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Companion Symposium

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Washington DC, May 20, 2010

AVOIDING COMMON MISTAKES AND PITFALLS IN DIAGNOSTIC SURGICAL NEUROPATHOLOGY
MARCH 20, 2010 (7 pm - 10 pm)
Program Chair: Tarik Tihan, MD
Local Chair: Elisabeth Rushing, MD

SYLLABUS

Daniel J. Brat, MD, PhD
Use of Radioimaging Information for Diagnosis of CNS Lesions

Bette K. DeMasters, MD
How to avoid problems in frozen sections

Gregory N. Fuller, MD, PhD
Common artifacts that impede reliable interpretation and cause misdiagnosis

Janet M. Bruner, MD
Practical interpretation of immunohistochemical stains in surgical neuropathology
Landmarks in Neuroimaging

- The German physicist Wilhelm Konrad Röntgen described the "x-ray" in 1895 and was given the first Nobel Prize in physics in 1901.
- Godfrey N. Hounsfield and Allan M. Cormack shared the 1979 Nobel Prize in Physiology and Medicine for developing Computed Tomography (CT).
- Paul C. Lauterbur and Peter Mansfield shared the 2003 Nobel Prize in Physiology and Medicine for discoveries that led to MR imaging.

Computed Tomography (CT)

CT displays bony anatomy and differentiates soft tissue densities. It is the technique of choice for evaluating skull base and calvarium. CT depicts cerebral tissue moderately well, especially when comparatively hyperdense material such as blood or hypodense material such as edema are present. CT does not differentiate tissues with similar densities, such as gray matter and white matter.

Contrast Enhancement: IV iodinated contrast material improves visibility of cerebral vessels and allows visualization of disrupted vasculature in disease.

Disadvantages of CT: Images are usually limited to axial plane. Artifacts (beam hardening with streaking) are significant problem in posterior fossa.

Magnetic Resonance Imaging (MRI)

A magnetic field and finely tuned radio waves produce cross-sectional images. Hydrogen nuclei are excited in presence of magnetic field, and then relax to baseline by releasing energy detected by coils. Relaxation consists of T1 and T2 components. Images emphasize either T1 or T2 properties, resulting in tissues taking on different
gray scale values. MR images resolve differences in the water content (gray vs. white matter). MR depicts anatomy in multiple planes. Artifacts typical of CT do not occur with MR.

T1-weighted images are excellent for displaying anatomic features. T2-weighted and fluid attenuated inversion recovery (FLAIR) T2-weighted images highlight free water changes in pathological conditions. Water, including CSF and edema, are hypointense on T1- and hyperintense on T2-weighted images.

**Contrast Enhancement**: Gadolinium-containing compounds are used with T1-weighted images.

**Disadvantages of MR**: Many medical appliances are not compatible with MR imaging because of potential motion and malfunction. MR does not image calcifications and cortical bone well. MR scans require more time and patient motion may degrade images.

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**Advanced noninvasive diagnostic imaging techniques**

**Diffusion-weighted MR imaging (DWI)**: images based on the random motion of water. Diseases that impede random motion, such as cytotoxic edema and cellular swelling in early cerebral infarction produce hyperintense diffusion-weighted regions on the scan. Cell packing in higher grade tumors restricts diffusion. Vasogenic edema distends the extracellular compartment with water and facilitates diffusion.

**MR spectroscopy (MRS)**: This modality noninvasively measures metabolites associated with neoplasms. Elevated Choline (Cho) implies cellular turnover. N-acetyl-aspartate, a neuronal marker and is reduced in infiltrating tumors. Lactate is produced in hypoxia and indicates necrosis. Lipid peaks typical of membrane destruction usually indicate high-grade gliomas.

**Perfusion MR** imaging uses techniques that assess tumor vascularity and permeability. Tumor grade in diffuse astrocytomas is correlated with rCBV. Functional MR imaging relies on the relative MR signatures of oxyhemoglobin and deoxyhemoglobin in activated cortex compared with inactive cortex during specific tasks. This technique cap map motor and language cortex prior to surgery.
Imaging patterns in neuroradiology

Differential diagnoses are based on patient age, clinical information, location and the imaging pattern of the lesion.

**The intracranial mass**: detected by distortion of normal anatomy or by abnormal density pattern on CT or MRI.

**Intra-axial vs. extra-axial mass.** Extra-axial: broad attachment to the inner skull or dura, adjacent calvarial changes (erosion or hyperostosis), buckling of the gray matter and white matter subjacent to the mass, widening of the ipsilateral subarachnoid space, a cleft of CSF separating mass from brain, and deviated cortical vessels between mass and brain. Common diagnoses: meningioma, hemangiopericytoma, solitary fibrous tumor, lymphoproliferative disease, sarcoidosis, granulomatous infection. Intra-axial: brain parenchyma surrounds lesion, cortical vessels compressed between mass and skull, thinned cerebral cortex between mass and skull. Common diagnoses: glial and non-glial neoplasms, tumefactive demyelination, infection, infarction and hematoma. Extra-axial vs. intra-axial may not be obvious: oligodendroglioma, PXA, GBM. Multiple masses suggest metastases or disseminated infection.

