practical and clinical aspects of malignant gliomas, all of which require multi-modality therapy, and are not likely to be cured by surgery alone. While "malignant glioma" does not constitute a specific pathological entity, the term identifies a group of aggressive tumors that require a multidisciplinary team approach.

Malignant gliomas include a range of neoplasms from astrocytoma to ependymoma, with rare anaplastic forms of circumscribed gliomas such as pleomorphic xanthoastrocytoma with anaplastic features (2). The overwhelming majority of malignant gliomas are WHO grade III (anaplastic) and IV (glioblastoma) astrocytomas. High grade oligodendrogliomas, oligoastrocytomas and ependymomas are less common tumors included in this general category.

Pathological features of malignant gliomas vary based on the tumor type (3). In addition to the typical architectural and cytological features, molecular and genetic distinctions are increasingly being made among types of malignant gliomas (4,5). It is well recognized that a significant number of infiltrating gliomas emerge as low grade tumors and eventually progress to high grade (6,7). An even larger group is malignant at diagnosis with only limited options for therapy and a short survival. The tumors in the latter group may have "low-grade" microscopic regions that are critical in the recognition of the type of malignant glioma. This large body of information will be briefly summarized for each tumor type with only limited references to the molecular alterations, since a detailed discussion is beyond the scope of this article.

ANAPLASTIC ASTROCYTOMA
(Infiltrating Astrocytoma WHO GRADE III-Fig. 1)

Intraoperatively, there may be little or no external abnormality, since most anaplastic astrocytomas are deep hemispheric lesions with only limited involvement of the cortex. Large

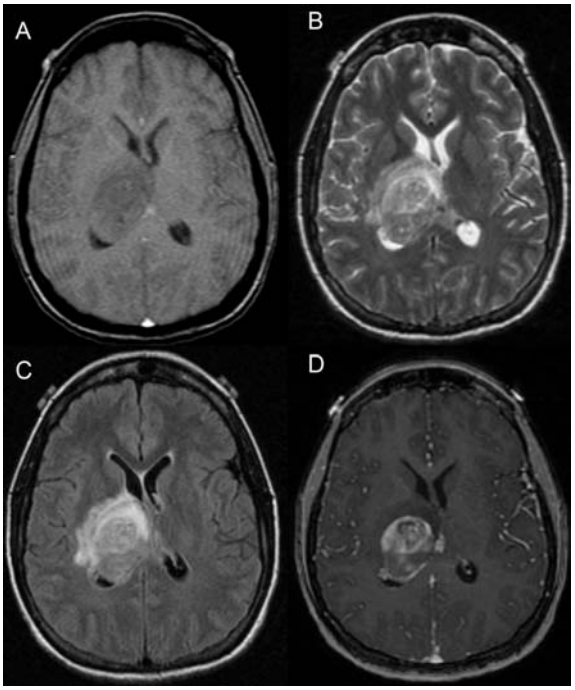

Fig. 1. Anaplastic astrocytoma, typical MRI appearance with limited mass effect and significant contrast enhancement. It is important to note that a significant percentage of anaplastic astrocytomas do not show contrast enhancement. A) axial T1-weighted image; B) axial T2-weighted image; C) axial FLAIR; D) axial contrast-enhanced T1-weighted image.

tumors may have a superficial component that expand the gyri or cause surface discoloration. In some examples, a striking demarcation can be observed on imaging studies. The infiltrative nature of a diffuse astrocytoma is most evident to the neurosurgeon, who usually finds its boundaries difficult or impossible to define intraoperatively. Some anaplastic astrocytomas in the brain stem produce enlargement of the pons without creating a discrete mass. Macroscopically, the anatomic details of the region are often lost, and the structures appear distorted or occasionally 'swollen' (Fig. 2a). Deep white matter tissue fragments from anaplastic astrocytomas are typically dusky, speckled gray with variable consistency from soft to almost gelatinous. Microcysts, which are often not visible, may give a spongy appearance to larger tissue fragments. Recognizing the extent of the tumor can be even more challenging in anaplastic astrocytomas of the spinal cord.

In recent years, successful use of the ultrasonic aspirator (CUSA) has made neuropathologists' task even more challenging, since the tissue obtained during these procedures are, at best, suboptimal for pathological typing or grading. In tissues obtained by CUSA, the macroscopic as well as microscopic inspection is often of little value. The gross evaluation post radiation treatment specimens is equally challenging. There is much more complexity to the texture and appearance of these specimens that prevent recognizing tumor tissue from reactive changes (8). One major caveat of CUSA material is the extensive "fried-egg" appearance of cells in tissue that can mislead to the diagnosis of oligodendroglioma.

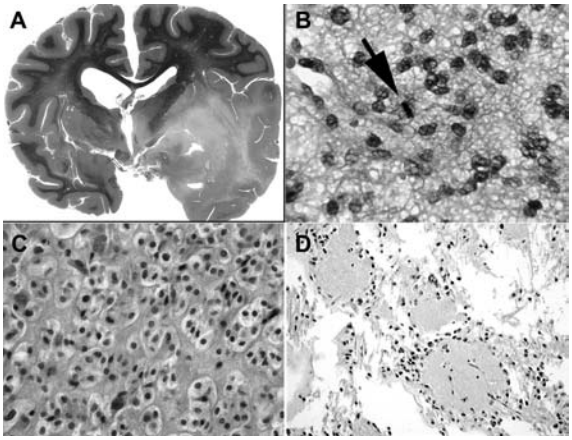

Fig. 2. A) Anaplastic astrocytoma, gross appearance of anaplastic astrocytoma that distorts and expands the midbrain; B) typical hypercellularity and scattered mitoses; C) granular cell pattern of anaplastic astrocytoma; D) glioneuronal tumor with neuropil-like islands (all images H&E original magnification x200).

Anaplastic astrocytomas often exhibit distinct hypercellularity with readily identifiable nuclear irregularities (9). The cell density is often a magnitude higher than the normal brain parenchyma, and a few fold of that seen in low grade infiltrating astrocytoma (Fig. 2b). Most anaplastic astrocytomas consist of cells with small amount of cytoplasm (except for gemistocytes), scant and short cell processes, and markedly hyperchromatic nuclei. Typically, numerous delicate processes in a glial cell are more indicative of reactive astrocyte. The morphological characteristics of the nuclei are critical in

diagnosis, yet any individual feature should be carefully interpreted, since there is considerable overlap between neoplastic and non-neoplastic processes in terms of nuclear size and shape.

A critical microscopic feature of anaplastic astrocytoma is the presence of mitotic figures (10,11). Finding even a single mitotic figure in a surgical specimen can be associated with a slightly poorer prognosis when compared to typical grade II diffuse astrocytomas (12). Nevertheless, presence of a single mitotic figure in a well-sampled tumor specimen (when most of the tumor is available for pathology) may not be sufficient to increase the tumor grade to anaplastic (13). However a cut-off value for mitoses has been elusive and there are different approaches. Our recommendation is to identify at least one mitotic figure for each large tissue fragment, and one can be confident about the diagnosis of anaplastic astrocytoma when a search readily identifies numerous mitotic figures.

