"CRITICAL" IMMUNOHISTOCHEMICAL AND GENETIC MARKERS IN GLIAL NEOPLASMS

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March 7, 2008
Introduction:

Pathologists have been inundated by published reports of new and potentially interesting diagnostic, prognostic, and putative predictive “markers” whose expression (or loss) hold great promise for more enlightened diagnoses and ultimately better patient care. The first findings of the NCI’s Cancer Genome Atlas project (1) suggest that although an understanding of therapeutically (and possibly diagnostically) relevant pathways of glioblastoma may be at hand, significant challenges remain (2). While some immunohistochemical and genetic tests have proved to be useful in day to day practice, the utility of many others awaits further study and validation. Yip and colleagues (3) have stressed the importance of critical review of the literature and careful consideration of practical issues such as test standardization, compliance, cost-effectiveness and availability.

Histological study of appropriately sampled tissue remains the gold standard for glioma diagnosis (4). The current WHO Classification of nervous system tumors (5) is based on histopathological diagnosis of defined entities and variants with confirmation by ancillary testing. A guiding principle of this presentation is that immunohistochemical and molecular studies supplement or complement conventional H&E histology. Ancillary studies are particularly useful in small samples from stereotactic biopsies that are used in surgical neuropathology. Information such as patient age, duration of clinical symptoms, and imaging findings showing the location (intra-axial vs. extra-axial) and appearance of the lesion typically provide a working differential diagnosis for subsequent histopathological analysis. An awareness of clinicopathologic conditions in surgical neuropathology and known variations or variants of histopathologic entities should guide the judicious use of ancillary studies (rather than the other way around).

Another reason to advocate a practical approach for ancillary testing in glioma diagnosis is that many patients will opt for an investigational therapeutic protocol. Most protocols today require tissue studies using the paraffin blocks. A requirement for as much as 200 mg of viable tumor will be necessary in some cases for the patient's inclusion in a clinical trial. Therefore, when the diagnosis of a glioblastoma (for example) is readily apparent by routine histology, it may be more prudent to ensure that sufficient tissue is available for such protocols rather than exhausting the block with many unnecessary or poorly validated immunostains.

Putting the “Multiforme” back into Glioblastoma (WHO Grade IV)

Since the 2000 WHO classification of nervous system tumors, the term “multiforme” has been excluded from glioblastoma multiforme (GBM), the most common malignant glioma of adults (6). However, GBM (the ‘M’ being retained for present purposes) is defined by significant variations in histology, both within individual tumors and among different patients. Molecular heterogeneity is well recognized in GBM (7) and some molecular genetic characteristics have been associated with certain histologic features (8-11). Molecular testing is now required for patient stratification in clinical trials for GBM but such has not yet reached ‘mainstream’ pathology. In this section, the utility of ancillary studies for the diagnosis of some important GBMs representing two ends of a histologic spectrum is discussed.

The presence of occasional multinucleated tumor giant cells is common in typical examples of glioblastoma multiforme. However, the predominance of giant cells along with cohesion, distinct cell borders, and a reticulin-rich stroma are histologic features of the giant cell variant of GBM (GCGBM) (5). This variant accounts for up to 5% of all glioblastomas. The average age of presentation is 42 years, which is less than for GBM overall (55 years), and the former has a wider age range. Historically referred to as “monstrocellular sarcoma”, the advent of immunohistochemistry and strong reactivity of the tumor cells for glial fibrillary acidic protein (GFAP) defined the glial lineage of this neoplasm. GCGBM may mimic a metastasis because both tumors typically show gross circumscripton with associated peritumoral edema, a subcortical location, and highly atypical tumor cells with cohesive cell clusters and distinct cell borders. However, presence of a single lesion on imaging studies in a relatively young patient would favor a primary CNS tumor. The finding of GFAP reactivity in GCGBM is especially useful because of the known cross-reactivity of some cytokeratin antibody cocktails (CAM5.2, MAK6) in glioma cells and even reactive astrocytes (12,13). This potential pitfall should be kept in mind when the differential diagnosis is between glioma and metastasis.
Molecular and genetic analysis has revealed that GCGBM occupies an intermediate position between primary (de novo) GBM and those that are believed to arise from sequential anaplastic transformation of lower grade tumors (so called "secondary GBM") (5,10,14,15). Like the primary GBM, GCGBMs tend to arise de novo, have a short clinical history, and have mutations of the PTEN gene in about one third of cases. However, p53 mutations have been demonstrated in 75-90% of GCGBM while EGFR amplification is rare. The opposite is true of 'primary' GBM (5). Frequent p53 mutations and the lower age at diagnosis are features shared by GCGBM and secondary GBM. While this variant of glioblastoma has been reported to have a better prognosis than more typical GBM, this may be related to the higher proportion of younger patients with the giant cell variant.

At the other end of the size spectrum, small neoplastic cells are also common in glioblastomas. However, malignant astrocytic gliomas composed primarily of small neoplastic cells have been recently defined (8,9). The "small cell glioblastoma" (WHO Grade IV) is important to recognize because of its histologic resemblance to the anaplastic oligodendroglioma (WHO Grade III). Histologically, glioblastomas showing predominantly small cell architecture are highly cellular and cytologically monotonous (8). They are primarily composed of small astrocytic cells with oval, mildly hyperchromatic, and deceptively bland nuclei that contain occasional small nucleoli (9). Mitoses are typically frequent and cytoplasmic borders are inconspicuous. While vascular endothelial proliferation and necrosis are present in the small cell GBM, as they are in other forms of GBM, Perry and colleagues emphasized that gliomas falling within the histologic spectrum of WHO grade III anaplastic astrocytomas could also show small cell histology (9). Such tumors can mimic the radiologic and histopathologic features of anaplastic oligodendrogliomas, but have a much worse prognosis (11 months median survival). In addition to nuclear and cellular uniformity, chicken-wire vasculature, clear haloes, perineuronal satellitosis, and microcalcifications were observed, thus further simulating oligodendrogial tumors. 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.

Astrocytic features of small cell glioblastomas and anaplastic astrocytomas are readily demonstrated by immunohistochemistry for GFAP, which reveals strong reactivity of thin cytoplasmic processes. A high MIB-1 labeling index is consistent with the frequent mitotic figures that characterize these tumors. Molecular characteristics help to further define the small cell glioblastoma. A high proportion of GBMs with small cell phenotype show amplification of the epidermal growth factor receptor (EGFR) (8,9,11). About 50% of tumors are immunoreactive for EGFR variant III, while about 80% are at least focally positive for wild type EGFR. Additional molecular changes include gains or polysomy of chromosome 7 and chromosome 10q deletions. In marked contrast to oligodendrogial neoplasms, none of the small cell astrocytic tumors showed 1p deletions and only one case was deleted for chromosome 19q. These findings lead Perry et al. to conclude that, in addition to 1p and 19q testing, analysis of EGFR and 10q status may improve diagnostic sensitivity in difficult cases (9).

Infiltrating vs. Focal Processes

Diffuse gliomas encompassing WHO Grades II-IV all invade the central nervous system. Pleomorphism, mitoses, vascular endothelial proliferation/hyperplasia, and necrosis are used for grading gliomas but all will have infiltration (4,5). Thus, diffuse astrocytomas (WHO Grade II) are composed of GFAP-immunoreactive, differentiated fibrillary or gemistocytic, astrocyte-like cells that infiltrate gray matter producing 'perineuronal satellitosis' with subpial and perivascular tumor cell accumulations (i.e., "Scherer's secondary structures"). Infiltration of white matter by tumor may occasionally be more subtle but can be demonstrated by immunohistochemistry using antibodies that recognize phosphorylation-dependant epitopes of neurofilament protein (NFP) to visualize axons. This may be useful for small biopsies or in situations where more focal forms of neoplasia are being considered. However, even "circumscribed" tumors such as pilocytic astrocytoma or ganglioglioma may at least focally infiltrate adjacent brain tissue.

Bizarre giant neoplastic cells, nuclear pseudo-inclusions, prominent intratumoral collections of lymphocytes, and cytoplasmic lipidization are features of the pleomorphic xanthoastrocytoma (PXA). This
tumor primarily affects younger individuals with superficially located solid or cystic brain lesions and a long history of seizures. PXA can be distinguished from the giant cell glioblastoma by the paucity of mitotic figures and relative lack of necrosis. Bizarre-appearing giant cells are also a feature of the subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis. However, the clinical history, periventricular location, and absence of mitotic activity or spontaneous necrosis of the SEGA distinguish it from the GGBM. The PXA and SEGAs share in common a tendency for individual neoplastic cells to express neuronal lineage antigens such as synaptophysin and neurofilament protein subtypes. Glioneuronal tumors will not be discussed formally here. However, the availability of several quite dependable antibodies that recognize neuronal lineage antigens will be useful in the evaluation of such tumors and their distinction from the more common gliomas.

**Cell Proliferation in the Diffuse Gliomas**

A critical decision point in glioma diagnosis is the distinction between a WHO Grade II diffuse astrocytoma and a grade III anaplastic astrocytoma. The finding of mitoses by H&E is the most defining diagnostic criterion (4,5,16-18). However, technical considerations, subjectivity, and the experience and diligence of the pathologist are all limiting factors in accurate mitotic counting. The immunohistochemical detection of phospho-histone H3, which is expressed during chromatin condensation during mitosis, was recently shown to be an independent predictor of survival in the diffuse astrocytomas and shows great promise for increased accuracy in detecting mitotic figures (19). Ki67 is a non-histone, cell-cycle-associated antigen that is expressed during G1, S, and G2/M phases. 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 (4,20-26).

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 low grade 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)(5). Issues such as individual laboratory technique (manual vs. automated counting), interobserver variation, and regional heterogeneity within diffuse gliomas have precluded the establishment of definitive cutoffs between low and high grade gliomas at this time.

**Molecular Diagnosis in Gliomas: A Work in Progress:**

Current recommendations for molecular testing for the malignant gliomas have been addressed thoroughly in several outstanding reviews (3,4,27). Only a brief update will be provided here. Temozolamide is the first-line chemotherapeutic agent of choice for malignant gliomas that damages DNA by adding methyl groups to the O6 position of guanine. This effect can be reversed by the endogenous DNA repair enzyme MGMT (O6-methylguanine-DNA methyltransferase), thus providing a mechanism of chemoresistance to alkylating agent chemotherapies. Several studies have suggested that epigenetic 'silencing' of MGMT by methylation of the gene's promoter region, which occurs in 40-50% of glioblastomas, is associated with improved survival and, by implication, a better response to temozolamide and other alkylating agents (28,29). Assessments of MGMT promoter methylation are currently being performed in clinical trials, and strategies to modulate MGMT activity to make tumors more responsive to therapy are under investigation (29).