**Calcified mass:** Calcium is best detected on CT scans as hyperdensity, but also on MR scans as signal voids. Supratentorial intra-axial calcified mass: oligodendroglioma, ependymoma, astrocytomas, AVMs. Extra-axial: meningioma. Suprasellar region: craniopharyngioma, meningioma, germ cell tumor, chondrosarcoma, chordoma, or even aneurysm. Pineal region: teratoma or pineocytoma. Intraventricular: choroid plexus papilloma, ependymoma, meningioma, neurocytoma.

**The cystic mass:** Benign non-neoplastic cysts, such as the arachnoid cyst and Dandy Walker cyst display no contrast enhancement and a homogenous fluid content. Neoplastic cysts, such as with pilocytic astrocytoma, ganglioglioma, PXA and hemangioblastoma, display a contrast-enhancing mural nodule. The fluid signal of neoplastic cysts may not be similar to CSF.
**The hemorrhagic mass:** Hemorrhage is identified easily on CT as hyperdensity. Differentiation from calcium is aided by clinical context and other subtle clues. On MR, imaging features of hemorrhage are more complex. Benign causes of hemorrhage, such as hypertension, are often difficult to distinguish from an underlying neoplasm and may require waiting for resolution of blood products. Hemorrhagic metastases are often seen at gray-white junction and are usually heterogeneous and surrounded by extensive edema compared to hematoma. Benign hematomas tend to evolve from the periphery of the clot centripetally to the center, whereas tumor-associated hemorrhages are poorly organized. Also, tumoral hemorrhages tend to recur and produce blood products of various ages on CT and MR imaging. Hemorrhagic neoplasms usually show contrast-enhancement.

**The rim enhancing mass:** Intravenous contrast agents used in CT and MR imaging identify blood-brain barrier breakdown. Contrast is sequestered in the extracellular space due to leaking capillaries. An avascular (therefore non-enhancing) central component implies necrosis or acellular debris. The rim enhancing pattern embraces several prominent diagnoses, such as high-grade astrocytoma and metastasis, as well as infections, such as abscess. Infarcts, radiation necrosis, resolving hematomas, and tumefactive demyelination may also produce a ring configured pattern with the administration of intravenous contrast and therefore careful correlation with the patient’s history and past imaging is required. Diffusion imaging is very useful in that bacterial abscesses tend to have restricted diffusion manifested by hyperintensity on DWI, whereas necrotic cavities of most tumors
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How to avoid problems in frozen sections

Topics to be covered

- **Role of Intraoperative Consultation---what is the goal of the neurosurgeon?**
  - Why aren’t they able to get where they are supposed to be??!!
  - What is the yield for neoplastic and non neoplastic biopsies?
- **Role of Intraoperative Consultation---why is the pathologist there and what are our goals?**
  - Assess adequacy of sampling based on what they anticipate by clinical and neuroimaging data—what are they aiming for??!!
  - To generate a diagnosis using optimal techniques available to us
  - To guide triage of tissue

**Role of Intraoperative Consultation---what is the goal of the neurosurgeon?**

The goal of the neurosurgeon is to obtain representative tissue that will yield a diagnosis on permanent sections. If the first frozen section (FS) specimen is not diagnostic, the neurosurgeon’s goal is to obtain feedback/guidance from the pathologist in obtaining additional specimen(s) that are diagnostic. Depending on the specifics of the case and what exactly is the problem with the first FS, the pathologist may need larger amounts of tissue (often generated by obtained more cumulative volume (i.e. more stereotactic cores), or may need tissue from a different depth or location than the first biopsy. Some processes (tumors, abscesses, post-chemotherapy and radiation therapy high grade gliomas) are extensively necrotic and the pathologist may find that the first FS is entirely necrotic. This is obviously abnormal tissue, but not diagnostic; hence the pathologist needs to convey that less necrotic tissue is necessary for diagnosis. In some cases, the lesion in question is located in a specific area that is easily recognized on FS, such as choroid plexus or the process which they are seeking is predominantly in the white matter (most diffusely infiltrating astrocytomas) and they have obtained gray matter; the pathologist conveying to the surgeon that she/he is in gray matter not white matter can be highly helpful in changing the neurosurgeon’s depth of biopsy.
It can be difficult for the pathologist to understand why the neurosurgeon isn’t able to get where they are supposed to be. Modern neuroimaging and intraoperative MRI techniques have improved targeting for neurosurgeons, but the brain moves during the procedure, due to swelling induced by the surgical procedure itself. And what the neurosurgeon may consider the TARGET (FS 0, T) may not be the most informative for the pathologist. This is largely due to the known heterogeneity that exists in all grades and types of gliomas and infectious and inflammatory processes that affect the CNS. Lymphomas and demyelinating lesions are also relatively heterogeneous. Indeed, with the exception of metastases, most CNS processes show heterogeneity and small volume biopsies may land on under-representative areas.