Necrosis or vascular endothelial proliferation is not expected in anaplastic astrocytomas. It must be noted that focal ischemic type necrosis and linear type vascular proliferation can occur in an astrocytic neoplasm independent of its aggressiveness. Especially in the setting of prior adjuvant therapy or surgical procedures (e.g. stereotactic biopsy) such focal changes should be interpreted with caution.

Anaplastic astrocytomas vary in architectural and cytological composition, as to give the tumor a "variably variable" appearance. One common architectural element is a microcyst containing mucinous material, typical of low-grade astrocytomas (11). The presence of microcysts is not indicative of a particular tumor grade, even though earlier reports claim a prognostic significance to the microcystic change (14). Other uncommon architectural patterns include perineuronal satellitosis and cortical calcifications, which are more typical of oligodendrogliomas. Focal myxoid/mucinous change and cartilaginous metaplasia have also been reported in anaplastic astrocytomas, and production of cartilage had been related to the ability of tumor cells to secrete mucopolysaccharides (15).

One common cell type is the so-called gemistocytic cell that appears swollen with round to oval but distinctly eosinophilic cytoplasm, small number of truncated processes, and a hyperchromatic, eccentric nucleus. Gemistocytic cells can be present in any infiltrating astrocytoma, yet the tumors composed of >20% such cells have been classified under the 'gemistocytic astrocytoma' category (16). It is important to note that any given anaplastic astrocytoma may contain gemistocytes, and the 20% cut-off value currently accepted by WHO 2007 for "gemistocytic astrocytoma" is almost entirely arbitrary. Gemistocytic cells have a low proliferation index, while the small neoplastic astrocytes that invariably accompany them show higher proliferative capacity. Even though suggestion was made to implicate the gemistocytic morphology as a marker of poor prognosis, there is insufficient data to suggest that gemistocytic morphology is an independent prognostic indicator (3). Gemistocytic cells can be confused with the "minigemistocytes" commonly seen in a subgroup of oligodendrogliomas. The minigemistocytes of oligodendroglioma have significant overlap with the typical gemistocytes in astrocytomas, but are smaller with more concentric collection of intermediate filaments and strong GFAP staining. There is almost no definitive criterion to separate minigemistocytes of oligodendroglioma from astrocytic gemistocytes, and the diagnosis should be made by the overall histological features of the neoplasm.

A less common cytological variation is the neoplastic cells with a highly granular cytoplasm (Fig. 2c). Granular cells can be seen in many astrocytomas, but tumors that are predominantly composed of such cells are rare (17). Infiltrating astrocytomas with granular cell features (the so-called granular cell astrocytoma) can resemble macrophage infiltrate or a reactive

lesion (18).

"Glioneuronal tumor with neuropil-like islands" recently described by Teo et. al. (19), has been recognized as an aggressive glioneuronal tumor. Most of these tumors are supratentorial, but a case has been described in the spinal cord (20). These uncommon infiltrating tumors contain microscopically well defined, round to oval islands composed of a delicate, neuropil-like matrix with synaptophysin positivity. The neuropil-like islands are surrounded by oligodendrocyte-like cells in a rosetted fashion (Fig. 2d). These cells as well as more atypical cellular elements show immunoreactivity for neuronal antibodies. The glial component consists of irregular atypical cells and GFAP-positive fibrillary and gemistocytic elements (21). Currently the WHO 2007 recognizes this neoplasm as a histologic pattern within anaplastic astrocytoma. While this may not be the final designation for these uncommon neoplasms, their biological behavior is similar to infiltrating astrocytomas of comparable grade.

Smear preparations from anaplastic astrocytomas are often suggestive, if not diagnostic of an infiltrating astrocytic neoplasm. However, grading of infiltrating astrocytomas is not recommended during intraoperative consultations due to significant geographic variation in tumor composition, and the possibility of a higher grade area elsewhere not sampled for intraoperative analysis. Typically, frozen sections from infiltrating astrocytomas have substantial freezing artifact that prevent identification of mitotic figures or the extent of nuclear pleomorphism. Therefore, proper intraoperative smear preparations are vitally important in determining the cytological irregularities and mitotic figures. Intraoperative smears are also of great help in determining the 'background fibrillarity' indicative of incorporated neuropil, and the fibrillarity emanating from neoplastic cells that often implies an astrocytic phenotype. Florid reactive changes incited by inflammation or infectious agents such as the JC virus may cause significant atypia in astrocytic nuclei, and can easily be interpreted as astrocytoma. It is critical to have a fair understanding of the radiological and clinical features of the case, and discuss the findings with the neurosurgeon to avoid any misinterpretation.

Although the astrocytic processes are often evident on routine stains, the diagnosis is aided by identification of these processes on immunohistochemistry for glial fibrillary acidic protein (GFAP). GFAP staining should be considered a marker for glial phenotype and not as evidence of astrocytic differentiation. It should also be noted that some astrocytomas stain poorly with GFAP, and not all neoplastic cells in a given astrocytoma are GFAP positive. True gemistocytes are only weakly positive for GFAP, whereas the minigemistocytes of oligodendrogliomas show stronger staining. Neuronal stains are of little value in the characterization of anaplastic astrocytomas. The most important contribution is probably the immunohistochemical stains for neurofilament protein (NF) that can assist in the recognition of invaded neuropil and substantiate the degree of tissue infiltration. Both Vimentin (VIM) and S-100 protein stain most astrocytomas along with the incorporated cells and elements of neuropil. Astrocytic neoplasms, especially anaplastic astrocytomas have been reported to stain more avidly with antibodies against VIM in comparison to oligodendrogliomas, but a statistically significant difference could not be found (22). There is variable data on the intensity of VIM staining in different types and grades of gliomas (23-25). In practice, the greatest value of VIM is to recognize the suitability of paraffin tissue for immunohistochemistry. VIM should stain a significant number of normal CNS components, vessels, reactive as well as neoplastic glial cells. A completely negative VIM is unlikely to be accurate, and identifies tissue or the method as inappropriate for immunohistochemistry. Anaplastic astrocytomas can rarely stain for cytokeratins, which is often not a problem, but this may be challenging

in less differentiated glial neoplasms (26). As a surrogate marker for cell proliferation, Ki-67 (MIB-1 antibody) is widely used in clinical practice, although its impact on the final grading or prognostication is debatable. There are more studies than one could care to list on MIB-1 labeling of astrocytic neoplasms. The studies on paraffin samples are often confounded by the fact that the determination of a particular labeling index is highly method-dependent. Suffice it to say that MIB-1 staining that is readily perceivable on low power magnification is compatible with a high-grade astrocytoma, and is much more typical of anaplastic than grade II astrocytomas. The percentage of MIB-1 positive nuclei differs widely and can range from 2% to 10% or even higher for anaplastic astrocytoma.