However, routine laboratory testing for MGMT promoter methylation or expression is not recommended at the present time (3). First of all, assays for measuring MGMT promoter methylation are nontrivial and each method presents different advantages and disadvantages. There has not been good correlation between MGMT promoter methylation status and protein expression as determined by immunohistochemistry (30-32), and it was recently reported that MGMT promoter methylation status may change in serial glioblastoma samples (33). Furthermore, there are currently no good alternatives to temozolamide and radiation therapy in the treatment of glioblastoma, and even patients with non-methylated MGMT promoter regions demonstrate a survival benefit with this therapy.
Identifying molecular genetic pathways leading to glioma tumorigenesis holds great promise for diagnostic, prognostic, and predictive testing. Involvement of specific genetic alterations was suggested by the common cytogenetic findings of chromosome 10 loss and chromosome 7 amplification in glioblastoma. Subsequently, mutation or deletion of PTEN (chromosome 10) was identified as a mechanism for activation of the PI3K/AKT pathway, which is associated with increased invasion, proliferation, and tumor cell survival (27). Chromosome 7 gains correlate with amplification of the epidermal growth factor receptor (EGFR) that occurs in about 40% of GBMs (4). A subset of patients with glioblastomas that express a truncated, constitutively activated form of this receptor (EGFRvIII) and co-express PTEN (thus inhibiting the PI3K/AKT pathway) were shown to respond better to therapy with small molecule inhibitors (gefitinib, erlotinib) (34). However, limited availability of an EGFRvIII antibody and disappointing results of subsequent trials with small molecule inhibitors have argued against routine laboratory testing at this time (3). Continued study of EGFR and the PTEN/PI3K/AKT pathway may better patient stratification for therapy based on tissue analysis (35).

Oligodendroglial tumors: Mind your (1)P’s and (19)Q’s!

The peak incidence of oligodendroglioma (O, WHO Grade II) and anaplastic oligodendroglioma (AO, WHO Grade III) occurs during the fifth decade (5). One of the most significant discoveries in glioma diagnosis and treatment is the strong association between co-deletions of chromosomes 1p and 19q and oligodendroglioma histology and improved prognosis in AO (36-40). A specific 1p/19q translocation was recently described suggesting a possible mechanism of co-deletion in some oligodendrogliomas (41,42). The association of this genetic change with the 'oligodendrogial' phenotype is strongest when strict histopathological criteria are used (36,37,40). Namely, classic oligodendroglioma is defined by a highly uniform population of tumor cells with round/regular nuclei, and little cell variability. Non-specific but useful features include extensive involvement of the cerebral cortex, perinuclear cytoplasmic clearing ('haloes'), presence of nodules, microcalcifications, microcysts, and delicate branching (chicken-wire-like) capillaries (4,5,40). The presence of strongly GFAP-reactive 'minigemistocytes' and 'gliofibrillary oligodendrocytes' in some oligodendrogliomas does not permit a histopathological distinction between astrocytic and oligodendrocytic tumors on this basis.

Testing of diffuse gliomas with oligodendroglioma-like features for 1p/19q deletions is currently performed by fluorescence in situ hybridization (FISH), loss of heterozygosity assays, or array comparative genomic hybridization. Although there are advantages and disadvantages of each method, testing is robust and is recommended at this time for diagnosis (to complement histopathology) and prognosis (4,5). Such testing may be quite useful in the 'mixed' gliomas, a gray area in neuropathology where other than a lack of histological uniformity, a definitive diagnosis may be difficult (3-5). The utility of this test in distinguishing small cell astrocytic gliomas from oligodendroglioma neoplasms was discussed above. Also, several oligodendroglioma mimics such as central neurocytoma, dysembryoplastic neuroepithelial tumor, extraventricular neurocytoma, and clear cell ependymoma do not show the 1p/19q deletion. Finally, oligodendrogliomas are rare in children and typically do not show 1p/19q deletions in this population.

Important mimics: Active demyelination and lymphoma:

Immunohistochemical studies along with standard special histochemical stains may be useful in evaluating non-glial tumors and tumefactive non-neoplastic processes. Demyelinating disease is the most important of these since therapy will be much different from that of a malignant glioma. Identifying macrophages on intra-operative consultation 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. Although axonal stains (neurofilament IHC or Bodian) may show relative sparing of axons typical of a demyelinating disease, axons degenerate in advanced lesions. In suspected cases of progressive multifocal leukoencephalopathy (PML), characteristic oligodendrogial viral inclusions, recognition of the reactive nature of bizarre astrocytes, and immunohistochemical stains for polyomavirus large T antigen supports the diagnosis. One important caveat is that p53, which is immunoreactive in
about 50% of gliomas (especially astrocytic types), is also reactive in the bizarre astrocytes of PML (43-45).

While the histopathological appearance of atypical lymphoid cells showing an angiocentric growth pattern may suggest a primary CNS lymphoma, immunohistochemical studies for B- (CD20) and T-lymphocytes (CD3) will usually be required for definitive diagnosis.

**Ependymomas and ‘ependymal-like’ tumors**

Classic ependymomas with their uniform histology, perivascular pseudorosettes, and tapering GFAP-immunoreactive processes may not present a significant diagnostic challenge for experienced pathologists. However, several recently described gliomas show histological, immunophenotypic, and/or ultrastructural characteristics that suggest ependymal-like differentiation (46). The **tanyctic variant of ependymoma** primarily arises in the spinal cord but occasionally presents as a third ventricular or hypothalamic mass. They are typically well demarcated from the surrounding neural tissue. The highly spindled microscopic appearance of bipolar tumor cell processes creates an astrocytic appearance, which is further suggested by poorly defined or absent perivascular pseudorosettes. However, tumor cell nuclei are quite uniform, round to oval, and have salt and pepper-like chromatin similar to other types of ependymomas. Tumor cell processes are typically strongly immunoreactive for GFAP, S-100, vimentin, and CD99. Ultrastructural features including intercellular junctions, numerous slender surface microvilli, and even microvilli-lined lumina are distinctly ependymal. The **clear cell ependymoma** may mimic an oligodendroglioma in its cytologic uniformity. While a circumscribed (rather than infiltrative) border distinguishes this tumor from an oligodendroglioma, the demonstration of ‘ependymal’ features by EM may be required for definitive diagnosis in some cases.

**Astroblastoma** is a rare glial neoplasm primarily affects children and young adults and has the unifying histological feature of perivascular pseudorosette-like arrangements composed of broad, non-tapering cell processes (47, 48). There is no formal WHO Grade at this time. By definition, astroblastomas lack the histologic features of other infiltrating astrocytomas, gemistocytic astrocytomas, and typical ependymomas. The processes of astroblastoma are shorter and less spindled than those of ependymoma and sclerosis of vascular elements may be striking. Immunohistochemical and ultrastructural findings suggest both astrocytic and ependymal features. Tumor cell processes are typically reactive for vimentin and S-100, while GFAP shows variable positivity. Focal membranous pattern of EMA reactivity is well described while neuronal lineage is not typically seen. Ultrastructural features of basal body polarization, apical cytoplasmic blebs with microvilli, and lamellar cytoplasmic interdigitations have suggested a tanyctic histogenesis. A relationship between Ki-67 labeling has not been established as an independent prognostic feature but astroblastomas with high grade histology have higher labeling indices. Gross total resection is associated with a favorable outcome even in high grade tumors although the prognosis is better in patients with low grade histology.

Clinicopathologic features of the **angiocentric glioma** (WHO Grade I) (“monomorphous angiocentric glioma”, “angiocentric neuroepithelial tumor”) were independently described by two groups in 2005 (49, 50). Most of these tumors have arisen in children and young adults with a history of chronic intractable epilepsy. Common sites include superficial cortical regions of the frontoparietal lobe and the temporal lobes including the hippocampi and parahippocampal gyri. Imaging studies and low magnification histology suggest an infiltrating glioma with striking perivascular and subpial spread. However, higher power reveals the tumor to be composed of monomorphic, bipolar cells with elongated nuclei having distinctly speckled, “crisp” chromatin (49). The striking angiocentric pattern is produced by the circumferential or longitudinal orientation of tumor cells along large and small blood vessels. Radial perivascular arrangements of tumor cell processes may focally resemble that of conventional ependymoma or even astroblastoma. Mitoses are rare or absent and there is typically no vascular endothelial proliferation or necrosis. Tumor cells are immunoreactive for GFAP, S-100, and vimentin but are negative for antigens of neuronal lineage (49-51). A distinctive dot-like, microlumen pattern of immunoreactivity for epithelial membrane antigen is typical. MIB-1/Ki67 labeling indices are low (1-5%) although one anaplastic recurrence had a Ki-67 labeling index of 10% (49). A histogenetic origin from bipolar radial glia has been suggested. The prognosis is generally good with gross total excision. An anaplastic recurrence was noted in one subtotally resected tumor (49).
The chordoid glioma of the third ventricle was first defined by Brat and colleagues in 1998 (52) and this rare brain tumor is formally recognized as a codified entity by the WHO. This distinctive neoplasm typically arises in the anterior third ventricle in the region of the lamina terminalis (53). Adults are most often affected with a mean age of 46 years and an approximately 2:1 female predominance. Patients may present with hydrocephalus, headaches, and nausea due to ventricular obstruction. Endocrine dysfunction and visual disturbances may also occur due to compressive effects on the hypothalamus and optic chiasm, respectively. Imaging reveals a contrast enhancing, well circumscribed mass of the third ventricle. Classic histologic findings include cohesive cords and clusters of epithelial-like tumor cells that are dispersed within a myxoid stroma that contains a striking lymphoplasmacytic infiltrate. While these features might suggest a chordoma or chordoid meningioma, the finding of strong GFAP immunoreactivity of the tumor cells will confirm the diagnosis. Mitoses are rare or absent and MIB-1 labeling indices are typically low. Numerous Russell bodies can be observed within the stromal lymphoplasmacytic infiltrates. Tumor cells are also immunoreactive for vimentin and variably positive for S-100. Ultrastructurally, tumor cells contain intermediate filaments, intercellular lumina with apical microvilli, hemidesmosomes, basal laminae, and possibly secretory vacuoles. Derivation from specialized ependyma that covers the laminae terminalis and circumventricular organs has been suggested. The attachment of this WHO Grade II tumor to adjacent hypothalamic and suprasellar structures may not allow for complete excision and subsequent recurrence (5).

Future Directions in Glioma Diagnosis:

Gene expression profiling using high-throughput oligonucleotide array platforms holds great promise for improved diagnostic, prognostic and predictive testing, and for the discovery of novel therapeutic targets. Recent studies have indicated that such profiling is as good as histology in subclassifying diffuse gliomas and better than histology in predicting outcomes of mixed or otherwise ambiguous cases (27,54-57). Also, it was recently discovered that gains (tandem duplications) on chromosome 7q34 that involve the kinase domain of the BRAF oncogene occur in pilocytic astrocytomas (58,59) but not in other gliomas. These studies provided evidence for activation of a RAS-BRAF-ERK signaling pathway that could be targeted in the treatment of non-resectable cases. Finally, the identification and study of tumor initiating stem cells is yet another exciting area that may provide a better understanding of glioma tumorigenesis and the development of resistance during therapy (60,61).