When confronted with a non-diagnostic FS, the pathologist is tempted to blame themselves; the antidote to this is to know the literature on expected diagnostic yield generated from experts on the topic. Prayson and colleagues studied this issue over a decade ago (Frozen section evaluation of stereotactic brain biopsies: diagnostic yield at the stereotactic target position in 188 cases. Brainard JA, Prayson RA, Barnett GH. Arch Pathol Lab Med. 1997 May;121(5):481-4). Their abstract appears below (underline and italics added by BKD):

| OBJECTIVE: Use of the image-guided stereotactic brain biopsy has facilitated the diagnosis of previously inaccessible lesions with both safety and reliability. However, few studies have assessed the diagnostic yield of frozen section evaluation of the initial stereotactic target (FS-0). We describe our experience with 188 stereotactic brain biopsies in order to evaluate the diagnostic yield of FS-0. |
| DESIGN: Retrospective study of 188 stereotactic brain biopsies from 185 patients. |
| PATIENTS: One hundred eighty-five patients who underwent imaged-guided stereotactic brain biopsy over a 58-month period. RESULTS: The patients studied included 107 males and 78 females (mean age 48 years). Eleven (6%) biopsies were nondiagnostic. Diagnoses from FS-0 included a neoplastic condition in 96 (73%) of 131 cases and a nonneoplastic condition in 23 (50%) of 46 cases. In 119 (67%) of 177 cases, a diagnosis was reached at FS-0. A correct diagnosis was made on subsequent frozen section in 28 (16%) of cases, including 21 (16%) of 131 neoplasms and 7 (15%) of nonneoplastic conditions. In 15 (54%) of 28 cases, the correct diagnosis was made on the second frozen section; in 25 (89%) of 28, the correct diagnosis was made by the fourth frozen section. In 14 (11%) of 131 neoplastic cases, a sampling error relative to the lesion resulted in an inaccurate diagnosis at FS-0. A significant error in diagnosis occurred in three cases (1.7%). |
| CONCLUSIONS: We conclude that because 58 (33%) of 177 diagnosed cases in our series would have been potentially misdiagnosed if only one biopsy had been taken at the stereotactic target, frozen section evaluation or cytologic examination of material at the time of surgery should be performed routinely to ensure that adequate tissue has been obtained for purposes of diagnosis; taking up to four biopsies increases the diagnostic yield (from 67% to 89% in this series); and neoplastic lesions are more likely to be definitively diagnosed at FS-0 than non-neoplastic lesions. |

(BKD comment: stereotactic biopsies are ideal for neoplasms and neoplastic look alikes such as acute demyelinating lesion, abscesses, all of which share a defined, relatively localized, variably ring enhancing configuration. Open biopsies provide better yield for diffuse, ill-defined, non-enhancing processes. Even in 2010, the pathologist needs to work closely and patiently with the neurosurgeon to make sure they are in the house, not the yard).
Role of Intraoperative Consultation—*what are other goals of the neurosurgeon?*

The neurosurgeon will be speaking to the family immediately post-op and understandable wants to inform the patient/patient’s family of at least a tentative diagnosis at that time when they ask. Obviously we understand that FS diagnosis in some instances is preliminary or tentative and the *corollary is that the pathologist should always make certain that the neurosurgeon understands this as well. Some suggest that we should read any FS report verbatim to the neurosurgeon so there is no misunderstanding later if diagnosis has to be modified [Burger]*

Role of Intraoperative Consultation—*other select goals of the neurosurgeon*

In some cases, the goal may be to obtain cultures in potentially infectious cases, to document viable recurrent brain tumor when utilizing novel treatment protocols that require viable, not necrotic, tumor (example Gliadel wafers), to obtain fresh or frozen tissue for tissue banking, molecular studies, flow cytometry, cell culture, or drug sensitivity testing. Finally, although this isn’t the optimal use of the procedure, over the years all neuropathologists have encountered examples when it appears the FS is only for the surgeon’s curiosity. Whatever the goal of the neurosurgeon, the point that needs underscoring is the need for good communication between neurosurgeon and pathologist for your mutual patient.

What should the neurosurgeon, and pathologist, NOT expect at the time of the intraoperative consultation?

There are a number of things that both parties should understand cannot be provided conclusively at the time of FS—this is part of that ‘good communication”. This includes: exact grade of a glioma (unless it is already a WHO grade IV GBM or medulloblastoma), exact subtype of a glioma (oligodendroglioma component may be difficult to definitively identify at FS), exact grade of a meningioma (WHO grade II tumors in particular may have focal atypical features), assessment of margins for a glioma (being asked for less and less these days and of no practical use for individual infiltrating tumor cells at the edge of diffuse gliomas), type of infectious organism in an abscess or cerebritis (sometimes large fungi show up..), cause of a stroke (the offending vessel or intraluminal thrombus is in the leptomeningeal vessels that may not
be in the resection material), cause of an ill-defined, non-enhancing white matter lesion and exact diagnosis on any of the notorious "sampling error", ill-defined diseases in NP, including vasculitis, collagen vascular diseases, vasculopathy, leukodystrophies, leukoencephalopathies, histiocytoses, and sometimes lymphoma (especially in immunocompetent hosts).