A significant percentage of anaplastic astrocytomas, especially the gemistocytic variant is immunoreactive for p53 protein. Positive staining in large number of nuclei is typically indicative of a stabilizing mutation, even though most antibodies recognize both the wild type and mutant p53 protein. Since positive nuclei have been encountered in infectious as well as reactive conditions, rare scattered positivity should be interpreted cautiously. Furthermore, since a significant number of tumors are negative with this stain, absence of p53 staining does not exclude anaplastic astrocytoma.

GLIOBLASTOMA (Infiltrating Astrocytoma, WHO Grade IV-Fig. 3)

Glioblastoma (GBM) causes significant alterations in the brain parenchyma that are readily observed radiologically and macroscopically. Tumors expand the gyri, create texture and color alterations often familiar to the neurosurgeon. The tumor tissue is variable in color, ranging from grayish yellow suggestive of necrosis to dark brown hemorrhage or thrombosis. The intraoperative finding of thrombosed vessels is a well-recognized ominous sign cited by neurosurgeons. Despite the false impression of

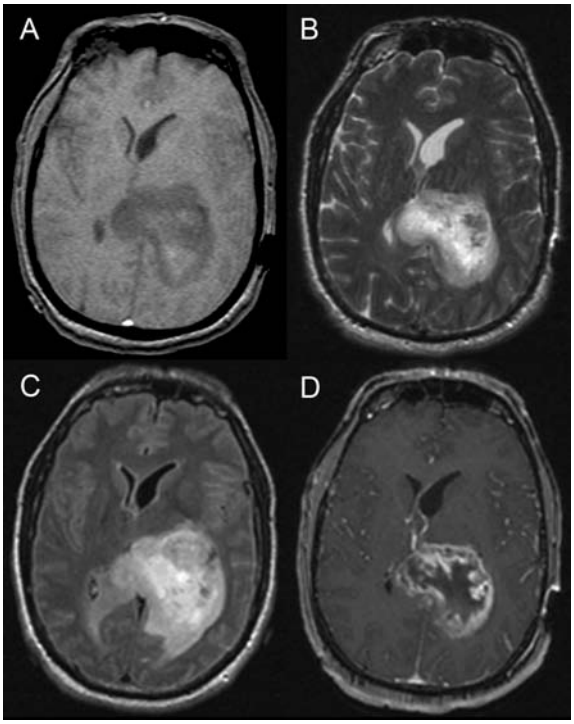

Fig. 3. Glioblastoma, typical MRI appearance: A) axial T1-weighted image; B) axial T2-weighted image; C) axial FLAIR; D) axial contrast-enhanced T1-weighted image.

circumscription intraoperatively, the tumor extends far beyond the visible abnormality. Normal appearing tissue submitted to pathology will harbor significant number of infiltrating astrocytes. GBM can extend to the contralateral hemisphere via the corpus callosum, a structure involved in many cases. Although most GBMs are not well circumscribed, demarcation is a feature of some giant cell GBMs and gliosarcomas. These two tumor subtypes can mimic metastatic carcinomas, and occasionally meningiomas radiologically and intraoperatively (27,28).

The cytological and architectural features of GBM are remarkably diverse, and there is virtually no limit to the variations one can describe within the spectrum of such neoplasms (Fig. 4). It is this diversity of phenotypic characteristics that implies GBM is not a single neoplastic entity, but the final, and most malignant culmination of glial neoplasms from diverse genetic and etiological origins. Typically, the fibrillarity and variable degree of cell processes are readily evident, confirming the glial nature of

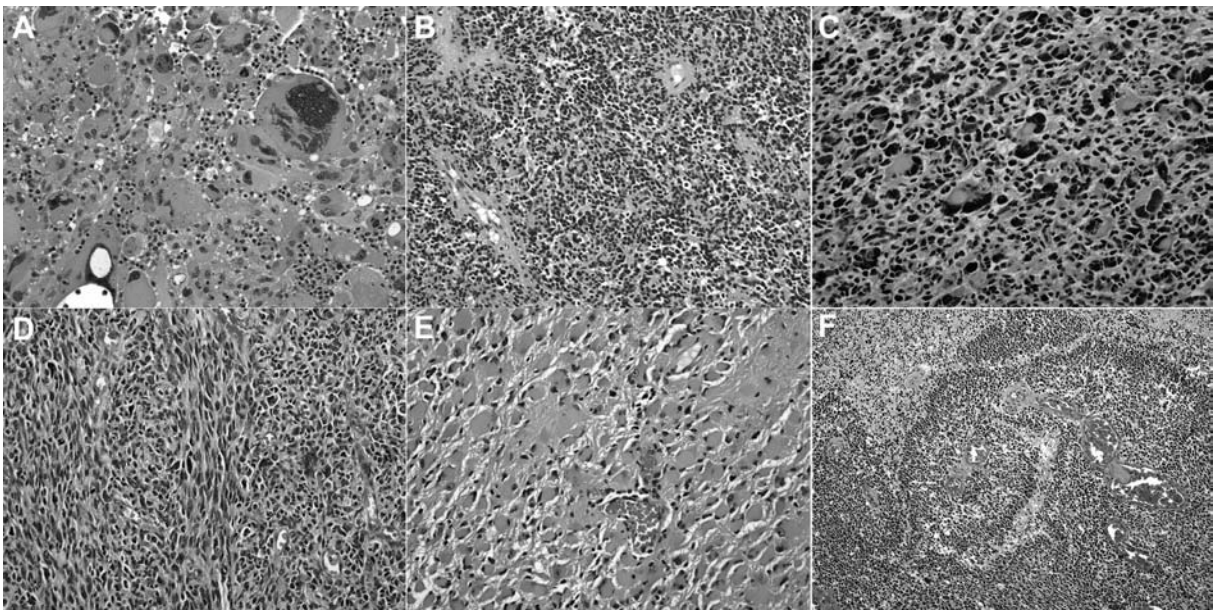

Fig. 4. Histological diversity of glioblastoma: patterns and variants A) giant cell ; B) small cell ; C) pleomorphic; D) sarcomatoid; E) gemistocytic F) PNET-like areas in otherwise typical glioblastomas (all images H&E original magnification x200).

GBMs. The tumor cells are markedly pleomorphic and hyperchromatic, and mitotic figures can be readily detected. The vascular endothelial proliferation in GBMs is polymorphous and includes not only endothelial cells but also smooth muscle cells and pericytes (Fig. 5a). A somewhat less well-defined term 'microvascular proliferation' has been used instead of vascular proliferation. An overwhelming majority of GBMs exhibit necrosis, and some may display a hypercellular ribbon of neoplastic astrocytes around the necrotic foci, referred as pseudopalisading necrosis. In some areas, necrosis is associated with a linear pattern of proliferating vascular structures. This linear pattern of vascular proliferation can also be observed around non-neoplastic cysts and is not a sign of aggressive biological behavior (Fig. 5b). The vascular proliferation and necrosis with pseudopalisading comprise the essential histological correlates that define GBM in addition to nuclear pleomorphism and mitotic figures (11).