References:


New Immunohistochemical Markers in the Evaluation of Primary Non-Glial Central Nervous System Tumors

Suzanne Z. Powell, M.D.
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Houston, TX

Recent Advances

- Germinoma
- Hemangioblastoma
- Meningioma
- Solitary Fibrous Tumor/Hemangiopericytoma
- Hereditary Schwannoma
- Craniopharyngioma vs. Rathke’s cleft cyst

Germinoma

- Occurs predominantly in the midline
- Suprasellar and pineal regions
- Biphasic pattern – large cells with prominent nucleoli admixed with lymphocytes
- When lymphocytes predominate, immunohistochemical analysis may be critical for diagnosis

Germinoma

- Placental alkaline phosphatase, PLAP – well-established marker; **membranous** pattern of staining
- C-kit proto-oncogene (CD117)
  - Present on cell surface in almost all seminomas and dysgerminomas
  - Rarely expressed in other germ cell tumors
  - CD30 and C-kit in combination have been useful in separating embryonal components in gonadal tumors
Germinoma

- **OCT4** (POU5F1, OCT3 or OTF3) – Nuclear transcription factor expressed in germ cells
- Regulation of “pluripotent” potential in germ cells
- Necessary for stem cell formation
- Almost 100% immunoreactivity in seminoma and embryonal carcinomas of the testes (Jones et al, Am J Surg Path 2004) negative in other germ cell components
- **Nuclear** staining pattern

Hemangioblastoma

- WHO 2007 grade I neoplasm of “uncertain histogenesis”
- Composed of capillaries and stromal cells – cerebellum and spinal cord
- Sporadic or associated with Von Hippel Lindau
- Necessity to differentiate from metastatic renal cell carcinoma in those patients with VHL
- Jarrel et al (J Neurosurg 2006) reported 8% of patients with VHL had metastases (most commonly RCC) within a hemangioblastoma
Hemangioblastoma – Alpha Inhibin

- Produced in Sertoli cells of the testes and granulosa cells of the ovary normally
- Expressed in sex cord stromal tumors and adrenal cortical tumors
- Hoang (Am J Surg Path 2003) demonstrated cytoplasmic immunoreactivity in stromal cells in hemangioblastoma and negative staining for renal cell carcinoma

Hemangioblastoma – Aquaporin 1

- Weinbreck et al (Am J Surg Path, 2008) found that a panel including alpha-inhibin and aquaporin 1 were reliable positive markers for hemangioblastoma
- Membranous pattern – aquaporin 1
- AE1/AE3 and CD10 were reliable markers of clear cell renal cell carcinoma (CCRCC)
- Combined use of AE1/AE3 and aquaporin 1 were reliable for the differentiation of hemangioblastoma and metastatic CCRCC

Aquaporin 1 in hemangioblastoma

Immunofluorescence
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**Meningioma**

- **Claudin-1**
- **PHH-3**
- **Secretory meningioma variant study**

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**Meningioma – Claudin-1**

- Tight junction-associated protein
- Identified in perineurial cells and soft tissue perineuriomas (Folpe et al Am J Surg Path, 2002)
- Bhattacharjee et al (AANP 2003) reported claudin-1 immunoreactivity in 85% of meningiomas with a punctate membranous pattern
- Hahn et al (AJCP 2006) found a similar pattern, but in a small percentage of cases, 53% studied

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**Expression of Claudin-1, a Recently Described Tight Junction-Associated Protein, Distinguishes Soft Tissue Perineurioma From Potential Mimics**

Andrew E. Folpe, M.D., Steven D. Billings, M.D., Jesse K. Mc Keown, M.D., Shari E. Walsh, M.D., Anuva Navsaria, M.D., and Shamsa W. Weiss, M.D.

Perineuriomas are rare benign soft tissue tumors having an immunophenotype resembling the neural perineurial cell (5,200 genes negative and 800 genes positive) and are categorized as perineuriomas. One of the main features of perineuriomas is the expression of claudin-1, a tight junction protein that has been shown to be expressed in a variety of normal and malignant tissues. The expression of claudin-1 in perineuriomas has been reported to be high and specific, distinguishing them from other soft tissue tumors. This study aimed to evaluate the expression of claudin-1 in perineuriomas and to determine its diagnostic utility.

Claudin-1 has been shown to be a useful marker in the diagnosis of meningiomas, as it is expressed in a punctate membranous pattern in up to 85% of meningiomas. However, Hahn et al. (AJCP 2006) reported a similar pattern in a small percentage of meningiomas, with 53% studied.

In this study, the expression of claudin-1 was evaluated in a series of 30 perineuriomas and compared to other soft tissue tumors. The results showed a high and specific expression of claudin-1 in perineuriomas, distinguishing them from other soft tissue tumors. This finding has important implications for the diagnosis and management of perineuriomas, as it provides a useful diagnostic marker.
Phospho-Histone H3 (pHH3)

A Mitotic Figure Immunostain

Phospho-Histone H3


Mitotic Figure Immunostain

Phospho-Histone H3 (pHH3)

Mitotic Figure Immunostain

Phospho-Histone H3 (pHH3)
Phospho-Histone H3 (pHH3)

Mitotic Figure Immunostain

Phosphohistone H3 (pHH3) Facilitates Rapid Reliable Grading of Meningiomas According to WHO 2000 Criteria

Teresa B. Block, MD, PhD; Tae E. McCluggage, MD; Kristopher D. Aldape, MD, PhD; Janet M. Brenner, MD, and Gregory N. Vollmer, MD, PhD


Table 4B
Multivariate Recurrence-Free Survival Analysis by Cox Hazard Regression in 99 Meningioma Cases

<table>
<thead>
<tr>
<th>Hazard Ratio (95% Confidence Interval)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03 (1.00-1.06)†</td>
</tr>
<tr>
<td>Extent of resection (subtotal resection)</td>
<td>2.19 (0.97-4.91)</td>
</tr>
<tr>
<td>H&amp;E MI</td>
<td>1.08 (1.03-1.14)‡</td>
</tr>
<tr>
<td>PHH3 MI</td>
<td>1.07 (1.06-1.10)‡</td>
</tr>
<tr>
<td>Ki-67 LI</td>
<td>1.03 (1.00-1.06)¶</td>
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H&E MI, mitotic index assessed in H&E-stained slides; LI, labeling index; PHH3 MI, mitotic index assessed in anti-phosphohistone H3-immunolabeled specimens.

† By the log-rank test.
‡ Hazard ratio per year of patient age.
¶ Hazard ratio per anastomosis.
Meningioma, General

- Cytokeratin expression depends on meningioma subtype
- Focal positivity except in microcystic and anaplastic tumors
- CK18 positivity commonly present in all types
- Negativity overall for CK20 (Mieettinen and Paetau, 2002)

Secretory Meningioma

- CK immunoreactivity is unique, confined to cells adjacent to secretory material ("pseudopsammoma bodies") – CK18, also CK7, CK8 and CK19
- CEA immunoreactivity well described in the secretory droplets
- Recent review of 6 cases (Caffo et al, J of Clin Neuroscience, May 2008) examining extracellular matrix proteins laminin, fibronectin and type IV collagen found staining for all three proteins in 2 of the 6 cases, and mild to absent immunoreactivity in the remaining 4 cases

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Solitary Fibrous Tumor to Hemangiopericytoma – A Continuum?

- Panel of immunostains including EMA, CD99, BCL-2 and CD34 for distinguishing meningioma from SFT and HPC
- SFT and HPC having overlapping histologic features
- SFT and HPC remain separate entities in CNS as opposed to soft tissues in which this diagnosis has merged and HPC is a “pattern”

SFT/HPC Immunohistochemical staining patterns

- BCL-2 and CD34 are diffuse and strongly positive in SFT; weak and focal in HPC
- CD34 is strongly positive not only in vessels, but in the cytoplasm in SFT
- Usually diffuse, with less staining in HPC
- CD99 often shows a similar pattern to CD34, but may be negative
- EMA is often negative, distinguishing these tumors from meningiomas, but may have focal positive staining areas
Hereditary Schwannoma

- *INI-1/SMARCB1* protein recently implicated in pathogenesis of schwannoma in a family with familial schwannomatosis
- Mutations of *SMARCB1* gene identified
- Mosaic loss of expression of INI1

RESEARCH ARTICLE

*Immunohistochemical Analysis Supports a Role for INI1/SMARC1 in Hereditary Forms of Schwannomas, but Not in Solitary, Sporadic Schwannomas*


Department of Pathology, Washington University School of Medicine, St. Louis, MO.

Department of Oncology, Massachusetts General Hospital, Boston, MA.

Department of Surgery, Dana-Farber Cancer Institute, Boston, MA.
INI-1 staining (Panel B) in hereditary schwannoma versus the diffuse positivity in a solitary, sporadic tumor (Panel A)

Sporadic Schwannoma

IN1 – Sporadic Schwannoma

Sporadic Schwannoma

Outpatient Clinic Building

TMH Outpatient Facility
Project Scope
24 Floor terrace
TPC $ 331 M
760,000 GSF
occupied space
846,000 GSF
parking – 1370 spaces

Rapid Communication
Evidence of a Four-Hit Mechanism Involving SMARCB1 and NF2 in Schwannomatosis-Associated Schwannomas

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Sellar/suprasellar masses with differing clinical outcomes

Purely cystic craniopharyngiomas vs. Rathke cleft cysts with squamous metaplasia and without significant ciliated epithelium may be extremely challenging

Craniopharyngioma may also rarely demonstrate ciliated epithelium, compounding an already difficult problem

Beta-catenin

Adamantinomatous craniopharyngiomas have been found to have an activating mutation in exon 3 of the beta-catenin gene in approximately 90% of cases examined (Buslei et al, Acta Neuropathologica, 2005)

Pattern of staining is inhomogeneous and may be clustered in “whorls”

Whorl-like immunoreactivity is associated with positive staining for CK8 (CAM5.2) and CK18
Betacatenin

- Rathke cleft cyst (RCC) and papillary craniopharyngioma immunoreactivity is exclusively membranous.
- Distinctive staining patterns may be useful in separating these entities, especially in small surgical specimens.
- NOT useful for distinguishing papillary craniopharyngioma from RCC with squamous metaplasia.

Cam 5.2 (CK8)

- Strong immunoreactivity for beta catenin in a "whorl".

CAM 5.2 (CK8)

- Beta – catenin – negative staining of "wet" keratin.

CK5-6

- Beta – catenin – negative staining of "wet" keratin.

CK7

- Cam 5.2 (CK8)
Beta-Catenin
membranous staining pattern in epithelium of papillary craniopharyngioma

Papillary Craniopharyngioma

Rathke’s cleft cyst

Membranous pattern as in papillary CP
Rathke’s Cyst

Pan Keratin

Utilizing the Differences

Cranioopharyngioma, Adamantinomatous Type
“Whorl-like” positivity for beta-catenin
Superficial staining for CK7, diffuse staining for CK5/6

Rathke’s Cyst
Membranous pattern of staining for beta-catenin as in Papillary craniopharyngioma
Similar patterns of staining as for adamantinomatous craniopharyngioma for CK7 and CK5/6 (CAM 5.2)

The Methodist Hospital System

Community Hospitals

San Jacinto Methodist Hospital
264 Licensed Beds
14,201 Inpatients
36,913 ER Visits

Methodist Sugar Land Hospital
154 Licensed Beds
5,204 Inpatients
33,495 ER Visits

Methodist Willowbrook Hospital
119 Licensed Beds
8,285 Inpatients
44,623 ER Visits

West Houston Methodist Hospital
200 beds
Spring 2010

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200 beds
Spring 2010
This symposium has several main objectives:

1. Demonstrate the global burden of disease and the disproportionate amount of infectious diseases that track with poverty, many of which, secondary to immigration and travel, must be diagnosed and treated in North America.
2. Highlight four infectious diseases affecting the CNS that are seeing global, local, or epidemic burdens increase in recent years.
3. Provide the working neuropathologist with a list of tools and resources to approach these and other difficult infectious diagnoses within the CNS.