Neuropathology frozen sections are uncommon in many practices, and hence can be stressful. A common response of the general surgical pathologist at the time of one of these frozen sections: “I’m not a neuropathologist, maybe the problem is ME & my inexperience, someone else could probably get this (where is Peter Burger…), I just don’t know, the neurosurgeon is getting agitated, s/he must be in the right place s/he’s the neurosurgeon, I’m sure I’ll get it on the permanents, let’s give up…” Antidotes to this problem for the surgical pathologist are that they should realize that if the clinical/nueroimaging team could not generate a short differential diagnosis BEFORE the biopsy, you—and everyone else (including Peter Burger)--- are more likely to have a difficult time at the FS. This is especially true for NON NEOPLASTIC conditions. The answer for the general surgical pathologist is to know the literature and quote a noted authority! (recall the Brainerd study from 1997, above), where:

N = 188 biopsies (131 tumors, 46 non-neoplastic, 11 not diagnostic)
Diagnosis obtained on 1 biopsy at what the neurosurgeon thought was the TARGET
  – 73% tumors
  – 50% non-tumors
  – 67% all cases
Diagnosis made on subsequent biopsies (16%) – up to 4
Sampling error with only 1 biopsy – 11%

A newer study by Prayson and colleagues specifically addresses the yield of FS for non neoplastic conditions (Plesec TP, Prayson RA. Frozen section discrepancy in the evaluation of nonneoplastic central nervous system samples Ann Diagn Pathol. 2009 Dec;13(6):359-66.). Abstract below (with added underlines and italics):

Frozen section (FS) for intraoperative evaluation of central nervous system (CNS) lesions provides the neurosurgeon with a rapid preliminary pathologic diagnosis. Diagnosis of nonneoplastic lesions is particularly challenging in this venue. To highlight common diagnostic pitfalls, we sought to identify discrepancies between FS and final diagnoses among nonneoplastic CNS samples via a
A retrospective review of 303 FS cases encountered from 1997 to 2006 (at Cleveland Clinic)

Thirty-nine (12.9%) discrepant diagnoses were identified, of which 27 were clinically suspected tumors. Final diagnoses in the discrepant group included the following: inflammatory lesions (n = 8, 20.5%), malformation of cortical development-cortical dysplasia (n = 5, 12.8%), gliosis (n = 5, 12.8%), vascular malformations (n = 5, 12.8%), demyelination/progressive multifocal leukoencephalopathy (n = 3, 7.7%), infarct (n = 3, 7.7%), hemorrhage/blood clot (n = 3, 7.7%), and no pathologic changes (n = 3, 7.7%).

The remaining 4 (10.2%) discrepant cases involved one case each of amyloid angiopathy, nonspecific vasculopathy, vasculitis, and meningioangiomatosis. Nonneoplastic lesions are often more challenging than neoplastic lesions at FS, particularly because they are less commonly sampled for FS and, therefore, less familiar to pathologists.

Does the frozen section diagnosis every truly change what the neurosurgeon is going to do?

Diagnoses made at frozen section that mitigate AGAINST gross total resection of a lesion include PCNSL, lymphocytic hypophysitis, acute demyelinating disease, rare leukodystrophies or systemic inflammatory disorders. ANSWER: yes.

Role of Intraoperative Consultation---why is the pathologist there and what are our goals?

To assess adequacy of sampling based on what they anticipate by clinical and neuroimaging data—for this we need to know, what are they aiming for?

Formulating a cogent differential diagnostic list (for the clinical team and for us as pathologists) is based on knowing: Patient age (infant, child, adult), gender; Duration and tempo of disease, i.e. time course in days, months, years; Previous surgery/diagnosis, systemic disease, underlying tumor predisposition syndrome, travel history, previous treatment; Location (esp. supra- vs. infratentorial vs. spinal cord vs. intraventricular); Imaging features (enhancement pattern).

Role of Intraoperative Consultation---why is the pathologist there and what are our goals?
We are there to generate a diagnosis using optimal techniques available to us.

Frozen Section versus Smear—which to use—depends on the experience of pathologist, expected pathology, quality of frozen section, quantity of tissue. Pros of smear: rapidity of technique, offers both cytological and architectural details. Cons of smear: that exact piece of tissue is not available for permanent sections or any further tests. Pros of FS: offers best architectural detail, tissue can be retained for permanent section and you can check your FS diagnosis directly with the permanent section of that same piece of tissue. Cons of FS: longer procedure, actual freezing can hamper SOME IHC. Quantity of tissue—very small pieces of total tissue we do not use for smear (stereotactic biopsies, spinal cord biopsies, brain stem biopsies, ACTH microadenomas).