The distinctive subtypes for GBMs include the giant cell GBM and the gliosarcoma, which are recognized by the WHO as GBM variants. The former is typically composed of ex-

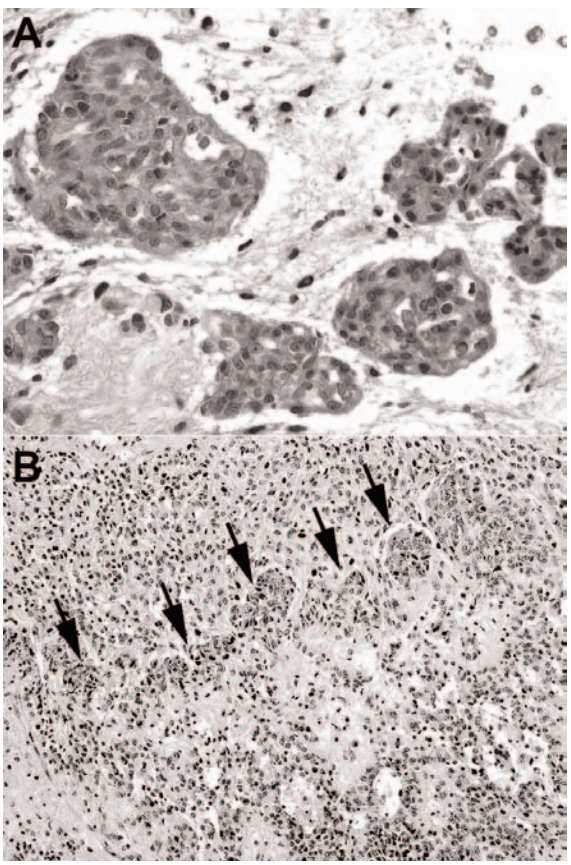

Fig. 5. Glioblastoma, A) bona fide vascular endothelial proliferation B) neovascularization around non-neoplastic cyst, not considered a valid criterion in grading (all images H&E original magnification x200).

tremely large cells with bizarre nuclei and abundant cytoplasm, admixed with smaller and more proliferative neoplastic astrocytes. Initial observations suggested that one should distinguish these neoplasms from the typical GBM (29). The gliosarcoma, on the other hand, is considered similar to typical GBM in terms of prognosis, but is distinguished by both morphological and immunohistochemical evidence of mesenchymal differentiation. Studies support the conclusion that gliosarcoma shares significant clinical and genetic similarities with GBM, and that the same principles should be applied for patient enrollment in research protocols and treatment for these two entities (30). Gliosarcomas can exhibit a plethora of mesenchymal patterns including cartilaginous (31), angiosarcoma-like (32), osteosarcoma-like (33) and MFH-like (34) features. Two added histological patterns of GBM are important to mention; the small cell GBM and the glioblastoma with oligodendroglial component. Recent work by Burger et al. concluded small cell GBMs constitute a significant percentage of primary GBMs, and are associated with a high rate of epidermal growth factor receptor (EGFR) gene amplification. Small cell GBMs exhibit striking cellular monomorphism not typically observed in other subtypes and may show little or inconspicuous positivity with the GFAP antibody. Small cell GBMs can be confused with anaplastic oligodendrogliomas, but are distinct from these neoplasms in discernable ways (35). The other important histologic pattern is the glioblastoma with oligodendroglioma component (36). This tumor has similar histologic appearance with anaplastic oligoastrocytoma except for the necrosis with or without palisading. Patients with this tumor have a worse prognosis than the patients with anaplastic oligoastrocytoma (37). However they have a better overall survival probability than the patients with conventional glioblastoma (38).

Focal, and sometimes prominent epithelioid features may be seen in some tumors, and these have been previously referred as 'lipid rich epithelioid GBMs' (39). These tumors are circumscribed cerebral tumors with diffuse cytoplasmic lipidization and a cohesive architectural disposition in epithelioid nests and sheets. These exceptional GBMs have been confused with metastatic carcinoma (39). In addition to the lipid-rich epithelioid pattern, some tumors with epithelioid features have been termed "adenoid GBM". These tumors may have branching trabecula of polygonal cells, mimicking epithelial tubules in a myxoid stroma (40,41). Rarely, one can observe intensely eosinophilic intracytoplasmic inclusions within the neoplastic astrocytes in a GBM, akin to those described for anaplastic astrocytoma (42).

Histological features previously considered among 'secondary structures' include satellitosis, perivascular and subpial aggregation of tumor cells, and may be prominent in some tumors. Perivascular pseudorosettes in some small cell GBMs may resemble the perivascular pseudorosettes of ependymomas. In some tumors, subpial aggregation of neoplastic cells extend into the leptomeningeal space or dura mater causing a secondary 'extra-axial' mass. Leptomeningeal involvement by neoplastic astrocytes can generate significant desmoplastic reaction and cause a more spindled appearance of the tumor. In such foci, tumor cells may retain their glial nature, but the phenotypic alterations suggest a mesenchymal differentiation, similar to gliosarcoma. Rare tumors demonstrate extensive leptomeningeal tumor spread without a discernable parenchymal mass (43-45).

Perivascular inflammatory infiltrates can be found in some GBMs, especially with the gemistocytic pattern, or in the giant cell variant (46). Most GBMs also harbor numerous macrophages both in association with necrotic foci and as scattered or isolated cells within the tumor. Presence of abundance of macrophages is not entirely incompatible with the diagnosis of GBM but one should exercise extreme caution not to over-interpret a macrophage-rich process

such as demyelination.

Recently published examples of glioblastoma with PNET-like features are not included in the current WHO classification (47). These tumors had foci resembling conventional glioblastoma, and separate foci with convincing evidence of neuronal or neuroblastic differentiation [36]. Nevertheless, there are very few published cases to determine the specific features of these tumors, and the WHO group has left the final decision on the fate of these rare tumors to the future revisions.

Intraoperative smear preparations of GBMs highlight the nuclear as well as cytoplasmic variations. Typically, nuclear features readily establish the neoplastic nature of the glial process. The tandem of pleomorphism and variable fibrillarity is very helpful in recognizing a malignant tumor as a glial tumor on smears. When adequate, smear preparations contain mitotic figures, which are also helpful in establishing an impression of a high-grade glioma. Furthermore, both smears and frozen sections can include clear evidence of vascular proliferation. This is extremely helpful in favoring a malignant glioma over lymphoma or metastatic carcinoma, since it is highly unusual to find true vascular (or microvascular) proliferation in the latter two malignancies.

A note of caution is highly appropriate for the interpretation of smears and frozen sections: none of the features in isolation can be deemed sufficient to diagnose a GBM. Intraoperative diagnosis of malignant gliomas is a gestalt impression of the collective features seen in all slides, and is invariably helped by the knowledge of the radiological and clinical features. Even though it cannot be a mandate (for practical purposes), we highly recommend a visit to the operating room and direct communication with the neurosurgeon during intraoperative consultation. This gives the best chance for understanding the critical details of the case. This is also helpful in cases where the initial material is non-diagnostic, or devoid of viable cells, precluding a diagnosis.