World Mapper is a data viewer (www.worldmapper.org) that plots different statistics proportionately on a global map. There are many dozens of maps available which are related to healthcare. In this particular view, the dollars spent on public health by each country are shown. When the total dollars spent is largely disproportionate to the population size (e.g. Africa, India, and China), situations of massive healthcare disparity occur. Several other World Mapper views are included at the end of this handout that show similar patterns for the burden of malaria, the number of doctors, etc. Within the context of central nervous system diseases, infections represent a small portion relative to the whole because the dollars spent on public health (which is an inverse metric for the burden of infectious diseases overall) reduce infections to rarities. In the modern world, a family from Africa who has lived in abject poverty can be in New York or Atlanta in less than 24 hours, bringing with them quiescent infections that may not present for weeks to years later. Rapidly expanding economies in India and China have newly wealthy business travelers and their families moving around the globe at rapid rates. Thus, as we consider the ever expanding human population, we must remember that when we view data presented above, that those dollars spent are only benefiting/injuring long term inhabitants of a country. © Copyright 2006 SASI Group (University of Sheffield) and Mark Newman (University of Michigan).

From the WHO’s 2002 data on population and DALYs (Disability Adjusted Life Years), we see the effects of the public health spending (and other health metrics) reflected in the massive differences in types of disease burden in different continents and regions. DALYs are not only life years lost when a person dies but effective life years lost due to disability. North Americans are very familiar with the leading healthcare issues in their own continent with DALYs lost attributed to neuropsychiatric, cardiovascular, cancer, pulmonary, and musculoskeletal diseases primarily (with accidents also contributing non-medical causes). The burden of infectious diseases in the Americas, Europe, and the Western Pacific is fourth or fifth
overall when considered as a group but individually never contribute more than 3%. In Southeast Asia, the Middle East, and Africa, we see the opposite with a third to 61% of all DALYs lost attributed to infectious diseases; moreover, as a category, infectious diseases dominate in these three regions. On an individual basis, malaria, HIV, and respiratory infections make up nearly 40% of the DALYs lost in Africa. These data are now 7 years old and there are many campaigns to reduce this deficit including ARV treatment for HIV, malaria control, vaccination, and elimination programs, and individual health treatment initiatives such as the Millennium Villages Project, Partners in Health, MSF, etc. However, economy is moving faster than these programs and the drive for peoples from these effected regions to move to other countries (especially North America) means that infectious diseases endemic in these countries are “emerging.”

Viral infectious of the CNS are by and large diagnosed on clinical grounds due to high clinical suspicion and confirmation with appropriate serology or antigen testing of either the peripheral blood or CNS. Rare is the report in the modern literature of a viral CNS infection diagnosed primarily on a histological biopsy as morbidity and mortality are nearly always increased with surgical intervention. In this handout, there are several long tables listing the many viral diseases of the CNS, some of which are certainly considering new and emerging or re-emerging. However, one of the most challenging viral infections to definitively diagnose pre-mortem is Rabies, a topic on which we will expand a bit.

A current discussion of rabies virus incidence, prevalence and new understandings of biology and immune evasion will be presented.[1, 2]

Unlike viral infections which can be difficult to localize, bacterial infections can be greatly augmented by radiology and microbiological culture. When a bacterial abscess is present, the differential includes fungal and parasitic infections as well as non-infectious causes. Unlike viral infections, surgical drainage and/or biopsies are more common in order to drain abscesses and especially for antibiotic resistance testing in prolonged lesions. Although the pathologist may be able to give a gram staining pattern and a morphology reading, culture is required for definitive diagnosis and antibiotic testing. 16S rRNA PCR and sequencing can be performed on formalin fixed tissue when no culture is available (e.g., the CDC); however, this does not provide antibiotic susceptibility in most cases. Fungal infections can be evaluated using several peripheral blood markers (Galactomannan and Beta-1,3-glucan). Parasitic infections (with the
exception of Toxoplasmosis) will usually have a clinical history of exposure; however, if patients are severely ill (i.e., coma) obtaining such a history may be difficult especially if family members are either not available or language is a barrier. Mycobacterial disease, especially disseminated tuberculosis, requires marked input from the neuropathologist with regards to the pattern of inflammation (granulomatous vs. neutrophilic), presence of AFB on special stains, etc. Let us further explore recent developments in the diagnosis of Mycobacterium tuberculosis in the CSF.

To begin, remember that aseptic or culture-negative meningitis can include a long list of organisms. Many of these organisms are aseptic because growth is not amenable to typical culture techniques and/or the specimen is not handled properly before arriving in microbiology. In some cases, however, growth may take an excessive amount of time (e.g., mycobacteria). Molecular techniques on both fresh tissue (if obtained) and CSF can be tremendously helpful in ruling in these organisms (though not the opposite) and a role for traditional serology is still common for both CSF and peripheral blood. From the perspective of a neuropathologist, very few of these lesions are ever going to produce a brain biopsy (nor should they!) with the exception of M. tuberculosis and Nocardiosis—the former due to bulky granulomatous disease and the latter due to the propensity to form abscesses eventually. Both organisms have AFB staining patterns but can be easily distinguished by morphology and milieu.

A current discussion of detection of mycobacterial disease in the CNS will be presented[3, 4].

Parasitic disease is a catchall expression for any non-viral/non-bacterial/non-fungal disease of humans which includes some TRUE parasites but many opportunistic or incidental infections which can not truly be thought of as parasitic. For example, in the diagrams above are several schematics of general life cycles of parasites. Diagrams A, D, and E demonstrate what can be thought of as true parasitic relationships with
examples being malaria, tapeworms, and roundworms or entamoeba, respectively. In these cycles, humans are REQUIRED for the parasite to complete their lives and a significant number of patients infected with the organism are asymptomatic. B and C represent zoonotic diseases such as African Sleeping Sickness and Toxoplasmosis respectively in which humans are either incidental hosts or dead end hosts. In the former, disease is very severe in humans (with 100% mortality if not treated for African Sleeping Sickness) because humans are not commonly part of the life cycle. In the latter, the role of the human as a reservoir that must be “consumed” by another organism such as a carnivore to complete the life cycle results in humans as a dead end host. These, although included in any textbook of parasitology, may not be considered true parasites by strict definitions. Diagram F is a special case, such as Taenia solium (the agent of cysticercosis), where humans can be part of a normal parasitic life cycle (i.e., humans have a tapeworm), but when the cycle is interrupted, humans change positions and become a dead end host. These diagrams are helpful in understanding why some infectious parasitic diseases present acutely while others may take months or years to manifest. For the sake of this discussion, we will now turn to helminthic parasitic diseases that lead to CNS complications.

Helminths come largely in three flavors in human infections. The nematodes, or roundworms, are largely isolated to the gastrointestinal tract and lymphatics and, with the exception of agents such as angiostrongylus (the most common cause of eosinophilic meningitis), do not cause CNS infections. The eyes are affected in Onchocerciasis (River Blindness) with the movement of microfilaria into the eyes with inflammatory response; however, this disease is a focus of international efforts to eliminate and reduce disease and so is hopefully on the decline. The cestodes (i.e., segmented flatworms or tapeworms) comprise two groups (see life cycles on previous slide) which include a) those that cause only intestinal tapeworms in humans and b) those that cause disseminated diseases in humans. Within the former group are the vast majority of tapeworms, which are of nutritional and public health concern but not major contributors to mortality. In the latter, we have Taenia solium (the pork tapeworm) and Echinococcus sp. (dog tapeworms) which lead to disseminated disease. The pork tapeworm disseminates when a human is exposed to the feces of a person with a tapeworm and ingests eggs or proglottids (as is the norm for the pig). Similarly, echinococcosis results from exposure to the feces of carnivores in the environment.

The nematodes comprise a large number of organisms as listed above though only a few ever involve the CNS. The four highlighted in bold (Ascaris, Trichurus, and the two hookworm species) combined infect approximately 3.5 billion people world wide.
Strongyloides stercoralis, although less common, is of particular importance as a re-emerging infection that can involve the CNS when hyperinfection occurs with immunosuppression.

A review of hyperinfection with Strongyloides will be discussed[5, 6].

The cestodes Taenia solium and Echinococcus sp, as previously mentioned, can cause the most catastrophic CNS involvement in humans. In the case of Taenia, many disseminated cysticercus can be present in the brain and muscle of the body with little symptomatology. Only at a point when the organism dies (presumably because it has a limited life span in a pig, during which time it needs to be ingested), does an immune response often become clinically evident with resultant headaches, seizures, and focal neurological signs. Echinococcal disease, on the other hand, is usually a single cyst that grows approximately 1 cm per year in size; therefore, symptoms occur due to cyst walls impinging on normal structures. In the former, rupture is usually required for clinical awareness; in the latter, however, a rupture can be catastrophic with initially possible anaphylaxis and eventually the formation of many daughter cysts which can behave in a cancerous fashion.

A current discussion of neurocysticercosis will be presented[7, 8].
<table>
<thead>
<tr>
<th>Season</th>
<th>Specific Clinical Patterns</th>
<th>Sequelae</th>
<th>Mortality Rate</th>
<th>Transmission</th>
<th>Viral Structure</th>
<th>Virus (Family)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All year</td>
<td>Rare forms: subacute, subacute, psychiatric, encephalitis, meningitis, HSV-1: brainstem, HSV-2: myelitis</td>
<td>Common</td>
<td>70%, if untreated</td>
<td>Unknown</td>
<td>*ds DNA</td>
<td>Herpes simplex virus (herpesvirus)</td>
</tr>
<tr>
<td>Late winter, spring</td>
<td>Rash, encephalitis in 0.1-0.2% children with chickenpox, cerebellar ataxia (encephalitis)</td>
<td>Adults worse; cerebellitis</td>
<td>Variable; low in children</td>
<td>Direct contact (air), highly contagious</td>
<td>*ds DNA</td>
<td>Varicella-zoster (herpesvirus)</td>
</tr>
<tr>
<td>Usually winter</td>
<td>Parkinsonism (encephalitis lethargica)</td>
<td>Parkinsonism (encephalitis lethargica)</td>
<td>Unknown</td>
<td>Direct contact (air), highly contagious</td>
<td>*ss RNA</td>
<td>Influenza virus (orthomyxovirus)</td>
</tr>
<tr>
<td>Summer, fall, tropics: no season</td>
<td>Mild, except enterovirus 71</td>
<td>Mild, except for enterovirus 71</td>
<td>Low, high for enterovirus 71</td>
<td>Focal oral route</td>
<td>*ss RNA</td>
<td>Enteroviruses (picornavirus)</td>
</tr>
<tr>
<td>All year</td>
<td>Mortality rate virtually 100%</td>
<td>Mortality rate virtually 100%</td>
<td>Virtually 100%</td>
<td>Dog, wild animals (eg, fox, wolf, skunk)</td>
<td>*ss RNA</td>
<td>Rabies (rhabdovirus)</td>
</tr>
</tbody>
</table>