Quality of frozen section—rapid freezing of tissue can reduce ice crystal artifact, make procedure more rapid, some large institutions make the frozen section so high quality that it is the final diagnostic material for some cases. Experience of pathologist—where you trained will influence what you use. Expected pathology: for example, touch preparation is a great technique for identifying: neutrophils in infectious cases; macrophages in infarctions or demyelinating lesions; metastatic tumor cells; germinoma; lymphoma. Macrophages show up much better on touch preparation than frozen section. Cytological features of some tumors show up much better on touch preparation than on frozen section. Touch preparation results can corroborate the diagnosis for mesenchymal or fibrotic lesions (touch off poorly), low versus high grade gliomas, based on the amount of tissue that exfoliates onto the slide (low grade gliomas show minimal exfoliation of tumor cells, high grade gliomas often show abundant cells on touch print). Touch Preparation—can corroborate, or may be the sole testing, for: pituitary adenomas of any size and could be the ONLY test possible for miniscule ACTH microadenomas.

TOUCH PREPARATIONS, unlike smears, ARE NOT EITHER/OR: frozen section or smear can still be performed after a touch preparation is prepared and quality of permanent section is not impaired when using a touch preparation, whereas smear preparations may exhaust the tissue and that exact same tissue fragment cannot be used again.

I have my FS slide—now what?
An Algorithmic Approach to Biopsies of the CNS is useful for both FS and permanent sections (Arch Pathol Lab Med 130(11):1630-8, 2006.)

**Triage of tissue at time of intraoperative consultation:**

Do I need Frozen tissue? Culture? Electron microscopy? Routine histology?

When is frozen tissue absolutely essential?: Muscle biopsies, Western blot analysis for neurodegenerative diseases, i.e., prion diseases (CJD), any enzymatic study, certain banking protocols. Frozen tissue is optimal for rare CNS infections since aldehyde fixation causes increased linkages that impede PCR techniques. PCR is performed best on frozen tissue>paraffin wax tissue> wet fixed tissue stored in formalin for any length of time. Thus, frozen tissue is optimal for DNA retrieval, but is not absolutely necessary since often DNA can be retrospectively successfully extracted from paraffin blocks.

What conditions necessitate placing tissue in culture medium (RPMI)?: Classic cytogenetic studies which should be especially utilized for sarcoma and lymphoma workups and flow cytometry. Flow should be conducted for any suspected lymphoma that involves CNS/PNS (put tissue up for flow, even if it is from an unusual site such as epidural space--never assume you are going to get more tissue from another traditional site!!). [FISH (fluorescence in situ hybridization) is performed off of paraffin embedded sections, with the optimal section selected after examination of the permanent material); this is technique used here for gliomas and medulloblastomas].

On which cases should I put aside tissue for electron microscopy?: Lesions with unusual/uncertain histological features at the time of frozen section, in which diagnosis is recognized to be potentially problematic, such as (but not limited to..) sarcomas, pituitary adenomas, pediatric neoplasms. EM can be performed from tissue extracted from paraffin blocks, but it is suboptimal. Since it takes such a small amount of the surgical specimen to conduct EM and that small amount of tissue does not compromise tissue volume for permanent sections, SET ASIDE TISSUE FOR EM IN ANY DIFFICULT CASE—”BETTER SAFE THAN SORRY”.

Parting point #1...
Sometimes the greatest impact on patient care is achieved by determining what the lesion IS NOT, not by determining WHAT THE LESION IS

Parting point #2 . . .
The pathologist can only work with what they are given

Parting point #3...
*Know the diagnostic yield from the literature on stereotactic biopsies for certain types of diagnoses (the best offense for the rare belligerent neurosurgeon is a good defense…)*

Parting point #4...
The neurosurgeon and pathologist need to talk to each other before, during, and after the surgical procedure to arrive at the best diagnosis for *their mutual* patient.

Common Artifacts That Impede Reliable Interpretation and Cause Misdiagnosis

Introduction

A large number of different types of artifacts can be seen with regularity in central nervous system surgical specimens. These can be broadly divided into three categories: 1) those that impede or even prevent diagnostic interpretation, 2) those that may be mistaken for bona fide pathological alterations, and 3) extraneous substances introduced into the tissue by physicians (Table 1). Some types of artifact, most prominent cautery and freeze, can either prevent reliable interpretation altogether, or can more subtly mimic pathological change depending on the severity of the artifact.