One can readily establish the presence of glial filaments within neoplastic cells using immunohistochemical stains for GFAP, which is neither required nor constitutes evidence of astrocytic differentiation. When positive, even focally, it is helpful establishing the glial nature of the neoplasm, and confirms an astrocytoma when coupled with the cellular morphology. Some GBMs show little or no GFAP positivity, or require additional antigen retrieval techniques not routinely used in clinical laboratories. Staining for neurofilament proteins often highlight the invaded neuropil within the tumor, and are especially helpful in establishing the tumor as an infiltrating glioma. Beyond this feature, neuronal stains are not of particular help in the diagnosis of GBM.

Immunohistochemical stains for cytokeratins, especially 'cocktail' antibodies can be positive in some GBMs, and can be misleading when metastatic carcinoma is within the differential diagnosis (26,48). Reactive astrocytes are often intensely labeled with cytokeratin antibodies with the exception of low molecular weight cytokeratins such as CAM 5.2. As mentioned above, some GBM can contain many macrophages and strongly stain for antibodies such as HAM-56 or CD68. Immunohistochemical markers of proliferation such as Ki-67 (MIB-1) has been used extensively, and the index has varied considerably (49-56). Due to considerable regional variation in staining, detection and counting methods and a lack of standardization, Ki-67 indices have been of little use in prognostication or grading, but a low Ki-67 staining may raise suspicion of the diagnosis of GBM.

Immunohistochemical stains for p53 and the EGFR proteins are increasingly being used to better define the GBMs as primary, or secondary, since alterations in these two molecules appear to define two distinct molecular forms of the disease based on amplification and mutation. (7,57). EGFR gene amplification and p53

mutation rarely seem to occur together. Among many variants of EGFR gene alterations, the one with class III deletion (EGFRvIII) is seen in more than half of glioblastoma patients. In addition, the increased use of drugs targeting growth factor receptors such as EGFR necessitates recognition of this molecular status in individual tumors. Two commonly used antibodies, one which recognizes the native EGFR molecule and another which recognizes the EGFRvIII mutant have become routine staples in the neuropathologists armament. It will be important to provide these stains in routine immunohistochemistry laboratories in the future.

ANAPLASTIC OLIGODENDROGLIOMA

(Oligodendroglioma, WHO Grade III-Fig. 6)

Oligodendrogliomas involve the superficial cortex and result in "swollen" gyri. The tumor has at least partially gelatinous texture and yellow-gray necrosis, hemorrhagic or thrombotic vessels. Intraoperatively, the affected cortex appears edematous with grayish discoloration and the architectural details become obscured. On cross sections, effacement of the cortex-white matter boundary can be readily identified. In some areas, cortical calcifications can be abundant enough to give the tumor a gritty texture.

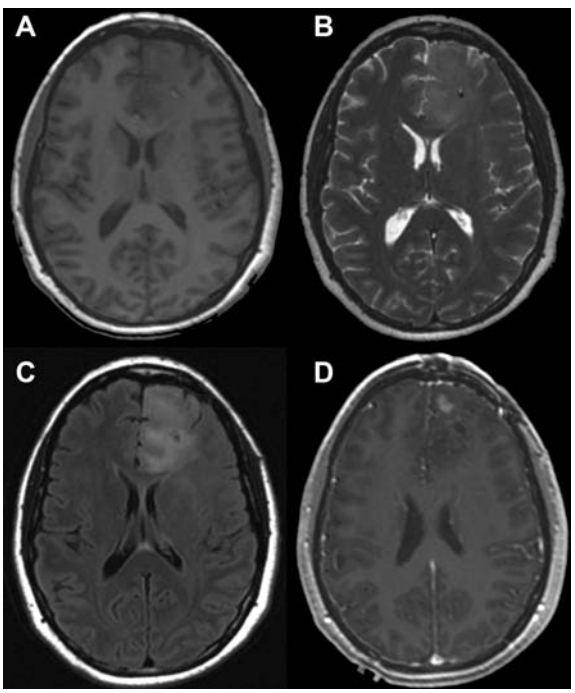

Fig. 6. Anaplastic oligodendroglioma, typical radiological features A) axial T1-weighted image; B) axial T2-weighted image; C) axial FLAIR; D) axial contrast-enhanced T1-weighted image.

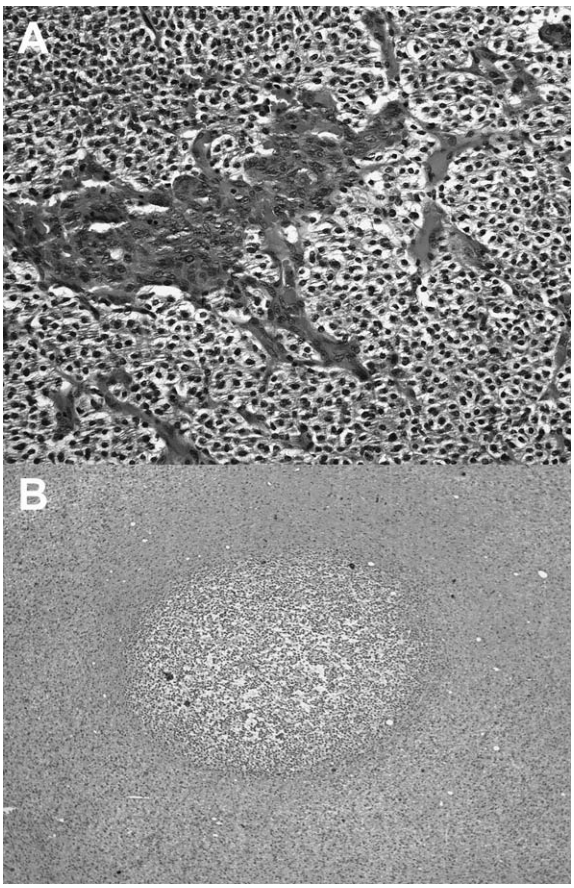

Fig. 7. Anaplastic oligodendroglioma, A) typical architectural pattern with mitotic figures and bona fide vascular endothelia proliferation (H&E original magnification X200; B) hypercellular nodules- not considered as a valid a criterion for grading (H&E original magnification x40).