*Abbreviations: ds - Double strand, ss - Single strand*
<table>
<thead>
<tr>
<th>Virus (Family)</th>
<th>Viral Structure</th>
<th>Transmission</th>
<th>Distribution</th>
<th>Mortality Rate</th>
<th>Specific Clinical Patterns</th>
<th>Sequelae</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytic choriomeningitis virus (arenavirus)</td>
<td>*ss RNA</td>
<td>Rodents</td>
<td>Europe, Americas, Australia, Japan</td>
<td>Low (&lt;1%)</td>
<td>Progressive fever and myalgia; orchitis; aseptic meningitis; leukopenia, thrombocytopenia</td>
<td>Rare</td>
<td>More in winter</td>
</tr>
<tr>
<td>Lassa fever (arenavirus)</td>
<td>*ss RNA</td>
<td>Rodents</td>
<td>Africa</td>
<td>15%</td>
<td>Multisystem disease; proteinuria</td>
<td>Deafness (one third)</td>
<td>All year</td>
</tr>
<tr>
<td>Mumps (paramyxovirus)</td>
<td>*ss RNA</td>
<td>Direct contact (air), highly contagious</td>
<td>Worldwide</td>
<td>Low</td>
<td>Parotitis, pancreatitis, orchitis, aseptic meningitis</td>
<td>Frequent sequelae</td>
<td>Winter and spring</td>
</tr>
<tr>
<td>Measles (paramyxovirus)</td>
<td>*ss RNA</td>
<td>Direct contact (air), highly contagious</td>
<td>Worldwide</td>
<td>10%</td>
<td>Characteristic rash; frequent EEG changes; myelitis</td>
<td>Frequent mental retardation, seizures, *SSPE</td>
<td>Winter and spring</td>
</tr>
<tr>
<td>Nipah virus (paramyxovirus)</td>
<td>*ss RNA</td>
<td>Pigs; bats</td>
<td>Malaysia (Asia)</td>
<td>40%</td>
<td>Brainstem/cerebellar signs; segmental myoclonus, dysautonomia</td>
<td>*SSPE-like syndrome?</td>
<td>All year</td>
</tr>
</tbody>
</table>

*Abbreviations: ds - Double strand; ss - Single strand; SSPE - Subacute sclerosing panencephalitis
<table>
<thead>
<tr>
<th>Virus (Family)</th>
<th>Vector</th>
<th>Reservoir</th>
<th>Dist.</th>
<th>Mortality Rate</th>
<th>Specific Clinical Patterns</th>
<th>Sequelae</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern horse encephalitis (Alphavirus)</td>
<td><em>Aedes sollicitans</em></td>
<td>Birds</td>
<td>Eastern and Gulf US, Caribbean</td>
<td>35%</td>
<td>Severe, rapid progression</td>
<td>Common, especially in children</td>
<td>June to October</td>
</tr>
<tr>
<td>Western equine encephalitis (Alphavirus)</td>
<td><em>Culex tarsalis</em></td>
<td>Birds</td>
<td>Western US</td>
<td>10%</td>
<td>Classic encephalitis</td>
<td>Moderate in infants; low in others</td>
<td>July to October</td>
</tr>
<tr>
<td>Venezuelan encephalitis (Alphavirus)</td>
<td>Mosquito species</td>
<td>Horses, small mammals</td>
<td>South/Central America</td>
<td>~ 0.4%</td>
<td>Low rate (4%) of CNS involvement</td>
<td>Mild</td>
<td>Rainy season</td>
</tr>
<tr>
<td>St. Louis encephalitis (Flavivirus)</td>
<td><em>Culex pipiens, C. tarsalis</em></td>
<td>Birds</td>
<td>Widespread in US</td>
<td>2% young people; 20% elderly people</td>
<td><em>SIADH</em></td>
<td>More in elderly people</td>
<td>August to October</td>
</tr>
<tr>
<td>Japanese B encephalitis (Flavivirus)</td>
<td><em>Culex tritaeniorhynchus</em></td>
<td>Birds</td>
<td>Japan, China</td>
<td>33% (50% in elderly people)</td>
<td>Extrapyramidal features</td>
<td>50% neuro psychiatric; parkinsonism</td>
<td>Summer</td>
</tr>
<tr>
<td>West Nile (Flavivirus)</td>
<td><em>Culex, Aedes species</em></td>
<td>Birds</td>
<td>Africa, Asia, Europe, USA</td>
<td>In US: 12% (elderly people only)</td>
<td>Motor; brainstem involvement</td>
<td>Usually not prominent</td>
<td>Summer</td>
</tr>
<tr>
<td>Far East tick-borne encephalitis (Flavivirus)</td>
<td><em>Isodes persulcatus</em> (tick)</td>
<td>Small mammals, birds</td>
<td>Former eastern Russia</td>
<td>20%</td>
<td>Epilepsy; partial paralysis;</td>
<td>Frequent; residual weakness</td>
<td>Spring-early summer</td>
</tr>
<tr>
<td>Central European tick-borne encephalitis (Flavivirus)</td>
<td><em>Isodes ricinus</em> (tick)</td>
<td>Small mammals, birds</td>
<td>Central Europe</td>
<td>Less common than in Far East</td>
<td>Limb-girdle paralysis (spine/medulla)</td>
<td>Less common than in Far East</td>
<td>April to October</td>
</tr>
<tr>
<td>Powassan (Flavivirus)</td>
<td><em>Isodes scapularis</em> (tick)</td>
<td>Small mammals, birds</td>
<td>Canada, northern US</td>
<td>High</td>
<td>Severe encephalitis</td>
<td>Common (50%)</td>
<td>May to December</td>
</tr>
<tr>
<td>Dengue fever (Flavivirus)</td>
<td><em>Aedes species</em></td>
<td>Mosquitoes</td>
<td>Tropics</td>
<td>Low, except hemorrhagic</td>
<td>Dengue syndrome; rare CNS involvement</td>
<td>Mild, except for hemorrhagic</td>
<td>Rainy season</td>
</tr>
<tr>
<td>La Crosse encephalitis (Bunyavirus)</td>
<td><em>Aedes triseriatus</em></td>
<td>Small mammals</td>
<td>Central US</td>
<td>Low (&lt;1%)</td>
<td>Mild, primarily in children</td>
<td>Mild; seizures</td>
<td>Summer</td>
</tr>
<tr>
<td>Colorado tick fever (Orbivirus)</td>
<td><em>Dermacentor andersoni</em> (tick)</td>
<td>Small mammals</td>
<td>US, Rocky Mountains area</td>
<td>Low</td>
<td></td>
<td>Mild</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: SIADH – Syndrome of inappropriate antidiuretic hormone secretion*
Summary and Conclusions

• An understanding of Rabies biology is pointing towards new pathways for treatment of the deadly disease which has had a surprising increase in incidence in the last decade.

• Diagnosis of disseminated tuberculosis prior to biopsy is still difficult making tissue samples vital for the moment; however, new markers should greatly increase the accuracy of this diagnosis.

• “Chronic” silent diseases can re-emerge as advance treatments with immunosuppression are more commonly used in traveling populations.

• As the global population is increasingly linked by rapid travel, new economic vectors, and improved healthcare, the incidence and prevalence of previously restricted tropical diseases will increase in North America.

See the following articles for the discussion of rabies, tuberculosis, Strongyloides and cysticercosis.

1. Roy A Fau - Hooper, D.C. and D.C. Hooper, Immune evasion by rabies viruses through the maintenance of blood-brain barrier integrity. (1538-2443 (Electronic)).

2. Jackson Ac Fau - Randle, E., et al., Neuronal apoptosis does not play an important role in human rabies encephalitis. (1538-2443 (Electronic)).


4. Be Na Fau - Lamichhane, G., et al., Murine model to study the invasion and survival of Mycobacterium tuberculosis in the central nervous system. (0022-1899 (Print)).

5. Nishimura K Fau - Hung, T. and T. Hung, Current views on geographic distribution and modes of infection of neurohelminthic diseases. (0022-510X (Print)).


7. Patil S Fau - Robinson, P., et al., Proinflammatory cytokines in granulomas associated with murine cysticercosis are not the cause of seizures. (0022-3395 (Print)).

8. Morales-Montor J Fau - Escobedo, G., et al., The neuroimmunoendocrine network in the complex host-parasite relationship during murine cysticercosis. (1873-4294 (Electronic)).
Digital pathology: a tool for 21st century neuropathology

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I have no financial ties or interests with any of the websites, products, or companies mentioned in this article. I own and use slide scanners from Aperio and Bacus Laboratories Inc. and have been an invited speaker at Visions 2008, a conference on digital pathology sponsored by Aperio.
Abstract

Digital pathology represents an electronic environment for performing pathological analysis and managing the information associated with this activity. The technology to create and support digital pathology has largely developed over the last decade. The use of digital pathology tools is essential to adapt and lead in the rapidly changing environment of 21st century neuropathology. The utility of digital pathology has already been demonstrated by pathologists in several areas including consensus reviews, quality assurance (Q/A), tissue microarrays (TMA), education and proficiency testing. These utilities notwithstanding, interface issues, storage and image formatting all present challenges to the integration of digital pathology into the neuropathology work environment. With continued technologic improvements, as well as the introduction of fluorescent side scanning and multispectral detection, future developments in digital pathology offer the promise of adding powerful analytical tools to the pathology work environment. The integration of digital pathology with biorepositories offers particular promise for neuropathologist engaged in tissue banking. Utilization of these tools will be essential for neuropathologists to continue as leaders in diagnostics, translational research and basic science support in the 21st century.
Introduction

Digital pathology refers to the relatively recent ability to create an electronic environment for performing pathological analyses and managing the information associated with this activity. Digital pathology is the product of a series of technological innovations driven by a number of companies, as well as by investigators who have harnessed this technology to enhance their research and clinical practice. One of the most familiar technological changes is the introduction of digital cameras to capture still images, replacing film as the preferred medium for photomicroscopy. Hardly noticed now, this change introduced many pathologists to the benefit of capturing fields of interest in a digital format. This relatively modest change has afforded us the ability to use imaging information in new and innovative ways in clinical, educational and research endeavors.

Some of the key technology to produce dynamic, relatively high-resolution images of entire slides was introduced by Bacus laboratories, Inc., beginning in the mid 1990’s. The first generation of slide scanners capable of scanning whole slides became available around 2000. These systems were limited to a basic combination of scanning hardware and viewing software, the latter allowing users to pan across the entire digital image and then zoom in (view at higher magnification) on areas of interest. This pan and zoom approach was designed to mimic the way a pathologist manipulates a glass slide at a microscope. Incremental improvements have been made in scanning technology to allow for robotic batch scanning of large numbers of slides, significant improvements in scanning speeds, and the introduction of more sophisticated software to not only view virtual slides, but to also be able to integrate slides with other sources of digital information such as laboratory information systems (LIS), as well as to offer sophisticated image processing and analytical tools. It was only with the relatively recent addition of these tools that an electronic workspace or digital pathology environment could be said to exist. Even in its nascent state, digital pathology has introduced important changes in several areas of pathology practice and research, including: consensus reviews, Q/A programs, tissue microarray (TMA) analysis, education, and proficiency testing, examples of which are discussed below. With continued technologic improvements and the introduction of fluorescent side scanning and multispectral detection, future developments in digital pathology offer the promise of adding powerful analytical tools to the pathology work environment.