Table 1. CNS Artifacts

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<th>Artifacts that impede interpretation</th>
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<td>Postal service artifacts (gross crush, paraffin melting)</td>
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<tr>
<td>Cautery artifact</td>
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<tr>
<td>Freeze artifact</td>
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<td>Crush artifact (forceps, embedding bag/sponge/cassette)</td>
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<th>Artifacts that mimic pathological alterations</th>
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<tr>
<td>Cavitron ultrasonic aspirator (CUSA) artifacts</td>
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<tr>
<td>Cautery</td>
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<td>Freeze</td>
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<td>Pseudomineralization</td>
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<td>Delayed fixation artifact</td>
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<td>Air-drying artifact</td>
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<td>Collapsed leptomeningeal vessel artifact</td>
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<td>Formalin pigment (“in situ hybridization” artifact)</td>
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<table>
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<tr>
<th>Extraneous iatrogenically-introduced material</th>
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<td>Preoperative Embolic Agents</td>
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Gelatin foam (Gelfoam®)
Particulate polyvinyl alcohol (PVA®)
Spherical polyvinyl alcohol (Contour SE®)
Acrylic polymer spheres (Embospheres®)
Ethylene vinyl alcohol with tantalum (Onyx®)
Color-coded polymer spheres (Embozene®)

Intraoperative Hemostatic Agents
Gelatin foam (Gelfoam®)
Gelatin Foam with human thrombin (Floseal®)
Oxidized cellulose (Surgicel®, Oxycel®)
Bovine collagen (Avitene®)

Freeze Artifact, in particular, can lead to misdiagnosis of diffuse oligodendroglioma as diffuse astrocytoma secondary to the nuclear distortion produced by freezing. An additional contributing factor to this potential pitfall is, paradoxically, the lack of a specific artifact! – the characteristic perinuclear halos, which constitute one of the hallmark diagnostic histopathologic features of oligodendroglioma, are an artifact of FFPE tissue and are lacking in frozen tissue, including frozen tissue that has been processed into paraffin. It is generally sufficient to render a diagnosis of “diffuse glioma” at the time of intraoperative consultation, with precise diffuse glioma classification and grading deferred to permanent sections.

Cautery artifact runs the gamut from complete tissue distortion incompatible with any meaningful evaluation at all, to milder, potentially misleading, forms in which nuclear elongation is the subtle result. Thus, with the latter situation, a predominantly round-cell tumor is transformed into a spindle-cell tumor. Careful attention to the vascular morphology usually provides a revealing clue in the form of purplish smudgy blood vessel walls.

Cavitational Ultrasonic Surgical Aspiration (CUSA, Cavitron) Artifact. The shearing forces and osmotic stress to which tissue resected by aspiration is subjected lead to several different types of artifact with which the surgical pathologist must be familiar. Nuclear smearing is very common and mimics necrosis. Saline, which is the irrigant used, can induce perinuclear halos and thus mimic
oligodendroglioma. In addition to these two major artifacts, extraneous material in the form of “bone dust” (microscopic fragments of cranial bone introduced into the surgical specimen by the neurosurgeon during craniotomy) and intraoperative hemostatic agents. Thus, CUSA specimens encompass the three major artifact categories of mechanical distortion (crush), osmotic (saline) distortion, and extraneous material introduction. A careful and judicious search of the CUSA tissue section will usually yield scattered well-preserved tissue fragments that are suitable for microscopic evaluation, but caution is the rule when dealing with this specimen type.

**Pseudocalcification.** Several forms of “pseudocalcification” may mislead the pathologist. The most common source, quite ubiquitous in neurosurgical specimens, is “bone dust” mentioned above with respect to its frequent presence in CUSA specimens. These microscopic fragments of cranial bone often become pressed into and surrounded by adjacent tissue fragments, thus mimicking dystrophic tissue mineralization. In re-operated patients, second resection specimens often contain bone dust fragments from the initial operation that have become entombed in a surrounding shroud of dense fibrous connective tissue, sometimes with an accompanying foreign body-type giant cell reaction.

**Pseudonecrosis.** Given the importance of necrosis as a cardinal feature of many CNS diseases, including tumors, it is important that the surgical pathologist be familiar with a number of necrosis mimics (Table 2).

**Table 2. Pseudonecrosis Etiologies**

- Lack of hematoxylin staining (simulates nuclei acid dissolution)
- Hemorrhage and/or fibrin deposition
- The normal cerebellar molecular and granular cell layers (simulate small cell tumor with necrosis on smear preparations and frozen sections)
- Degenerating hemostatic agent (particularly Avitene microfibrillar collagen)

**Preoperative Embolic Agents**
Interventional radiologists routinely introduce foreign substances into blood vessels to create embolic obstruction of flow to highly vascular lesions in order to minimize blood loss and attendant need for replacement during subsequent surgery. Preoperative embolization is typically performed on the day before surgery is scheduled to occur. Embolic agents may then appear on intraoperative cytologic
Intraoperative Hemostatic Agents