In addition to the monotonous infiltrative microscopic appearance of low-grade oligodendroglioma, anaplastic examples demonstrate vascular proliferation, markedly increased mitotic rate and occasional necrotic foci. Vascular proliferation can be seen as either in the form 'glomeruloid' vessels, or endothelial hyperplasia (Fig. 7a). Even though palisading necrosis can be seen, necrosis is more commonly encountered as the coagulative type without palisades. The typical cellular monomorphism of low-

grade oligodendrogliomas may not be readily evident in the anaplastic examples, especially if there is prior treatment. Similar to low-grade tumors, involvement of the cortical structures produces perineuronal satellitosis, subpial accumulation of tumor cells, and microcalcifications. Perineuronal satellitosis can be observed in reactive processes or in the normal cortex, but the extent is much more exaggerated in oligodendrogliomas, and the cells that satellite the neurons are atypical and more numerous than in reactive conditions. Other secondary structures of anaplastic oligodendrogliomas are also observed in the white matter. The most prominent of these is the so-called 'filing' of tumor cells along white matter tracts that give the impression of a beaded chain. Another feature that can be seen in the anaplastic examples is the hypercellular nodules with mitotic figures, apoptosis, and more pleomorphic nuclei (Fig. 7b). It is not clear whether the hypercellular nodules represent clonal growth centers with additional genetic aberrations or simply overcrowded foci. Some anaplastic oligodendrogliomas show densely cellular areas composed of minigemistocytes. The minigemistocyte, unlike the gemistocyte of astrocytomas, has not been linked to a more aggressive behavior (58), yet some anaplastic oligodendrogliomas may harbor significant number of minigemistocytes. In some tumor cells, striking eosinophilic granular structures, also called crystals can be seen within the cytoplasm, which can also be positive with periodic acid-Schiff (PAS) stain (59). These neoplastic cells are distinct from the minigemistocytes, and their presence has not been associated with any particular clinical variable except for uncertainty about the diagnosis of oligodendroglioma.

Even though formation of perinuclear haloes or the so-called 'fried-egg' cells are typical of oligodendrogliomas, this feature is neither unique nor uniformly present. Anaplastic oligodendrogliomas are less likely to show a predominantly fried-egg cell artifact. Since this is an artifact of delayed fixation, haloes are absent in tissues fixed promptly, but are especially prominent in CUSA material. CUSA specimens from any infiltrating astrocytoma can easily be misrecognized as oligodendroglioma.

Floridly anaplastic oligodendrogliomas can lose all evidence of their oligodendroglial nature. Even though the chicken-wire vascular pattern may be retained, recognition of such a neoplasm as anaplastic oligodendroglioma can be very difficult. In such cases, the most reliable feature is a low-grade oligodendroglioma in an earlier biopsy, or low-grade, typical oligodendroglioma elsewhere in the specimen.

While the current WHO categorizes oligodendrogliomas as either grade II or III neoplasm, one can encounter individual cases that complicate this grading scheme. A low-grade histology with increased mitotic activity or a well-differentiated tumor with necrosis are such examples. On the other hand, a cellular and focally pleomorphic tumor with few mitoses, and without vascular proliferation or necrosis force the boundaries of the anaplastic category.

Earlier studies suggested that necrosis or the mitotic count can be used to identify the "anaplastic" oligodendroglioma in the appropriate setting (60). A widely quoted study from the Armed Forces Institute of Pathology used a four-tiered grading scheme on the basis of a binary (yes-no) assessment of five variables (61). These included microvascular proliferation, necrosis, nuclear/cytoplasmic ratio, cell density, and pleomorphism. Significant differences in survival were noted between low and high-grade groups, but the survival curves overlapped for patients with the two intermediate grades. "Pleomorphism" was the only histological variable correlated with patient survival in this study (61). In other studies, the most compelling feature of anaplasia was glomeruloid or microvascular proliferation. A large study involving 7 neuropathologists identified 6 unfavorable prognostic factors by univariate analysis: older age, high cellularity, presence of mitoses, endothelial hypertrophy, microvascular proliferation, and

necrosis (37). Multivariate analysis reduced the significant factors, leaving only age and microvascular proliferation as independent variables. Mitoses were not significant for the group of pathologists as a whole, but were significant for individual pathologists' analyses. Thus, it is suggested that the designation of anaplastic oligodendroglioma can still be made in the absence of vascular proliferation if there is sufficient degree of cellular atypia and mitoses (13). One should exercise caution in using mitoses for evidence of anaplasia, since a universally accepted cut-off value for mitotic rate is not available.

In short, some of the histological features found to be of prognostic significance in one study are not validated in another. This discrepancy may be partly due to the variations in the diagnostic criteria and/or inclusion of oligodendroglioma-like neoplasms such as small cell astrocytoma (35).

There are significant challenges in recognizing anaplastic oligodendrogliomas in frozen sections and in smear preparations. The increased nuclear pleomorphism, loss of typical architecture and absence of fixation artifacts that are used to recognize oligodendrogliomas constitute the apparent reasons for this challenge. Furthermore, the anaplastic oligodendrogliomas may have more complex vascularity, and the typical 'chicken-wire' vascular network may be elusive. One of the most helpful tools during intraoperative evaluation is a well-prepared smear with undisturbed cellular and nuclear morphology indicating an oligodendroglial neoplasm. Usually, smear preparations reveal a more monomorphous tumor cell population with rounded nuclei harboring a speckled 'salt-and-pepper' chromatin pattern. Unfortunately, sufficient number of anaplastic oligodendrogliomas divert from this typical pattern.

The search for lineage specific markers that effectively distinguish oligodendrogliomas from astrocytomas has been fraught with major challenges. Antibodies that are presumed most useful in determining cell lineage, e.g. myelin basic protein or myelin-associated glycoprotein have proven to be of limited or no use as specific 'markers' (48). Although a well-differentiated oligodendroglioma is expected to show negative immunostaining with GFAP antibodies, many oligodendrogliomas contain GFAP positive tumor cells. Since oligodendrogliomas also integrate significant number of reactive, as well as normal astrocytes, the tumors may appear to be GFAP positive. This issue is further confounded by variable GFAP staining of astrocytic neoplasms, making the distinction based on this stain subjective. In addition to radiological and clinical information, neuronal markers are frequently applied to oligodendrogliomas in an effort to distinguish them from neuronal or glioneuronal neoplasms (62). Although synaptophysin stains the underlying neuropil that is invaded by an oligodendroglioma, it often fails to stain tumor cells. However, variable degrees of neuronal marker expression have been reported in typical oligodendrogliomas which can confound the diagnosis and should be interpreted with caution (63). It is unclear whether tumors that coexpress neuronal markers represent divergent neuronal differentiation, a distinctive form of glioneuronal neoplasm, or a reflection of histogenesis in oligodendrogliomas. Markers that are typically positive in mature neuronal cells, such as Neu-N are often negative in anaplastic oligodendrogliomas. Stains for neurofilament proteins can also be helpful in anaplastic oligodendrogliomas in highlighting the extent of neuropil infiltration.

Immunohistochemical stains for proliferative markers such as Ki-67 (MIB-1) show marked variability within anaplastic oligodendrogliomas, and the labeling indices are rarely helpful to determine the tumor grade. Some studies have found survival differences in oligodendrogliomas based on Ki-67 labeling index (64). Variation among studies may be due to methodological or interpretive differences (65). For practical purposes we do not recommend altering the grade of an oligodendroglioma based on

the Ki-67 labeling index.

Staining for p53 is present in some anaplastic oligodendrogliomas, and occurs much more commonly than low-grade examples (66). The staining is often variable in intensity and is present in a small fraction of the tumor nuclei. The p53 staining status does not appear to correlate with outcome in anaplastic oligodendrogliomas (66-69).