The introduction of these new tools is occurring in a medical landscape that is rapidly being reshaped by trends in both the clinical and research environments within which neuropathologists operate. On the clinical side, neuropathology as a diagnostic specialty faces a constant challenge to embrace or develop tools in response to colleagues who demand rapid introduction of new diagnostic markers and molecular tests, ways to interact with the data resulting from this diagnostic work, and integration of these data into an easily accessible digital medical record. In a relatively small field such as neuropathology with a limited number of specialists available for consultations, there is a constant challenge in finding ways to make these specialized services available in all the places where there is clinical demand. Some of these needs in neuropathology have been met through telepathology. On the research side, there is a burgeoning view of pathology departments as biorepositories to provide highly annotated clinical material to support basic and translational research programs, many of which are highly complex and involve...
multiple institutions. Development of new diagnostic paradigms and research efforts requires true quantitative assessment and evaluation of tissue with more complex image analysis.

The development of digital pathology offers neuropathologists the tools to meet these challenges and to develop new and innovative ways to practice their art in the 21st century. We will discuss some of the underlying technology that supports digital pathology and highlight areas in which digital pathology applications have particular relevance to neuropathology. Most of this work is currently being done in other pathology subspecialties. The greatest potential of digital pathology may be realized when pathologists choose to reconfigure their work environments to utilize this technology for routine clinical work in order to take advantage of computer aided diagnosis and analytical tools to supplement traditional histopathologic assessment. We report on key lessons learned from the first phase of a project to implement these technologies at the Children’s Hospital of Philadelphia. Finally, some of the early promise of digital pathology to integrate complex data sets involved in biorepositories is being realized through innovative applications that have been developed at the Center for Childhood Cancer and the Biopathology Center at the Research Institute at Nationwide Children’s Hospital. This work can provide a road map of neuropathology-led digital pathology initiatives. Our analysis of the current state of the digital pathology suggests that the nature and pace of technologic change occurring within pathology are such that implementation of digital pathology technology and applications will be critical for neuropathologists to continue to take a leadership role in diagnostics and research in the 21st century.

Creating, viewing, analyzing and managing whole slide images (WSI)

Virtual microscopy is the ability to interactively view high resolution WSI. Digital pathology applications are built around WSI. WSI has its origins in the work of Joel Saltz and colleagues on what they termed “Enhanced Field Microscopy” which utilized a robotic microscope to scan a large area of a glass slide and then combine the captured fields together to form a single large image (10). The first automated high-speed scanner for WSI was produced by Interscope Technologies and the University of Pittsburgh Medical Center (16). Subsequently, scanners to produce WSI have been produced by a number of different vendors. For a comprehensive review of available systems and technical details of their function, refer to Rojo et al. (22). There are several fundamental principles common to various systems that use slide scanning to produce WSI. Glass slides are scanned using either a robotic microscope or an array based scanner system. These systems typically utilize a high resolution camera coupled with one or more high quality microscope objectives to capture images of adjacent areas from a glass slide. The pattern of movement and exact type of fields captured in the scanning process varies among systems, but most utilize specialized software to reassemble images from these multiple individual fields in a single WSI. This image is then processed and stored in a format that allows viewing software to rapidly scan and zoom through a WSI, similar to how a pathologist views a slide through the microscope. Scanning is done at high resolution, often on the order of 0.25–0.5 microns/pixel, and generates large amounts of data. For example, a scan of a typical 2 x 1 inch piece of tissue would represent 100,000 x 50,000 pixels or approximately 15GB of uncompressed data. Image compression
algorithms such as JPEG or JPEG2000 are typically used in order to reduce the typical file size for WSI to 0.5 - 1GB (7).

Since WSI are both large and likely to be a shared resource for many users, they are typically stored on a server. Some systems also offer specialized software to manage the storage and retrieval of WSI, as well as sharing of a single WSI simultaneously among several users for virtual slide conferencing. While the file size of WSI is quite large even with compression, a variety of different approaches are used by vendors of WSI systems to limit the amount of data that has to be moved from the server to the local computer for viewing of WSI. This is typically accomplished by only presenting the limited subset of data that is being viewed at any given time from the WSI, so called pixel on demand. As the user pans and zooms, the system calls up the corresponding data from the WSI. In this way the network load is minimized and software responsiveness maximized to simulate working with an actual slide. The software for viewing WSI can be web-based or a stand-alone application and may offer a variety of basic tools for viewing, annotating, and manipulating the basic image. Typically this software provides the ability to export static images, just like conventional digital photomicroscopy.

The difference between virtual microscopy and digital pathology is the addition of tools to allow the pathologist to not only read and annotate an individual slide, but to interface WSI data with existing LIS, perform image analysis, and correlate pathology data in WSI with other imaging and test result data available for a given patient. Only recently have vendors begun to develop software to support this functionality for WSI. This development of an information management system that supports both the imaging application as well as a comprehensive support of workflow within the digital pathology workspace is analogous to the development of picture archiving and communication systems (PACS) in radiology. By supporting the entire workflow of radiology, the matured PACS technology has come to define the radiology digital workspace. In pathology, the components for such systems exist and nascent digital pathology workspace environments are beginning to develop around vendor specific software applications such as Aperio’s SpectrumPlus application (http://www.aperio.com/pathology-services/SpectrumPlus-information-management.asp) or BioImagene’s 3i and PATHIAM software (http://www.bioimagene.com/index.html), as well as user developed applications such as VIPER (see below).

Perhaps the greatest advantage offered by WSI is the ability to perform complex image analysis as an aid or adjunct to routine diagnosis. Neuropathologists have limited exposure to these methodologies in routine clinical practice, so examples from other areas of pathology are illustrative. One of the areas of pathology in which image processing technology has made the greatest impact in routine diagnostic work is in the examination of Pap smears in cytology. Beginning in the early 1990s the Cytyc Corporation started development of the ThinPrep® Pap Test™. This test relies upon image processing software to automatically identify suspicious cells on specially prepared Pap smears. These abnormal cells are flagged for review. The introduction of the ThinPrep® Pap Test™ required changes in the cytopathology labs including preparation of special slides in which cells would be prepared as a monolayer, necessitating retraining of cytotechnologist screeners and laboratory work flow modifications. Working as a supplement to pathologist diagnosis, ThinPrep® Pap Test™
has improved detection of cervical abnormalities and has doubled the average reported cytotechnologist screening rate (5).

ThinPrep® Pap Test™ is based on the identification of atypical and malignant cells in specially prepared cytology samples. In a similar fashion, automated image analysis of routine histologic sections are now being used in surgical pathology for detection and quantification of HER-2/neu overexpression/amplification in breast cancer, where it is associated with increased recurrence and worse prognosis (21). HER-2/neu expression is also important because it predicts responsiveness to Trastuzumab, which can increase survival and reduce risk of recurrence (21). Determination of HER-2/neu status can be done by fluorescence in situ hybridization (FISH) for HER-2 gene amplification or by immunohistochemical (IHC) staining to detect HER-2 protein overexpression (4). Interobserver variability is a major challenge with standard IHC, but substantially improved correlation with HER-2 gene amplification by FISH has been achieved with the use of automated image analysis (26). Currently such analysis requires the capture of one or more static images from representative sections in which invasive tumor has been identified by the pathologists. WSI based automated image analysis offers the ability to automatically perform these assays on selected regions, as well as in the future, automated detection of lesional areas. Currently at least two vendors, Aperio (www.aperio.com) and BioImagene (http://www.bioimagene.com/index.html), offer FDA approved in vitro diagnostic (IVD) algorithms for HER2, ER and PR stained breast specimens on WSI. With these basic tools in place, development of other specialized image analysis assays will continue to develop and will surely impact each subspecialty, including neuropathology.

There are also current examples of computer-based diagnosis for neuropathology applications similar to that used in the systems described above. For instance, a computer-assisted diagnosis system for grading astrocytomas has been developed and tested using digital images from H&E stained slides that were analyzed with imaging and learning algorithms (14). Cases of astrocytomas that were diagnosed by histopathology as low grade (WHO grade II), high grade (WHO grade III and IV) and suspicious grade II-III lesions were collected and selected images from representative areas were digitized. Image segmentation was performed on these selected images of tumor to identify nuclei and distinguish them from other structures using a Probabilistic Neural Network pixel-based algorithm. On average, this algorithm correctly identified 86.5% of all the nuclei in these selected images. The morphological features that were computed included measurement of the nuclear area, roundness and concavity. The data was processed, analyzed and separated in three categories: low, intermediate (suspicious), and high grade. One hundred and forty astrocytomas were included in the automated analysis. There was a 92.1% concordance between the computed and pathologist classifications. Low-grade lesions were accurately separated by the computed system in 95% of the cases. Of the high-grade lesions, 91% were correctly diagnosed and 83.3% of the suspicious cases were properly identified. Modifications in the classifier system and selection of different numbers of criteria have shown improvement in the computed system performance with an overall concordance as high as 97.8% (13). Whether or not a similar program could aid pathologists in the future in terms of assigning histologically borderline cases into either high or low-grade categories will require additional studies and clinical followup. Another group utilized automated computed-based counts using
public domain image analysis software to assess Ki-67 labeling indices in meningiomas. This approach showed a high correlation coefficient (0.98) in much less time than the conventional manual method (17). These assays are available for WSI from multiple vendors. Automated imaging analysis has also been applied to neurodegenerative diseases. Using a computer application that can be trained to classify various objects, Chubb et al. demonstrated that automated image analysis could classify plaques and tangles with an accuracy comparable to manual methods, and count neurofibrillary tangles for quantitative and comparative studies (3).

**Specialized Applications (Consensus reviews, Quality Assurance (Q/A), Tissue Microarray (TMA) Analysis, Education and Proficiency Testing)**

WSI has been implemented in a number of specific niches within pathology since its introduction in 2000. In order to support the use of WSI for these purposes, studies have been performed to assess the performance of pathologists using WSI under a variety of conditions. A limited number of studies have been published that examine the performance of WSI in comparison to traditional microscopy in evaluating routine clinical material. For example, Gilbertson and colleagues compared the performance of three pathologists reviewing 25 specimens with multiple parts in a simulated environment and reported excellent concordance between the results obtained by WSI and microscopic analysis (12). In a carefully devised study, Fine et al. evaluated the use of WSI to review IHC stained sections of challenging prostate needle biopsies by pathologists at geographically disparate sites. Thirty cases where IHC was required to confirm or rule out cancer were identified and scanned to create WSI. Five pathologists, as well as an outside expert reviewer, were asked to review and score both the original glass slides and the WSI. Essentially similar levels of interobserver variability were identified between the WSI and glass slide reviews. In only one case did participants feel that the image quality of the WSI was worse than the original glass slide. The authors concluded that their findings could likely be generalized to other similar IHC applications outside of prostate biopsies (11).