Neurosurgeons liberally use both non-resorbable and resorbable hemostatic agents to control bleeding during surgery and after closure of the craniotomy. Non-resorbable agents, such as kites and cottonoids, are removed and counted prior to closure, whereas a number of resorbable agents are designed to be left in place to mitigate chances of potentially catastrophic hemorrhage into the resection cavity following closure. Upon subsequent re-operation of recurrent tumor, residual resorbable hemostatic agent is frequently resected with the surgical specimen and thus comes to the attention of the pathologist on frozen or permanent sections in various stages of resorption with an attendant inflammatory response. As with the pre-operative embolic agents, there are several different chemical classes of agent currently employed, and most of these are recognizable by virtue of their unique morphology. One agent class, bovine collagen (Avitene) is notable for its propensity to elicit a robust eosinophilic infiltrate. This reaction is not seen in every case, but may be quite pronounced in some. Any of the various hemostatic agents may be associated with an exceptionally robust host inflammatory response during resorption that may lead to significant edema and even neurologic symptoms. Even in asymptomatic cases, the appearance on scheduled follow-up surveillance imaging studies can be striking, with new contrast enhancement and associated parenchymal vasogenic edema, to the extent that recurrent tumor is suspected and surgical resection performed. Thus the surgical pathologist must keep this type of inflammatory pseudotumor in mind when confronted with a new contrast-enhancing mass lesion arising in a previously treated brain tumor patient. Such iatrogenic inflammatory pseudotumors are referred to as “textilomas” or “gossypibomas”. Their occurrence is by no means confined to the central nervous system and has in fact been widely documented throughout the body, usually in an inadvertent “retained sponge” setting, rather than as a hemostatic agent deliberately left in place to control postoperative bleeding.

Intraoperative therapeutic wafers. Gliadel wafers are synthetic polymer wafers impregnated with a chemotherapeutic agent that are placed into the surgical cavity by the neurosurgeon following tumor (typically glioblastoma) resection to treat local tumor recurrence. The wafers are held in place along the walls of the cavity by a covering layer of hemostatic agent (typically Surgicel and/or Gelfoam). If subsequent local tumor recurrence occurs, resection of the recurrent tumor together with the wafers and hemostatic agent is often performed. Hence the surgical pathologist is likely to see these materials,
which may or may not be readily identifiable grossly, in the frozen and/or permanent tissue sections. Gliadel wafers have a very characteristic rectangular shape, resist staining with H&E, and are refractile when viewed in “tissue sections” under the microscope.

**Recommended References**


Brat DJ, Schniederjan MJ. *Biopsy Interpretation of the Central Nervous System*. Philadelphia: Lippincott Williams & Wilkins (coming in 2011)

Questions, Comments, Correspondence:

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General Points
Know the patterns of labeling for each antibody
Use your control slides

Patterns – know them
Cytoplasmic
Nuclear
Membranous
Perinuclear dot
Extracellular
Combinations

Patterns of stain make a difference:
e.g. Epithelial membrane antigen in a dot-like perinuclear pattern in ependymoma

Perverse reactions: When a negative = positive
e.g. INI1 staining is lost in atypical teratoid rhabdoid tumor (ATRT)

Confusing results: they are not what they seem
e.g. Keratin labels reactive gliosis and astrocytomas. Synaptophysin can label oligodendrogliomas

What is really useful to know about each antibody?

Keratin AE1/AE3
Staining is cytoplasmic. Never use this to differentiate an astrocytoma from a metastatic carcinoma. If a glial fibrillary acidic protein (GFAP) stain is positive in a tumor, this might be positive, also. A robust intermediate filament marker. The cross-reactivity in reactive astrocytes and astrocytoma is well known. Use some other keratin antibody in brain to confirm metastatic carcinomas.
Epithelial Membrane Antigen (EMA)

Usually shows membranous labeling in epithelial tumors. Frequently positive in meningiomas, but usually cytoplasmic, not just membranous. Can be patchy. Other staining patterns occur, such as the “dot-like” positivity in ependymomas, astroblastomas, angiocentric gliomas. Also “ring-like” positivity in ependymomas, which is said to be highly specific.

Remember that EMA is strongly positive in the cytoplasm of most plasma cells and in many plasmacytomas/multiple myelomas. EMA stains normal or reactive arachnoidal cells.

Glial Fibrillary Acidic Protein (GFAP)

A cytoplasmic stain only; one of the intermediate filaments. Very robust. If you have nuclear staining, there is something wrong with the staining procedure. This can be negative in oligodendrogliomas, but is often positive in specific patterns: cytoplasmic rim in gliofibrillary oligodendrocytes (GFOs), cytoplasm with stubby processes in minigemistocytes. It is strongly positive in reactive astrocytes, but the stain emphasizes the “star-like” processes, not the cell bodies.

This can be very useful for showing microvascular proliferation (MVP) as negative foci in relief in high-grade gliomas. It is also useful for showing the marbleized pattern in gliosarcomas (but S-100 protein is often better for that).

Also useful for defining brain invasion by meningioma. The GFAP-positive brain will be intimately mixed with the meningioma and clearly show the invasion.

