Introduction:

In surgical neuropathology, as in other areas of pathology, before making a diagnosis of “tumor” the possibility of a non-neoplastic process should be fully considered and excluded. “Gliosis” is a non-specific change that is encountered quite often in routine practice and may accompany both neoplastic and non-neoplastic conditions. Because there is usually some type of pathological process that stimulates gliosis, correct interpretation of biopsy or resection specimens from the central nervous system must begin with knowledge of basic clinical information (age, duration of symptoms, location of the lesion), correlation with imaging studies, and evaluation of any clinical laboratory analyses. Only after a tumor diagnosis is established with certainty can there be an enlightened assignment of glioma type (infiltrating vs. focal; astrocytic vs. oligodendroglial) and grade (low vs. high).

IS IT A TUMOR OR NOT?

Types of Gliosis: Despite its aforementioned “non-specificity”, the process of gliosis (or “reactive astrocytosis”) involves rather stereotyped morphological changes in the two major astrocytic cells types: fibrous (or fibrillary) astrocytes (primarily located in the white matter) and protoplasmic astrocytes (located in the gray matter). Fibrous astrocytes become “hypertrophic” in early stages of reactive gliosis where they develop prominent cell processes and homogeneous eosinophilic cytoplasm on routine H&E staining. Reactive fibrous astrocytes tend to be rather “evenly-spaced” within an area of gliosis and this change can be demonstrated quite well by immunohistochemistry for GFAP. During the late stages of reactive fibrous gliosis long astrocytic cell processes predominate with formation of a “glial scar”. In the cerebral cortex, protoplasmic astrocytes “react” by developing classic nuclear clearing seen in “Alzheimer type II” cell change; the inciting cause being hyperammonemia associated with chronic liver disease.

Gliosis Associated with Non-Glial Tumors: Gliosis can be quite prominent adjacent to brain metastases or in association with central nervous system lymphomas. Attempted stereotactic biopsy of a firm brain metastasis can occasionally fail when the tumor is pushed away by the needle. A diagnosis of “gliosis” should be suspect in the setting of discrete enhancing brain lesion(s) on imaging studies. Primary central nervous system lymphomas may present an infiltrative histological pattern thus mimicking a glioma. The latter is further suggested by the presence of hypertrophic astrocytes within the background giving the impression of glial lineage for the tumor. Correlation with clinical data and imaging studies and the identification of atypical lymphoid cells showing an angiocentric growth pattern suggest a diagnosis of lymphoma, which can be confirmed by immunohistochemical studies for B- (CD20). Cases in which patients with CNS lymphoma have been treated with corticosteroids prior to biopsy are particularly challenging since steroids are lympholytic and can cause transient “disappearance” of the tumor in imaging studies or in tissue sections!!
One of the most striking features of chronic astrocytic gliosis is the development of elongated, granular, hypereosinophilic, proteinaceous deposits called “Rosenthal fibers”. The term “piloid gliosis” is often used when Rosenthal fibers are prominent because such structures are characteristic of (but not specific for) the pilocytic astrocytoma. Furthermore, striking piloid gliosis with Rosenthal fibers commonly occurs in the walls of cystic non-glia neoplasms that may mimic the appearance of pilocytic astrocytoma by imaging. Hence, cerebellar hemangioblastomas and suprasellar craniopharyngiomas typically have Rosenthal fiber-containing, densely gliotic cyst walls. Syringomyelia, with or without an associated tumor, and benign cysts of the pineal gland are also associated with brisk piloid gliosis. Rosenthal fibers are particularly widespread in the brains of patients with Alexander’s disease.

**Gliosis Associated with Some Brain Tumor Mimics**: Atypical reactive gliosis is a feature of some common glioma mimics, namely tumefactive non-neoplastic processes that will have markedly different prognosis and therapies. The differential diagnosis of a “ring” or “rim”-enhancing lesion on neuroimaging includes such disparate clinical entities as glioblastoma, metastasis, abscess, tumefactive demyelination, radiation reaction, and resolving hematoma. Reactive gliosis may occur at the periphery of any of these. Demyelinating disease is important to consider because astrocytic reactions can appear quite atypical and bizarre. Creutzfeldt cells may form at the gliotic edges of active demyelinating lesions and contain fragmented nuclear material that can mimic mitoses. In progressive multifocal leukoencephalopathy (PML) virally infected astrocytes may not only present highly bizarre transformed-appearing morphologies, they may be immunoreactive for p53 thus further simulating a neoplasm. Identifying macrophages is critical to distinguish a neoplastic process from a demyelinating (or other destructive non-neoplastic) disorder. The discohesive character of macrophages is best demonstrated on touch or squash preparations. Immunohistochemical staining for CD68 may be used for confirmation. Axonal stains (neurofilament IHC or Bodian) may show relative sparing of axons typical of a demyelinating disease. Even in cases without characteristic oligodendrogial viral inclusions, recognition of the reactive nature of bizarre astrocytes and immunohistochemical stains for polyomavirus large T antigen supports the diagnosis of PML.