One of the most helpful molecular features in the recognition of anaplastic oligodendroglioma is the recently recognized deletions of chromosome 1p and 19q that also implies a chemoresponsive tumor (70). The detection of this combined deletion is almost sine qua non for oligodendroglial tumors and is also shared by most oligoastrocytomas (71). A number of methods can be easily applied to detection of this genetic alteration, but in our opinion, the one that is most suitable for the pathologist's interpretation is the fluorescent in-situ hybridization (FISH) technique. While the chromogenic alternative, CISH appears to be equally sensitive, the former test is currently better standardized and should be performed in all tumors where an oligodendroglial component is suspected. A few words of caution are warranted for this test: 1) it is important to identify regions of tumor in samples before this test is executed, 2) it is critical not to consider hyperploidy (i.e. tumors with multiple copies of both arms) as "relative" deletion. Most anaplastic oligodendrogliomas will retain this genetic alteration, but occasionally it may be helpful to retrieve an earlier, low grade sample from the same patients to perform the analysis. We strongly recommend addition of this analysis as a part of routine testing in surgical pathology practice.

ANAPLASTIC OLIGOASTROCYTOMA
(Oligoastrocytoma, WHO Grade III)

Typical oligodendrogliomas and astrocytomas have distinguishing features and can be readily recognized, but there is considerable histological overlap in some infiltrating gliomas with features of both or neither. To some, this is a true 'mixed' glioma, with discrete areas corresponding to either phenotype, or a haphazard intersection of a typical astrocytoma with a typical oligodendroglioma. The former is an exceedingly rare occurrence wherein distinct fields of classic oligodendroglioma and diffuse astrocytoma coexist (72), while the diagnostic criteria for the latter is virtually non-existent. Thus, there are no definitive histological criteria for the diagnosis of "mixed glioma". Most tumors designated "mixed gliomas" are grade II or III neoplasms that contain cells resembling the rounded nuclei of oligodendroglioma, but has an undefined percentage of tumor cells with the nuclear and cytoplasmic features of astrocytomas. Some consider this vague, and highly subjective category as a tumor with bidirectional differentiation, even though attempts to identify to genetically distinct neoplastic cell groups have been unsuccessful. Since there are significant number of tumors in which histological features are neither of the two well-defined infiltrating gliomas, it is difficult to be critical of the oligoastrocytoma concept. However, the greatly variable incidence of oligoastrocytomas in different institutions, and increase and decrease of the incidence of such tumors after an arrival or departure of a pathologist from an institution imply that there is tremendous subjectivity in the definition and diagnosis of oligoastrocytomas. Some reserve the "mixed" designation for truly biphasic tumors with fibrillary or frankly gemistocytic cells (73). Some consider this category when tumors have features of neither oligodendroglia nor astrocytes. Recent studies suggest that the occurrence of mixed gliomas is not indicative of a tumor with bi-clonal origin but rather reflects altered gene expression, leading to a change in the balance of growth factors influencing glioma differentiation (74). The nosological and therapeutic problems created by these neoplasms are still unresolved.

There is no established percentage of "as-

trocytes" in a mixed glioma. The oft-quoted figure of 20% has no scientific or experimental basis. Fortunately, at present, the distinction between oligodendroglioma and "mixed glioma" makes little initial therapeutic difference, since "oligoastrocytomas" and "pure" oligodendrogliomas are treated similarly. For the patient, however, the difference can be highly significant given the more aggressive nature of diffuse astrocytic neoplasms. Furthermore, the designation of an astrocytic neoplasm as a mixed glioma may generate undeserved prognostic optimism. One might expect that "mixed gliomas" with a 1p/19q loss would behave in an indolent or chemo-responsive fashion expected of an oligodendroglioma, whereas those without this profile act biologically like astrocytic tumors, i.e. more aggressively and more prone to high grade transformation (75). The current WHO scheme classifies high grade tumors showing mixed glial features and necrosis as "glioblastoma with oligodendroglioma component".

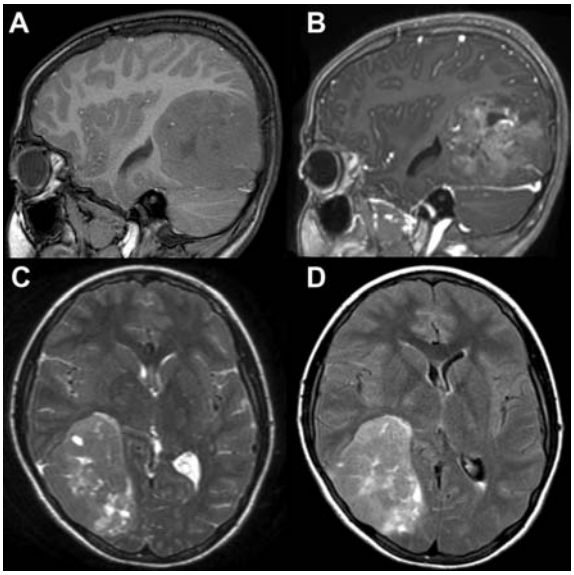

Fig. 8. Anaplastic ependymoma, typical radiological features A) sagittal T1-weighted image; B) sagittal contrast enhanced T1-weighted image; C) axial T2-weighted image; D) axial FLAIR image.

ANAPLASTIC EPENDYMOMA

(Ependymoma, WHO Grade III-Fig. 8)

The ependymal neoplasms often appear sharply circumscribed, and their textures vary from soft to rubbery. Some tumors are firm and gritty due to calcification. Most tumors have variegated color with hemorrhagic areas. Intraoperatively, one can observe involvement of the subarachnoid space where the tumor extends to encase cranial nerves and blood vessels. Anaplastic ependymomas are less well circumscribed, much more hemorrhagic and may have necrotic areas.

Anaplastic ependymomas exhibit moderate to high cellularity, mitotic activity, and vascular endothelial proliferation (76). Evidence of ependymal phenotype is often critical in recognizing anaplastic ependymomas, and the most convincing proof is found in the typical perivascular pseudorosettes. Even though histological grade has been considered to be prognostically significant, there is controversy in the use of histological criteria. In addition, studies report conflicting results on the prognostic significance of histological grading in ependymoma (77-79).

Almost all ependymomas may contain mitotic figures that are more evident in anaplastic examples. While there is no standard cut-off value in the frequency of mitotic figures, some studies report mitotic count as a significant prognostic indicator (80,81) . Vascular proliferation is also considered a strong evidence of high-grade ependymoma (Fig. 9a), while the presence of necrosis has been controversial. Necrosis was considered inconsequential in one study, while others have found it among the prognostically important factors (76,78,82). Necrosis in isolation is of little value since in our experience, most low-grade ependymomas in the posterior fossa contain necrotic regions, when sampled adequately.