S-100 Protein

A general “glial” marker, so stains oligodendrocytes and astrocytes. It is nuclear and cytoplasmic. Useful for oligodendrogliomas when GFAP is so negative that you wonder if this tumor is even a primary glioma. It labels oligodendrogiomas. Also sensitive for the marbleized pattern of gliosarcomas. Not good for distinguishing melanoma from glioma (both will be positive). Doesn’t work in tissue that has been previously frozen, so don’t try it and then interpret the result as “negative.”
Synaptophysin
A cytoplasmic stain that can be membranous or granular. It is said not to label normal cerebral cortical neurons. It strongly labels the cytoplasm of neuronal tumors, even primitive ones, such as medulloblastoma. Also labels central neurocytomas. Use for detecting neurons in ganglioglioma, but sure they have abnormal distribution or morphology, in addition.

Somewhat useful for oligodendrogliomas. A significant number of lower-grade oligodendrogliomas, especially those with 1p/19q deletion, will be positive for synaptophysin.

Synaptophysin diffusely stains the background neuropil of the brain. Make sure the labeling you are interpreting is actually inside the tumor cells.

Neu-N
Labels nuclei of abnormal neurons in tumors. Also labels normal neurons, but in cortex it can label both the nuclei and proximal perikarya. It stains more “mature” neuronal forms, so not as likely to stain primitive tumors. Does not label oligodendrogliomas.

MIB-1/Ki-67
Nuclear label. Stains all phases of the cell cycle except interphase, so will label some cells that are not in mitosis. For infiltrating gliomas, the “index” is likely to be falsely low, as many of the unlabeled cells are not tumor cells. Normal brain and reactive gliosis are essentially negative (one or two cells will stain on a section.) You have to be careful not to count “non-glial” cells: vessels, inflammatory cells, granulation tissue.

Phosphohistone H3 (PHH3)
Specifically labels the chromatin (nuclei) of cells in mitosis. You must be able to see the chromatids and confirm the mitotic figure to count this as “positive.” More sensitive than counting mitoses on an H&E stain. Useful for lower-grade gliomas and for meningiomas. The numeric criteria for grading may be different than on an H&E stain because you can detect the mitoses more easily.
p53 Protein

Any nuclear positivity is abnormal. The protein is unstable in tissue and is only labeled by immunohistochemistry if it is abnormally stabilized or bound to another cellular component. About 60% of astrocytomas of all grades will stain positively in more than 5-10% of the tumor cell nuclei. Thus a positive stain is meaningful, a negative one is not. The tumor can still be an astrocytoma. Oligodendrogliomas rarely (or never) label with p53. Cytoplasmic staining can’t be counted, only nuclear. The p53 stain is also positive in the nuclei of progressive multifocal leukoencephalopathy (PML). This is the only benign condition with positive staining. (The virus binds to the protein and stabilizes it.)

Use p53 to detect infiltrating astrocytoma nuclei in brain at the edge of a diffuse astrocytoma. Use p53 to distinguish reactive gliosis (not stained) from a low-grade astrocytoma or a gemistocytic astrocytoma or to detect radiation effect vs recurrent astrocytoma. Remember that p53 can be positive in low-grade diffuse astrocytomas. Use p53 IHC to help distinguish “ambiguous” gliomas or oligodendrogliomas from astrocytomas with rounder nuclei and little pleomorphism, or to distinguish small cell malignant astrocytomas from oligodendroglioma, BUT remember that a negative result does not eliminate astrocytoma. A strongly and diffusely positive tumor is unlikely to be an oligodendroglioma. (We still do the 1p/19q testing.)

In our experience, it does not label the nuclei of the circumscribed astrocytomas (pilocytic astrocytoma, pleomorphic xanthoastrocytoma, ganglioglioma).

It will stain nuclei of some pituitary adenomas, those thought to be potentially more aggressive.

INI1 (BAF47)

The staining that counts is only nuclear. Labels the INI1 gene product, which is normally present in all cells, but absent in malignant rhabdoid tumors due to gene deletion. Look for largely “negative” reactivity in atypical teratoid rhabdoid tumors, with retained labeling of the vascular endothelium.

Beta-catenin

Nuclear positivity; must be strong and diffuse to be counted as positive. The protein is stabilized by a gene mutation and moves to the nucleus in medulloblastoma.
Focal nuclear or cytoplasmic reactivity is not counted. Overexpression suggests more favorable prognosis in medulloblastoma.

**CD20 vs CD79a in treated B-cell large cell lymphoma**

Some patients with systemic lymphoma may have been previously treated with Rituximab, an antibody that binds *in vivo* to CD20. This will block any immunoreactivity for CD20 in their tissues. You must use another B-cell antibody to confirm the type of lymphoma. CD79a is a good one.

CD45 can sometimes stain weakly in primary central nervous system lymphomas. CD20 or CD79a can be useful in that situation.

**SUMMARY**

Know why you are using each antibody.
Know how both a “positive” and a “negative” reaction look.
Make sure your control slides are valid.
Know what a positive reaction will mean in your application.
Know what a negative reaction will mean.
Know what pattern you should expect to see.
Know whether this is a “hard” or “soft” marker.
Determine the reaction and see if it confirms or supports your diagnosis.