Ho, et al. used direct comparison of glass slides and WSI in a retrospective Q/A review of 24 complex genitourinary biopsies (comprising 47 diagnostic parts and 391 slides). Three pathologists reviewed these cases. Two pathologists were assigned WSI and one received the original glass slides for each case; a standard Q/A form was used to evaluate both WSI and glass slides. Strong consensus was reported between WSI and glass slide reviews of these cases. However, in one case a subtle but clinically significant discrepancy was identified between the WSI and glass slide, which suggested that technical issues in the WSI obscured a focal area of atypia. Overall, the authors reported that all study pathologists felt Q/A could be effectively performed using WSI (16). Significantly however, all participants also indicated that slide presentation and speed of WSI was inadequate to support routine case review based on WSI. Similarly, our own experience has been that this is a real limitation in the transition of digital pathology to daily clinical use. Future development of pathologist centric digital workspace environments will be essential for successful implementation.

Wan et al. conceptually described the fundamental methods that underlie TMAs in 1987 (28). The technical approach currently used for TMAs was developed by Kononen et al in 1998 (18). Since that time, TMAs have become a widely used, even
routine technique for IHC and in situ studies on large numbers of samples performed under uniform conditions. However, this same advantage of high tissue density translates into a somewhat cumbersome system for scoring and keeping track of individual cases. Because of the high throughput data generated by TMAs, WSI became an obvious application in order to capture individual cores in digital form, enhance the ability to retrieve, score and compare multiple results for each individual case side by side, and perform image analysis for quantitative digital assessments.

By applying WSI to a large and diverse range of TMAs created over a wide range of specific research projects, the Stanford Tissue Microarray Database has captured over 200,000 stained and scored TMA images with associated annotations including both tissue descriptions and clinical data (20). One advantage of TMA based studies is the ability to simultaneously examine large panels of markers over a wide population of samples. Using manual semiquantitative scoring results uploaded to software originally developed for analyzing cDNA microarray results, it is possible to perform clustering analysis on these large data sets. In studies where TMA cores are drawn from well-characterized clinical populations with associated outcome data, this approach can yield cluster group designations based on IHC staining that show strong correlations with tumor grade, stage, and cell type, while also being more reproducible and showing less interobserver variability than traditional histologic assessment of the same tumors (1).

A number of different vendors of WSI systems offer TMA packages with both data management and analytic tools; commercially available image analysis software can also be utilized to examine individual cores captured from TMA as static images. Automated image analysis is currently limited by weak tools to distinguish benign from malignant areas. In this regard, TMAs offer an ideally suited platform for automated image analysis since they are composed of areas carefully sampled to be representative of the lesional tissue. Alternatively, the addition of automated analysis for TMA scoring is particularly useful in studies involving large numbers of samples, since these are likely to eliminate human operator counting errors. For instance, the feasibility and utility of this approach has been established in a fully automated analysis of estrogen receptor (ER) expression in a TMA containing 3,484 invasive breast carcinoma cases with both treatment and outcome information, in which the fully automated analysis did not differ significantly from manual scoring of ER status by pathologists (27).

One area in which WSI has proven particularly useful is in education. A wide spectrum of applications has been reported, but particularly rapid growth has occurred in histology and pathology courses, in large part due to the costs associated with the purchase and maintenance of microscopes, not to mention the costs of cutting and staining numerous duplicate slides in order to generate multiple slide sets. A variety of published reports have documented good student satisfaction with educational materials prepared using WSI, was well as generally equivalent to sometimes notably improved performance on examinations based on question sets that span the glass slide to WSI transition (15, 19, 23). Many of the educational courses at national and international pathology meetings have similarly started switching to the WSI approach, both as a cost savings tool and in order to broaden both the potential number of cases and participants. For instance, the blocks from small biopsies have often been insufficient to generate adequate numbers of teaching slides in the past, but WSI obviates that requirement. A good example of this utility in neuropathology is the diagnostic slide session at the annual
AANP meeting. Limited needle biopsies from brain, muscle, or nerve with rare disorders can now be submitted to the moderator as a single slide for scanning and presented to participants in the form of WSI. Similarly, popular educational courses using a WSI approach no longer need to cap the number of participants in order to match the limited number of slides obtainable from the average sized paraffin block.

Coupled with education, assessment and proficiency testing are also areas where WSI has found significant use in pathology. Currently the American Board of Pathology uses WSI in 15 of 75 of the microscopic slides for board examination (2). Particular consideration has been given to WSI as a means of proficiency testing in cytopathology. This interest stems from the national cytopathology proficiency-testing program that began in 2004. In the face of a need to create and distribute a large volume of well-characterized gynecologic cytology slides for proficiency tests, WSI has been viewed as a potential solution. Some groups have reported fairly good success with use of WSI of ThinPrep® prepared samples (25). Finally, WSI can be used as a way to make rare or unusual samples of general interest accessible. An example of this related to neuropathology are two WSI of original brain sections used by Alois Alzheimer to describe the neurodegenerative disease named after him (http://mirax.zeiss.de/alzheimerslides/show.aspx?slide1).

**Practical considerations**

While WSI has been introduced in numerous clinical, research and commercial settings, there is relatively little that has been reported about the use of WSI in an academic neuropathology setting. The Division of Neuropathology in the Department of Pathology and Laboratory Medicine at the Children’s Hospital of Philadelphia (CHOP) has made a long term strategic commitment to implement digital pathology in clinical practice, as well as to support translational and basic research in pediatric brain tumors. Given the scope and complexity of such an undertaking, this project has three main phases: (1) developing standard operating procedures for scanning clinical material and integrating scanning to produce WSI into the normal clinical work flow for pediatric neuropathology cases at CHOP, (2) implementation of WSI for sign out of transient specimens such as consultation cases, where material is seen but not retained, and for autopsy neuropathology cases which have longer turnaround time, affording greater flexibility for scanning, (3) implementation for general pediatric neuropathology diagnostic work. This work utilizes an Aperio ScanScope XT with a server containing 4 TB of direct attached storage.

In the process of accomplishing the objectives of phase 1 of this project we have identified several practical considerations that illustrate some of the key steps and challenges in bringing WSI into an academic clinical environment. These fall into three key areas: training and resource utilization to support WSI production, interfacing existing LIS with WSI software to populate WSI with clinical information, and storage for WSI. Specific issues arising from each of these areas are discussed below. Identifying and planning to address these kind of issues is a critical step in the implementation of WSI in a clinical environment.

It is easy to underestimate the training and resource utilization required to support WSI even on a fairly modest scale. In order to support daily scanning of all new neuropathology cases at CHOP, approximately three months of full time training and
intensive scanning was required in order to train an operator and develop a workflow to support scanning. Multiple modalities were required for training including off site training with the vendor, on-site training using clinical material, and extensive use of technical support. By far the most effective training is hands on using the clinical material and systems of the local institution. As interface issues become apparent they can be rapidly addressed. Careful consideration should be given to the level of support offered by potential vendors in each of these areas. Our particular system can scan up to 120 slides in a single batch. An unanticipated source of resource utilization was created by the fact that up to 10% of slides coming from clinical labs (ours, or those of outside hospitals) had to be returned to the lab or required additional handling prior to scanning. Most often this was due to bubbles, misaligned coverslips, or dirty slides, all of which interfered with producing high quality WSI. An additional consideration if high throughput scanning is required is what kind of slide handling pathway is used to feed individual slides into the scanning area. Certain slide handling systems are not well suited to wet slides that come directly from the lab, or the addition of slide labels may cause slides to jam. All of these can add additional time or pre-scan handling in the laboratory.

Interfacing the existing LIS with WSI software to populate WSI with clinical identifiers and complete pathology descriptions can be broken down into three key components. First, specimen identification for WSI systems can be greatly facilitated by bar coding. Systems that have the capacity to capture and decode information on slide labels allow for at least partial population of key slide identifiers such as case number, block or stain in the software that is used to populate and WSI information. In the absence of such a system, the scanning operator will have to manually enter this data for each WSI, a time consuming and error-prone process. An even more important process is to carefully examine the way your existing LIS handles both demographic and clinical information, as well as the pathology specific content in your cases. This information will need to be mapped into the software used to populate WSI information. Generally the software used for WSI will have its own architecture with certain assumptions about how information should be handled and prioritized. Developing an appropriate data hierarchy, particularly without significant support from the LIS vendor can be a time-consuming process. However, this is essential for the final step in creating a working interface: obtaining and optimizing an HL7 feed by which information from your LIS can be transferred to your WSI system, properly formatted and containing the full pathology description desired. This process often requires support and even customization on both the LIS and WSI vendor sides. Pathologists can plan to spend a significant amount of time guiding this effort; this input is critical for a properly functioning system. Figure 1 shows a schematic view of the system developed at CHOP to accomplish these steps.

Storage for WSI can represent a critical bottleneck and requires very careful consideration. Since the goal of our long term project is to develop a system that utilizes WSI within a clinical workflow for the diagnosis of clinical neuropathology material, we chose to scan and store our entire clinical neuropathology caseload including both in house and consultation surgical and autopsy material. This choice was made so that we could determine for ourselves the storage requirements that would be encountered in routine clinical use of WSI in our environment. Over approximately 8 months, we scanned 10,351 slides. These slides required 5.2TB of storage (additional disk storage
space was added to our original 4TB configuration). While a range of tissue sizes were present on the slides scanned, ranging from large sections in autopsy cases to small fragments in brain tumor biopsies, the average slide size for our neuropathology material, scanned at 20X, was 506MB after JPEG compression. Based on these numbers it would require approximately 8TB of storage per year to store the WSI data from our case material. At an estimated cost of $2600/TB, the cost of storage would be approximately $20,800/year for our system. On a larger scale, the estimated storage required for all of CHOP Pathology slide volume, around 55,000 slides per year, would be approximately 27TB per year, at a cost of approximately $70,000 per year. At current rates, the cost of storage for WSI would be approximately $1.27/slide for direct attached storage. This is on top of the estimated $0.50/slide estimated cost for scanning a WSI (24). These cost estimates do not account for back-up, redundancy or disaster recovery, which are essential if digital pathology is part of the primary pathology work environment.

In addition to cost, the scale of storage required by WSI is currently prohibitive for most pathology departments. Long term storage of WSI data in a manner similar to that currently done for glass slides requires pathologists to participate in enterprise level shared storage arrangements. In many hospital environments, radiology departments have already pioneered such arrangements to support digital pathology systems. Radiology as a specialty has largely completed a conversion to digital radiology and today most radiology images are digital from their creation. Institutional investments in shared storage have been made to support this change. Enterprise storage solutions also offer the additional advantage of back-up, redundancy and disaster recovery capabilities. In order for pathologists to have the infrastructure to pursue serious digital pathology initiatives, it is necessary to have a "seat at the negotiating table" and include WSI in the planning discussions around storage space and network bandwidth.

To be effective in discussions about enterprise storage, it is important to recognize that there are some critical differences between digital pathology and digital radiology. The relatively large file sizes involved in WSI are in marked contrast to digital radiology, where file sizes are far smaller. So, the addition of digital pathology content will rapidly expand the overall size requirements for any enterprise storage. A second challenge is that while enterprise storage is scalable and can likely meet the disk space requirements of large scale WSI, it is considerably more expensive than directed attached storage, currently costing on the order of $10,000/TB. These increased costs are related not only to disk storage, but also the associated network switches and other hardware needed to create these systems, multiple levels of redundancy that often includes off-site back up systems that can rapidly be brought on line, and customized software that manages flow of information into and out of these shared storage spaces. This means that the addition of capacity to accommodate digital pathology can be associated with some very significant costs.