Ependymomas show wide variation in cell density and differentiation. Some tumors may appear quite cellular with hyperchromatic nuclei and hypercellular nodules (Fig. 9b), while ot-

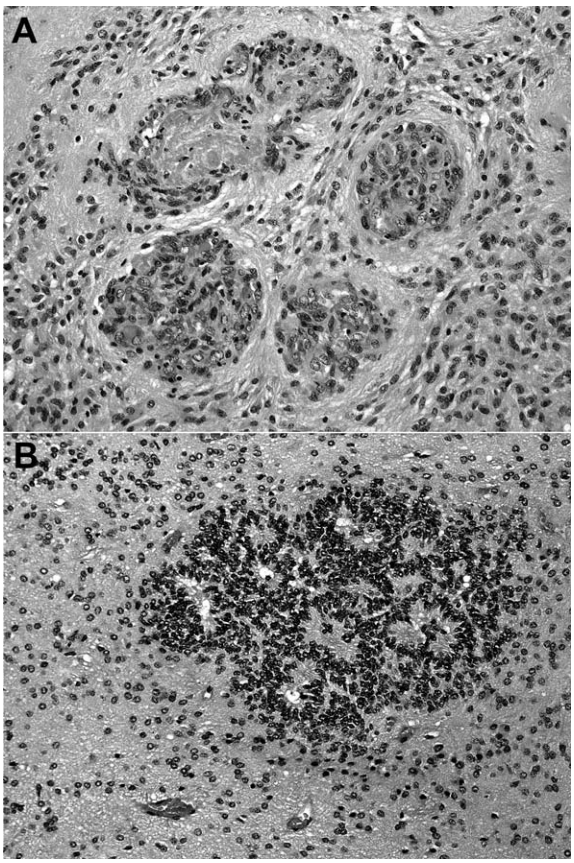

Fig. 9. Anaplastic ependymoma, typical histological features A) vascular endothelial proliferation B) hypercellular nodules- not considered a valid criterion for grading. (all images original magnification x200).

hers may be only slightly more cellular than subependymoma. Hypercellular nodules often demonstrate pleomorphic nuclei and mitotic figures. Such nodules may not justify the diagnosis of anaplastic ependymoma, but their presence raises doubts about a favorable prognosis (83).

The progression of anaplastic ependymoma to GBM is problematic and largely semantic. Unlike GBM, high-grade ependymomas usually exhibit a solid, non-infiltrating growth pattern. On the other hand, GBMs rarely show unequivocal ependymal features, and then only in the form of focal and vague perivascular pseudorosettes. In some cases, an infiltrating malignant glioma with focal, vague ependymal features is much more likely to behave as a typical GBM than an ependymoma.

Recognizing ependymomas in frozen sections can be challenging since perivascular pseudorosettes and the ependymal differentiation may be difficult to recognize. The freezing artifacts may accentuate the fibrillarity and cytoplasm of cells resulting in an impression of an astrocytoma. On the other hand small-blue-round-cells with mitotic figures and a lack of distinctive architecture can raise the possibility of medulloblastoma, especially in the setting of a posterior fossa tumor in a child. The intraoperative smear of ependymomas highlight two distinctive features: the glial quality of the cells and the monotonous nature of the nuclei. Angiocentric arrangement of cells may be conspicuous in smears, and can also be observed in frozen sections but this is less common in anaplastic ependymomas. Vascular proliferation can suggest a high-grade neoplasm, but grading of ependymomas during intraoperative consultations is discouraged unless there is unequivocal evidence of high-grade features.

Ependymomas are variably GFAP positive. Typically, there is an accentuation of staining in the perivascular spaces corresponding to cellular processes. Soma anaplastic examples may show limited GFAP positivity. Others are diffusely positive for GFAP as well as S-100 protein. Keratin cocktails (AE1/AE3) can be reactive in a large percentage of cases, the pattern being similar to that of GFAP (84). The frequency of staining for other keratins is quite variable and limited to rare individual cells and processes. Epithelial membrane antigen (EMA) stains up to half of the anaplastic ependymomas and particularly highlights true rosettes, canals and individual cells. Diffuse strong staining for other keratins or for CEA is inconsistent with a diagnosis of ependymoma. Vimentin staining is often diffuse and strong, but this only confirms the suitability of tissue for immunohistochemical analysis.

Recent studies suggest that high Ki-67 (MIB-1) and p53 immunostaining might be objective indicators of high grade in ependymomas that do not otherwise meet routine histolo-

gical criteria (85,86). A similar study proposed a cut-off value of 20% for ependymomas with more aggressive behavior (87). Others found no correlation between Ki-67 labeling index and outcome (82). The practical value of these stains in grading is currently limited.

OTHER MALIGNANT GIOMAS

PXA with Anaplastic Features

While PXAs have a more favorable outcome, the neoplasm has a significant rate of recurrence, some becoming histologically malignant in time (88). Some PXAs show anaplastic features at initial surgery. The relationship between histological features and outcome is still debated, as reviewed recently in a number of retrospective studies (88-91). These studies reveal that high mitotic rate and the extent of surgical resection appear to be the most valuable prognostic information, while necrosis is not of critical importance. Typically, one should not observe brisk necrosis, easily identifiable mitotic cells or vascular proliferation in PXA. The presence of these features should be viewed with concern and should prompt a closer scrutiny of the diagnosis.

Currently, despite the presence of such aggressive PXAs, the WHO classification does not recognize a separate anaplastic variant, and recommends the use of the term "PXA with anaplastic features". Whether this distinction is relevant to the management of the patient, and necessitates further treatment is not clear. Obviously, the concept of a grade IV PXA is even more controversial. This concept is also often contrasted with giant cell GBM that can be confused with a PXA with anaplastic features. Ultrastructurally, PXAs often reveal various epithelial properties, such as cell junctions and interdigitations, and prominent basal laminae surrounding tumor nests, often distinctive from GMB. As a rule, PXAs with anaplastic features lack the microvascular proliferation or a predominant infiltrative component common to GBM. The predominance of epithelioid cells differs from the composition of giant cell GBMs with bizarre mitoses. The differences between PXA with anaplastic features and giant cell GBM are not entirely clarified, but in our experience, the former can still be considered less aggressive.

Pilocytic Astrocytoma with Malignant Transformation

The validity of grading pilocytic astrocytomas is controversial, but there are well-documented cases wherein an apparently pilocytic astrocytoma demonstrates a very aggressive clinical course along with high grade histological features (92-94). The classical grading schemes used for infiltrating gliomas are of little value in pilocytic astrocytomas. There are, nevertheless, tumors that exhibit high cellularity, brisk mitotic activity, microvascular proliferation and/or necrosis with pseudopalisading, for which the term "malignant" or "anaplastic" is unavoidable. One should always suspect the diagnosis in an older patient with atypical radiological features, especially in the absence of a well documented prior pilocytic astrocytoma. Small samples of malignant deep seated gliomas are potentially likely to be misrecognized as pilocytic astrocytoma, only to be correctly diagnosed in subsequent surgery. Histological malignancy in pilocytic astrocytoma is quite rare and less reliably correlates with prognosis than in patients with fibrillary astrocytomas (95).

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