**Gliosis vs. Glioma**: This distinction may be difficult by histology alone, especially in small biopsies from the infiltrating edge of a glioma or the gliotic margin of a non-gliomatous process. As noted previously, reactive fibrous gliosis features uniformly distributed astrocytes that show regular spacing within the tissue and abundant processes that can be well demonstrated by GFAP immunohistochemistry. In contrast, diffuse infiltrating gliomas will be less uniformly distributed, tend to form cell clusters, and have much more variable degrees of perinuclear cytoplasm and processes. Individual tumor cells in many infiltrating gliomas will appear as isolated nuclei and tend to aggregate around neurons (perinuronal “satellitosis”) or beneath the pia. Large, hyperchromatic nuclei are also frequently found in gliomas. The cytoplasm and cell processes of glial neoplasms are much highly variable than those of reactive astrocytes. Tumor cells may have little discernable cytoplasm or contain large amounts such as “gemistocytic” forms.

Ancillary studies may be useful in helping to distinguish a gliotic vs. gliomatous lesion but all have certain drawbacks. GFAP helpful to identify evenly spaced cell processes or cell processes making contact with blood vessels. However, GFAP immunohistochemistry does not allow for the definitive diagnosis of gliosis vs. tumor nor does the pattern of GFAP immunoreactivity allow for the distinction between an oligodendroglioma and astrocytoma. Ki-67 (MIB-1) is a non-histone cell-cycle-associated antigen that is expressed during G1, S, and G2/M phases in proliferating cells regardless of whether they are neoplastic or not. It often shows increased labeling of atypical cell nuclei in infiltrating gliomas but like GFAP is not tumor-specific. Some tumor-associated antigens have shown promise in identifying infiltrating tumor cells within a background of normal or gliotic brain. P53 accumulates in nuclei of about 60% of diffuse astrocytomas and about 80% of gemistocytic astrocytomas. In contrast, gliosis is usually negative (except for virally infected astrocytes in PML) and only a minority of oligodendrogliomas are immunoreactive.
Therefore, a positive p53 can be quite useful although a glioma is not excluded if p53 is negative. A subset of glioblastomas have a mutation of the NADP+-dependant isocitrate dehydrogenase-1 (IDH1) gene, which mostly affects a single codon (IDHR132H). This mutation has been identified in 50-80% of secondary glioblastomas but in only 3-12% of primary glioblastomas and occurs in around 70% of diffuse WHO Grade II gliomas (astrocyomas, oligodendrogliomas, and mixed gliomas). Antibodies are currently available that recognize mutated variants of IDH1 (R132H) that may distinguish infiltrating glioma cells from native CNS elements. As with p53, a negative stain is non-revealing. Finally, initial excitement over the putative tumor specificity of antigens like WT-1 and Nogo-A have been tempered by their subsequent demonstration in reactive conditions.

MAJOR ISSUES IN GLIOMA GRADING:

Focal vs. Infiltrating Gliomas: The histopathological grading of diffuse infiltrating gliomas remains rather well defined, depending on the presence or absence of pleomorphism, mitoses, vascular endothelial proliferation/hyperplasia, and necrosis, all assessed in routine H&E-stained tissue sections. Ancillary immunohistochemical and molecular studies are used to support a primarily histopathological diagnosis. However, focal primary WHO Grade I glial tumors should be excluded before applying grading criteria for the diffuse gliomas (WHO Grade II) or above. For example, pilocytic astrocytomas (WHO Grade I) may contain areas with vascular endothelial proliferation and even have limited mitotic activity without influencing grade. Therefore, special attention must be directed towards localized lesions of children with cystic cerebellar tumors (pilocytic astrocytoma) or children and young adults with temporal lobe tumors and long epilepsy histories (ganglioglioma, WHO Grade I). Awareness of more recently recognized entities such as the WHO Grade I “angiocentric glioma” in young people with epilepsy will prevent “over-grading” along with “over-treatment”. On the other hand, a suspicious infiltrating tumor in an older adult is more likely to be a malignant glioma on final pathology review.

Occasionally, it is unclear whether a glioma is truly focal or diffuse and the pathologist must make this determination by histology alone. This may become an issue when the tumor is deceptively circumscribed on imaging or in unusual locations such as the midbrain-diencephalic region in younger patients. The identification of eosinophilic granular bodies or an abundance of Rosenthal fibers in astrocytic or glioneuronal tumors is useful because such “reactive” changes are most characteristic of focal gliomas like pilocytic astrocytomas and gangliogliomas. Axons within a glioma, as demonstrated by immunohistochemistry using antibodies that recognize phosphorylation-dependant epitopes of neurofilament protein (NFP), may support white matter infiltration by tumor. This study may be useful for small biopsies or in situations were more focal forms of neoplasia are in the differential diagnosis. An important caveat is that some ganglion cell tumors (ganglioglioma for example) may express neurofilament protein in elements of the tumor itself so this method must be used with care and in the appropriate clinical and radiological context. It should also be noted that even “focal” tumors such as pilocytic astrocytoma or ganglioglioma may at least focally infiltrate adjacent brain tissue.