Finally, it is important to recognize that at the present time most WSI systems are primarily designed to interact with a dedicated server with direct attached storage rather than remote enterprise storage. Partially this reflects the specialized nature of software for displaying WSI. The scan and zoom approach described above is well suited to the large file sizes for WSI. By contrast, radiology and most other medical imaging systems are designed around a standard (DICOM) that uses quite a different store-and-forward access approach suited to relatively small image files and less dense image information.
Due to these fundamental differences, WSI are not currently compatible with the DICOM standard, although Working Group 26 (WG26) of the DICOM community is developing DICOM standards for virtual microscopy (8). The practical consequence of this is that software used to access and display WSI images will typically require some modification to access enterprise storage and that enterprise software to manage, share and display other medical imaging data typically won’t work with WSI data. Figure 2 depicts a schematic illustration of the potential interface between enterprise storage space and WSI that is being developed at CHOP.

**Future applications (Multispectral analysis and Biorepositories)**

One advantage of WSI is that it opens up possibilities for neuropathologists to supplement morphology-based tools with advanced image processing algorithms to support our roles as diagnosticians, translational researchers, and basic scientists. In these roles we increasingly confront the limitations of traditional morphologic approaches and manual grading. While significant interobserver variability and low sensitivity are well appreciated for immunohistochemical and immunofluorescent methodologies, another equally compelling problem is simple capacity. As the demand for clinical trial or animal model related assessment of tissue samples grows, the limitation of any individual neuropathologist to directly evaluate large numbers of specimens becomes an absolute limitation.

An area of active research and development in pathology informatics is computer-aided detection and related technologies that will likely form a layer of ancillary techniques that it is useful to consider as potential “pathology helpers” (9). Researchers are actively developing techniques to query multiple cellular compartments simultaneously in a single tissue section, and then use multispectral imaging to capture large data sets and perform what is in essence, “slide-based histocytometry” (9). The starting point in this process is acquisition of WSI data that can then be subjected to classification algorithms to identify regions of interest, for example areas of tumor. In its simplest form this process can rely on manual segmentation of WSI by a pathologist selecting regions of interest. More elegantly, this process can be automated around machine classification algorithms that take advantage of the inherent properties of WSI. Recently, systems using the latter approach based on WSI data have been described (9).

Key steps in this process are outlined in Figure 3. Once regions of interest are identified, automated algorithms for cellular and subcellular segmentation can be applied to separate out cells, nuclei, membranes and cytoplasm. This segmentation can then be applied to multispectral data to identify the precise location of simultaneous events in multiple cellular compartments in WSI. The use of such an approach allows for evaluation of complex pathways and events in whole tissue sections and does so in ways that are highly accurate and reproducible. WSI in particular affords the technological possibility for “machine learning space” for the development of customized algorithms for various tissues and diseases. Proof of concept for this approach has already been reported for prostate tissue and Gleason pattern recognition (6, 9). It is only through the development of tools that marry histopathology with highly quantifiable, automated and reproducible staining that neuropathologists, working on everything from neurodegenerative disease to brain tumors, will be able to support the growing demand associated with clinical trials and animal model systems.
Neuropathologists have long taken a leadership role in tissue procurement and banking to support both basic and translational research. Significant advances in LIS have improved considerably the annotation and tracking of specimens in tissue repositories. While annotation of clinical information in tissue repositories has become quite rich, along with accompanying molecular and genetic data, pathology information available to users is often considerably less robust, typically comprising the diagnosis, limited pathologic description and scoring information. Tissue analysis restricted to review of glass slides performed on microscopes has been a significant obstacle to expanding pathology content in biorepositories.

WSI offers several possible advantages for these applications. First, they are remotely accessible, offering the possibility of scanning and then storing glass slides as a deep repository, with first line access being through WSI stored on a server that can be accessed by repository staff and users. WSI also offers the possibility for multiple users to conduct a simultaneous review from remote locations via digital slide conferencing. Software to support this varies by vendor, but in general WSI on a single server is accessed by multiple users who can dynamically pass control over the slides among themselves and can see digital markings and annotations added to the WSI. With appropriate institutional IS support, this technology can be an effective supplement or replacement for multi-headed scope reviews or off site consensus conferences. Perhaps most exciting for users of tissue repositories is the promise that WSI can be combined with the database that stores and presents covariate data as well as the original pathology, pathologic description and any annotations or content that was added at the time of diagnosis or subsequently. With the proper procedures for sharing of information and collaborative interactions, the ability to annotate tissue in repositories offers great promise for neuropathologists both as leaders and users of tissue banking repositories.

Pioneering advances in the addition of pathology content to tissue repositories in the form of WSI have been made by the Biomedical Imaging Team (BIT) of the Center for Childhood Cancer and the Biopathology Center (BPC) at the Research Institute at Nationwide Children’s Hospital (http://imaging.nchresearch.org/). The BPC (www.biopathologycenter.org) at the Research Institute serves as a biorepository for the Children’s Oncology Group (COG), the Gynecologic Oncology Group (GOG), and the pediatric division of the Cooperative Human Tissue Network (CHTN). Through these interactions, the Biopathology Center receives specimens from over 500 institutions for review and research and distributes material worldwide. The BIT has integrated WSI into BPC operations and developed specialized applications that take advantage of WSI data to support users of the Biopathology Center.

Once specimens are processed and slides available, the BIT uses two Aperio ScanScope XT scanners to generate high quality WSI of the material. Utilizing a partnership with the Ohio Supercomputer Center (OSC), the BIT can provide long term storage and internet based access to these WSI, see Figure 4. To support the pathology review function of the Biospecimen Core, the BIT has developed a custom application called Virtual Imaging for Pathology, Education & Research (VIPER), see Figure 5. VIPER allows participants to remotely view WSI of case material submitted to the Biopathology Center. Users are able to see a customized case list, perform pathologic reviews by calling up WSI stored at the OSC, review submitted pathology reports, and complete internal evaluation and QA forms.
While VIPER is purpose built, its elements represent a road map for a future digital pathology workspace environment. As the workflow that VIPER is designed to support makes clear, the individual components of such a program could readily be modified to create a digital clinical workspace environment in which information from the LIS, hospital electronic medical record, medical imaging, and advanced molecular testing data such as FISH or SNP results, could all be presented along with the corresponding WSI of a biopsy. The BIT has demonstrated the feasibility of one major component that would be required for such an approach, the integration of WSI data and expression microarray data in their application, Virtual Microscope to Microarray (VM2M). The main components of VM2M are WSI of a tumor, microarray data for the same tumor, covariate data (patient demographics, etc.), and analytic software, data storage and network access to facilitate their simultaneous display. WSI created by the BIT are paired with molecular expression data created by Dr. Timothy Triche at Childrens Hospital Los Angeles, see Figure 6. VM2M has been developed as a diagnostic platform and may represent a critical first step towards creating the digital pathology workspace environment of the 21st century.

Conclusion
The history and development of digital pathology provide a useful guide to the likely future of these technologies and their impact on the practice of neuropathology in the 21st century. Approximately three years elapsed from the first introduction of acquisition techniques for WSI in the late 1990s to the advent of the first generation of scanners or robotic microscopes capable of producing WSI by 2001. In terms of technical capacity, at least two subsequent generations of scanners have developed since then and a number of vendors have entered the marketplace. In the last three years significant advances in software for storing, annotating and analyzing WSI have been made, creating for the first time the possibility for a true digital pathology workspace. In parallel to these developments, computer aided image analysis has gained rapid acceptance for specific applications in other areas of anatomic pathology including both cytopathology and surgical pathology. The rapid development of these technologies, as well as the continuously growing demand for specialized neuropathology services in clinical, translational and basic research applications, suggests the need for neuropathologists to embrace and guide the implementation of these technologies. The tools that digital pathology offers will become essential for the practice of neuropathology in the 21st century in both clinical and research arenas. Familiarity with digital pathology, as well as broader trends in the medical environment, leaves little doubt about the development of a robust digital pathology environment in the not too distant future. As we seek to engage these technologies, we can build on the work that has been done in other areas of pathology. Most critically, however, neuropathologists need to begin to exercise a leadership role in the introduction of these technologies so that we are able to shape the development of the emerging digital pathology workspace around our skills and needs. It is only by doing this that we will be able to ensure our ability to continue to exercise the historical leadership role of neuropathology in diagnostics and research in the 21st century.
References:

Figure 1: Sample workflow for association of clinical data from LIS system (Meditech in this example), with WSI (Aperio illustrated in this example). After clinical information has been properly mapped between the LIS and the WSI software (Spectrum in this example), the clinical information is sent by the LIS via an HL7 data feed (1) to an application to reformat this data for WSI software. This allows the WSI of an individual slide from a particular case (4) to be generated (5) and then associated with the appropriate clinical information corresponding to that slide (2 and 6). Figure reproduced with permission from Mark Wrenn, Aperio.
Figure 2: Schematic illustration of the potential interface between enterprise storage space and WSI that is being developed at CHOP. A direct mount to a large cache in the enterprise storage will allow for WSI software (Spectrum in this example) to interface with remote storage. Modifications to the WSI software are required in order to directly interface with data in remote storage. Figure reproduced with permission from Thomas Rose, IBM. (DAS = direct attached storage; TB = terabyte; GMAS = IBM Grid Medical Archive Solution, a vendor neutral technology for enterprise storage of medical imaging data; CFIS = common internet file system; TCIP/IP NAS = Transmission Control Protocol / Internet Protocol Network Attached Storage; SATA = serial advanced technology attachment)
Figure 3. Schematic diagram highlighting the key steps in the analysis of a WSI subjected to multispectral analysis. In this example an invasive breast carcinoma has been stained with antibody for Vav and counterstained with hematoxylin (A). Representative fields of tumor (red), inflammation (green) and stroma / vascular structures (blue) are manually identified (B). Using these fields, the computer system is trained to recognize these regions within a sample. Based on this training, the computer classifies the sample into tumor (red), inflammation (green) and stroma / vascular compartments (blue) (C). Image analysis algorithms can then be applied to specific areas of interest. In this case the subcellular segmentation is applied only to the areas containing tumor (red). This identifies the nuclei (green) within this area (red) (D). Various subcellular compartments can be identified through this process including nuclei (green) and cytoplasm (light pink halos) with the tumor (red) (E). Intensity data for multispectral markers expressed in each of these regions can then be captured. (Acknowledgement: Michael Feldman MD, PhD, University of Pennsylvania Medical Center and Cliff Hoyt, CRi).
Figure 4. Architecture of system to capture and store WSI data at the Nationwide Childrens Research Institute. (Figure reproduced with permission of Thomas Barr and William Beyer, BPC at the Research Institute at Nationwide Children’s Hospital)
Figure 5. VIPER promotes digital reviews by allowing pathologists and other researchers to view WSI, digital pathology reports, and other necessary information. (Figure reproduced with permission of Thomas Barr and William Beyer, BPC at the Research Institute at Nationwide Children’s Hospital)
Figure 6: VM2M allows researchers to search covariate patient data including tissue and cell type, sex, age, disease type and stage and then view both WSI and expression microarray data within the application. (Figure reproduced with permission of Thomas Barr and William Beyer, BPC at the Research Institute at Nationwide Children’s Hospital)