Diffuse low grade astrocytoma (WHO Grade II) vs. diffuse anaplastic astrocytoma (WHO Grade III): A critical decision point in glioma diagnosis and management is the distinction between a WHO grade II diffuse astrocytoma and a WHO grade III anaplastic astrocytoma. The finding of mitotic activity by H&E is the defining diagnostic criterion (WHO 2007) for the anaplastic tumor. However, technical considerations, subjectivity, and the experience and diligence of the pathologist are all limiting factors in accurate mitotic counting. Ancillary testing with the immunohistochemical detection of phopho-histone H3, which is expressed during chromatin condensation, has recently been shown to increase the accuracy of detecting mitotic figures and holds promise as an independent predictor of survival in diffuse astrocytomas. Immunohistochemistry using the Ki67/MIB-1 monoclonal antibody is the most reliable and technically feasible method to measure cell proliferation or tumor growth fraction and has been extensively investigated
in the gliomas. Several studies have documented a significant positive correlation between Ki67 labeling indices and tumor grade, and an inverse correlation with survival. In practice, Ki67 is often useful in limited biopsy samples where the differential diagnosis involves diffuse astrocytoma vs. anaplastic astrocytoma. Here, an estimation of proliferative rate can confirm or support the diagnosis when clinical, radiological, and histologic findings have been considered. Measurement of Ki67/MIB-1 is not included in the WHO grading criteria, although Ki67 labeling indices for diffuse astrocytomas (WHO Grade II) are "usually less than 4%" (WHO 2007).

Diffuse “Small-Cell” Glioblastomas and Astrocytomas vs. Diffuse Anaplastic Oligodendrogliomas:

Although small neoplastic cells are common in glioblastomas, malignant astrocytic gliomas composed primarily of small neoplastic cells have been defined. The “small cell glioblastoma” (WHO Grade IV) and small cell types of anaplastic astrocytoma (WHO Grade III) are important to recognize because of they represent particularly aggressive glioma variants and have a histologic overlap with anaplastic oligodendrogliomas (WHO Grade III), which carry a much better prognosis. Histologically, small cell glioblastomas are highly cellular and cytologically monotonous. They are primarily composed of small astrocytic cells with oval, mildly hyperchromatic, and deceptively bland nuclei that contain occasional small nucleoli. Mitoses are typically frequent and cytoplasmic borders are inconspicuous. As with other forms of GBM, vascular endothelial proliferation and necrosis are present. Importantly, Perry and colleagues emphasize that gliomas falling within the histologic spectrum of WHO grade III anaplastic astrocytomas may also show small cell histology. Such tumors can mimic the radiologic and histopathologic features of anaplastic oligodendrogliomas (WHO Grade III), including nuclear and cellular uniformity, chicken-wire vasculature, clear haloes, perineuronal satellitosis, and microcalcifications, yet have a much worse prognosis (11 months). The findings of oval or elongated nuclei and inconspicuous cytoplasm in small cell astrocytic tumors are perhaps most useful in distinguishing them from anaplastic oligodendrogliomas, which are typically composed of cells with uniformly round nuclei and well-defined clear to amphophilic cytoplasm. Thin cytoplasmic processes of small cell astrocytomas are strongly immunoreactive for GFAP and such tumors typically display a high MIB-1 labeling index although these features will not definitively distinguish such tumors from the anaplastic oligodendroglioma. In marked contrast to oligodendroglial neoplasms, no small cell astrocytic tumors showed co-deletion of chromosomes 1p and 19q but frequently show EGFR amplification and chromosome 10q deletions, thus showing utility of molecular diagnosis in this important differential diagnosis.

Glioma Heterogeneity and Sampling: Ultimately, careful sampling of representative areas of a glial neoplasm will provide the pathologist with the best opportunity to make the right diagnosis. Once again, the pathologist should correlate clinical characteristics and imaging findings with the sample provided to ensure that appropriate sampling has occurred. This is best done at the time of intra-operative consultation. For example, a specimen that looks like “neuroglial tissue with gliosis” does not correlate with imaging findings of “a large rim-enhancing lesion with peri-lesional edema” and another specimen should be requested. On the other hand, a glial tumor may appear to be low-grade on imaging (bright on T2 and FLAIR sequences but non-enhancing after contrast) and still have mitotic activity that forces an “anaplastic” (Grade III) diagnosis. In this case, the old adage that “the slides do not lie” holds true! Good communication (either direct or during interdisciplinary tumor boards) between the operating neurosurgeon, neuro-oncologist, and neuroradiologist will promote optimal patient care.
Selected References:


