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Intraoperative Consultations of Central Nervous System Tumors: A Review for Practicing Pathologists and Testing of an Algorithmic Approach

Emel ÇAKIR[1,2], Gülbin ORAN[2], Gül Emek YÜKSEK[2], Cristine DING[2,3], Tarık TİHAN[4]

Department of Pathology, [1]Karadeniz Technical University Faculty of Medicine, TRABZON, TURKEY, [2] Visiting Scholar, University of California, SAN FRANCISCO, USA, [3]Tan Tock Seng Hospital, SINGAPORE, and [4]Neuropathology Division, Department of Pathology University of California, SAN FRANCISCO, USA

ABSTRACT

Intraoperative consultations or frozen sections for central nervous system (CNS) tumors present a significant challenge for surgical pathologists because of their relative rarity and diversity. Yet, such lesions are encountered by every surgical pathologist, and a basic understanding of clinical, radiological and genetic information is critical to successfully evaluate CNS frozen sections. It is often beneficial to have a systematic approach or an algorithm, and to be aware of the common pitfalls and mimickers when dealing with these lesions. We propose such an algorithm in an effort to construct a sensible approach to CNS frozen sections that considers recent developments in the WHO CNS tumor classification. The algorithm was developed for surgical pathologists who are occasionally faced with making diagnosis of CNS tumors on frozen sections. To test the algorithm and its practicability, we selected a group of tumors among a total of 3288 consecutive intraoperative consultations performed at UCSF between 2013 and 2017. The selected cases represented lesions that may be encountered in everyday surgical pathology and constituted a fair reflection of the main group. The algorithm was used by three of the authors who did not have formal neuropathology training and had been in surgical pathology practice for at least 3 years. There was a very high level of concordance among the authors' diagnosis (interobserver concordance: 0.83-0.97-kappa value) using the algorithm with high intraobserver reliability (concordance 93%, $p<0.001$). We suggest that an algorithmic approach is an effective means for the surgical pathologists, and may help reach diagnosis during frozen sections.

Key Words: Algorithm, Central nervous system tumors, Frozen section, Intraoperative consultation, Surgical neuropathology

INTRODUCTION

Intraoperative consultation, commonly known as the Frozen Section (FS), is a critical function of surgical pathologists to help guide the management of patients undergoing surgical procedures. One of the main reasons for performing FS in patients with central nervous system (CNS) disease is to determine the course of action during surgery. In this capacity, the surgical pathologist is a key ally of the neurosurgeon to help select the most appropriate patient management. A successful FS procedure requires an understanding of the essential phases of the process while recognizing the pitfalls and limitations. A number of precautions have been historically recommended (1-6) in order to avoid mistakes and provide the greatest benefit to the patient: 1-good communication with all those involved, 2- recognition of the clinical and radiological information by the pathologist, 3-determination of sample adequacy, 4-appropriate tissue handling and accurate interpretation.

The approach to FS always demands adequate communication between the neurosurgeon and the surgical pathologist to understand the complexities of the case and the specific issues about the patient. At the initial step, the request is to render a diagnosis and help the surgeon decide the course of action, but in many occasions, there may be other questions directed to the pathologist (1-3). The pathologist needs to acquire the critical clinical and radiological information at this initial stage and anticipate questions that may be critical for the specific case.

The second step is appropriate tissue processing and assessment of tissue adequacy. Tissue processing typically involves preparing a smear and performing tissue sections with the help of the cryostat. Evaluation of the prepared smears and tissue sections can be made using an algorithm that helps the pathologist incorporate all the relevant information in order to reach the appropriate differential diagnosis (4). Previous studies have proposed methodical assessment of the information as well as evaluation of cytological and histological features for accurate diagnosis (1,2,5,6).

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Correspondence: : Emel ÇAKIR
Karadeniz Technical University Faculty of Medicine,
Department of Pathology, TRABZON, TURKEY
E-mail: emelnur7@gmail.com Phone: +90 462 377 55 20

The combined interpretation of smears and tissue sections is of critical importance in order to reach the appropriate differential diagnosis. This combination was shown to significantly improve the diagnostic accuracy leading to a high rate of concordance with the final diagnosis (1,5,7-10). Smears are useful, especially if the amount of sampled material is difficult to freeze or if the lesion is in vulnerable anatomic regions such as the brain stem, spinal cord and deep grey structures, precluding large or repeated samples (1). Standard H&E staining (Table I) has been the method of choice for both smears and tissue sections (2).

Several studies correlating FS and final diagnoses identified challenges in the correct recognition of tumors. These challenges include difficulties in distinguishing diffuse gliomas from reactive or inflammatory lesions, differentiating spindle cell tumors (e.g. meningioma vs. schwannoma), recognizing primary versus metastatic malignancies, defining grade or aggressiveness of meningothelial and glial tumors (5,9,11-13). In addition, differential diagnosis of astrocytoma versus oligodendroglioma, lymphoma versus other small-blue-round-cell tumors, and diffuse versus non-infiltrating low grade glial neoplasms have been historically challenging (5,7,11,14,15). Non-neoplastic lesions have often been overlooked since they are less commonly sampled for FS and therefore less familiar to surgical pathologists. The most common non-neoplastic lesions often mistaken for neoplasms include demyelinating lesions, infections, dysplastic lesions such as focal cortical dysplasia, radiation-associated changes, vascular malformations, and hemorrhages (16).

Recent developments in the classification of CNS tumors underscore the need to revise the decades old approach to FS of CNS tumors, and to reconsider what could be reliably reported during the intraoperative consultation, and what old habits should be reconsidered for a more realistic characterization. The most recent WHO classification scheme has adopted the integrated diagnosis for which molecular or genetic information must be considered before a final diagnosis is rendered (17). This approach affects the specificity of FS diagnosis for some entities in at least two aspects; first, there should be little need to subclassify glial tumors as oligodendroglioma or astrocytoma and to provide WHO grading during FS interpretation. Second, obtaining additional tissue for diagnostic molecular studies should be paramount for providing the appropriate care. These considerations require surgical pathologists to be familiar with the necessary testing (Table II) and the degree of specificity of the FS diagnoses.

This introduction provides a stepwise description of key issues in the pre-analytical, analytical and post-analytical stages. It is aimed at pointing out the challenges at each stage of FS, and to encourage the reader to consider the critical stages of the process.

Stage 1- Pre-Analytical Phase

Awareness of the pertinent information that could affects FS interpretation is critical in the pre-analytical phase of FS. The clinical as well as radiological features are extremely helpful in constructing the list of possible diagnoses as well as those that would be improbable in a specific clinical setting. Integration of patient demographics with imaging helps to narrow the diagnostic possibilities, and collaboration with expert neuroradiologists should be the first choice of action. If a neuroradiologist is not available, the surgical pathologist is left on her/his own resources for the interpretation of radioimaging studies. This is neither optimal nor advised, and inaccurate interpretation of radioimaging characteristics can lead to significant mistakes or unrealistic diagnoses.

Table I: Standard H&E staining protocol for smears and frozen sections.

• Fix the smear or tissue section slide on methanol/ethanol mixture for at least 30 seconds and transfer into slide holders	
Station 1	Immerse the entire tissue into Hematoxylin solution for a minimum of 30 seconds with agitation- then thoroughly rinse in water
Station 2	Immerse in 1% HCl to remove excess hematoxylin for 10 seconds (5-10 dips) - then thoroughly rinse in water
Station 3	Place into Ammonia solution to develop blue color of hematoxylin for 10 seconds (5-10 dips)
Station 4	Stain with Eosin solution for a minimum of 15 seconds (slow 10-15 dips)
Station 5	Clear the slide in 95 % ethanol twice for 10 seconds (two jars) and then twice in 100% ethanol for 10 seconds (two jars)
Station 6	Wash in Xylene twice (two jars) until completely clear changing jars (2 x 5-10 dips)
• Immediately cover the slide using mounting media	

One of the most critical issues in every stage of the FS is clear, unambiguous and effective communication among the stakeholders of this consultative process. This communication should involve the neuropathologist and the neurosurgeon in as much direct fashion as possible. The most efficient ways to achieve this communication is an actual visit to the operating room by the pathologist, where he/she can directly communicate with the neurosurgeon, understand the critical issues concerning the case, find out about the clinical details, past history, and prior treatment(s). Access to diagnostic imaging studies is always possible in the operating room, which saves the pathologist the extra effort to learn the imaging features of the case. The combination of clinical, radiological and operative findings is sometimes pathognomonic, and will only require simple visual microscopic confirmation by the surgical pathologist. On the other hand, clinically, radiologically and surgically challenging cases will often be a harbinger of difficulty at the microscope.

Communicating the critical patient information to the pathologist is very important and the surgeons and clinicians should recognize the need to provide their perspective, since the surgical pathologists are not experts in clinical, neuroradiological or neurosurgical practices and nuances. While the surgical pathologist must actively seek this information, he/she should also educate others about the importance of providing the pertinent information for an accurate FS diagnosis.

Another critical issue in the first stage is the correct recognition of the tissue sample and patient identification. Each system must develop a reliable chain-of-custody procedure to ensure timely and efficient delivery of the correct tissue samples from the operating room to the pathology laboratory.

Stage 2- Analytical Phase

The surgical pathologist should be familiar with the situations that may lead the process astray in the analytical phase, and must have a good understanding of the tissue processing steps in order to identify/avoid errors. The critical elements of this stage include appropriate tissue procurement, processing and interpretation. Many studies report that the use of intraoperative smears significantly improves the diagnostic accuracy (1, 7). Therefore, use of smears and the ability to interpret the features in smears are critical. Certain nuclear and cytoplasmic details of tumor cells are best appreciated on smear preparations. However, smears do not permit a detailed assessment of architectural features and the nature of the tumor-brain interface. Tissue sections provide better information on the architectural features, cellularity and extracellular environment.

Ideally, a well-prepared smear, a good tissue section and sufficient material for subsequent critical studies (final diagnosis) must be obtained prior to the analytical process. Sufficient material for special studies and molecular analyses are required as a standard of care for increasingly

Table II: CNS Tumors that require molecular analysis for integrated diagnosis (therefore require extra caution when making the diagnosis during frozen sections).

Tumor Type	Molecular Marker Required
Diffuse Astrocytoma, IDH-mutant including Glioblastoma, IDH-mutant	IDH1, ATRX, p53 immunohistochemistry, IDH1/2 sequencing (if necessary)
Diffuse Astrocytoma, IDH-wildtype including Glioblastoma, IDH-wildtype	EGFR, PTEN, TERT alterations, Chr7 gain and Chr 10 loss
Oligodendroglioma	IDH1 immunohistochemistry, IDH1/2 sequencing (if necessary) Chr 1p/19q co-deletions
Diffuse midline glioma	H3 K27M, H3K27me3 immunohistochemistry
Medulloblastoma, WNT-activated	B-catenin, ALK, LEF1, YAP 1 immunohistochemistry
Medulloblastoma, SHH-activated	GAB1, YAP1 immunohistochemistry
Atypical Teratoid/Rhabdoid Tumor	SMARCB1 (BAF47), SMARCA4 (BRG1) immunohistochemistry
Embryonal Tumor with Multilayered Rosettes	LIN-28A immunohistochemistry C19MC sequencing
Solitary Fibrous Tumor/ Hemangiopericytoma	STAT6 immunohistochemistry

large number of tumor types in the molecular era (1). There is an ever growing list of tumors that will benefit from molecular analysis and a larger number of genetic alterations that are helpful for the diagnosis of tumors, yet a short list can be generated for tumors that MUST be characterized in terms of their molecular characteristics required for the WHO 2016 integrated diagnosis (Table II). In circumstances where the tissue is too small to be sufficient for all of the above, the priority should be given to preserve the tissue for an accurate final diagnosis using permanent sections.

Difficulties of interpretation can be due to multiple factors including limited sampling, inadequate information, processing problems, and inexperience. Any tumor can be morphologically heterogeneous, and accurate diagnosis highly depends on adequate sampling. Insufficient tissue, necrotic material or non-representative tissue often does not permit an accurate diagnosis and further material should be sought. When large specimens are submitted, gross visual examination and recognition of the normal gray and white matter as well as the abnormal tissue allows a better and more appropriate sampling for FS diagnosis (1). To prevent sampling errors, multiple smears and tissue sections may be necessary, as long as there is sufficient material for final diagnosis and molecular studies. Some studies show that a minimum of 4 tissue samples may be necessary to ensure a high diagnostic yield (1,18,19).

Preparation of a good smear is a crucial part of sample preparation. A tissue fragment, not to exceed 2 mm should be used. This tissue fragment is placed on the glass slide closer to the label side, and the fragment is "smeared" by gentle pressure using another glass slide and dragged along the length of the slide. The slides should be dipped and fixed in 95% ethanol for at least 15 seconds immediately after smearing. No air drying should be permitted.

The frozen section should be performed with utmost care to avoid over-freezing or drying. In addition, the tissue should not be allowed to "bathe" in water or saline, and preferably should be placed on a tissue paper wetted with isotonic solutions. A frozen metal block and optimal cutting temperature (OCT) compound could be used to mount the tissue on the cryostat and no thicker than 5 micron sections should be obtained containing the entire cut surface of the tissue sample. One important point about the OCT compound is the ability of this mixture to inhibit the PCR reaction, and to render the tissue inappropriate for some molecular studies. The staining for smears and tissue sections can be made with the same standard H&E protocol. Since different H&E staining protocols may influence the quality of the interpretation, an optimal staining protocol is provided on Table I.

Once the smears and tissue sections are optimally prepared, the surgical pathologist should begin the interpretive task using a set of standard questions (Figure 1) and a systematic assessment of microscopic features; i.e. an algorithm (Figure 2A,B). The initial question should focus on the normal and abnormal elements in the sample (**Is the tissue abnormal and are there normal cells?**) followed by the inquiry as to whether the sample is actually representative of the process (**Is the specimen sufficient and representative?**). A negative answer to either of these questions require an early remediation such as repeating the smear or the tissue section or requesting additional tissue from the operating room. Once both questions are answered affirmatively, the next step in the algorithm is to distinguish between a neoplastic and non-neoplastic process (**Is it neoplastic or non-neoplastic?**). Once the lesion is recognized as neoplastic, the algorithm (Figure 2A,B) can be employed to further characterize the process. Naturally, since the probabilities vary between the pediatric and adult patients, the algorithm should be modified accordingly (Figure 2A,B). The step in which the neoplastic/non-neoplastic decision is made can be deceptive and unexpectedly difficult.

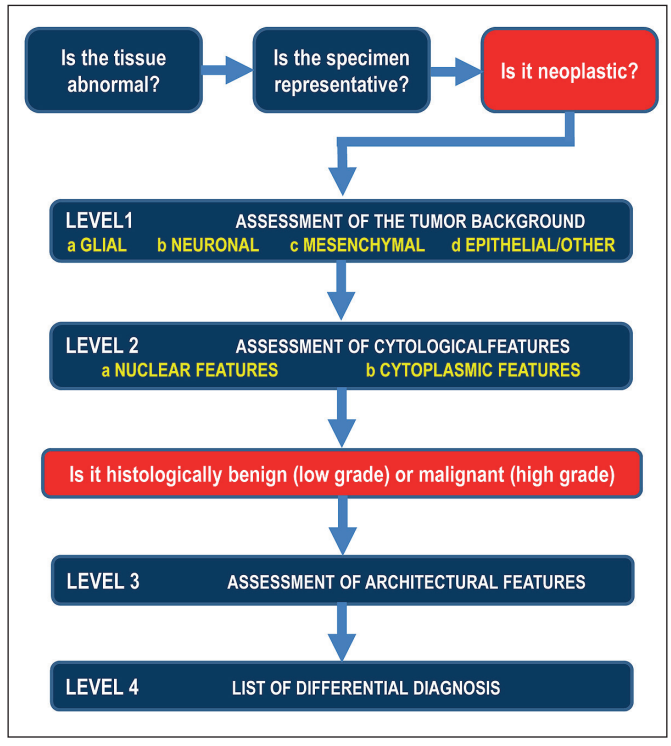

Figure 1: The algorithm of intraoperative consultation specimens for CNS tumors.

The first step of the algorithm (Level 1) is to recognize the smear and frozen section background as belonging to one of the four basic patterns; predominantly glial, neuronal, mesenchymal or epithelial (Figure 2A,B). The second step (Level 2) is the analysis of cytological features of the "abnormal" cells in terms of nuclear morphology (Level 2a) and cytoplasmic characteristics (Level 2b). Nuclear morphology is evaluated in terms of hyperchromasia, pleomorphism, nucleoli, structural anomalies such as grooves or inclusions. Cytoplasmic characteristics include definition of cellular membranes, processes, cytoplasmic structural anomalies (folding, inclusions, vacuolization). The third step includes review of architectural features in the smear and tissue sections (Level 3).

The recognition of the smear and tissue background features can allow the surgical pathologist to create shorter lists of differential diagnosis and help construct a practically relevant interpretation for the surgeon (Is the tumor glial or non-glial). In addition, level 1 categories can be further subdivided into diagnostically relevant subgroups based on

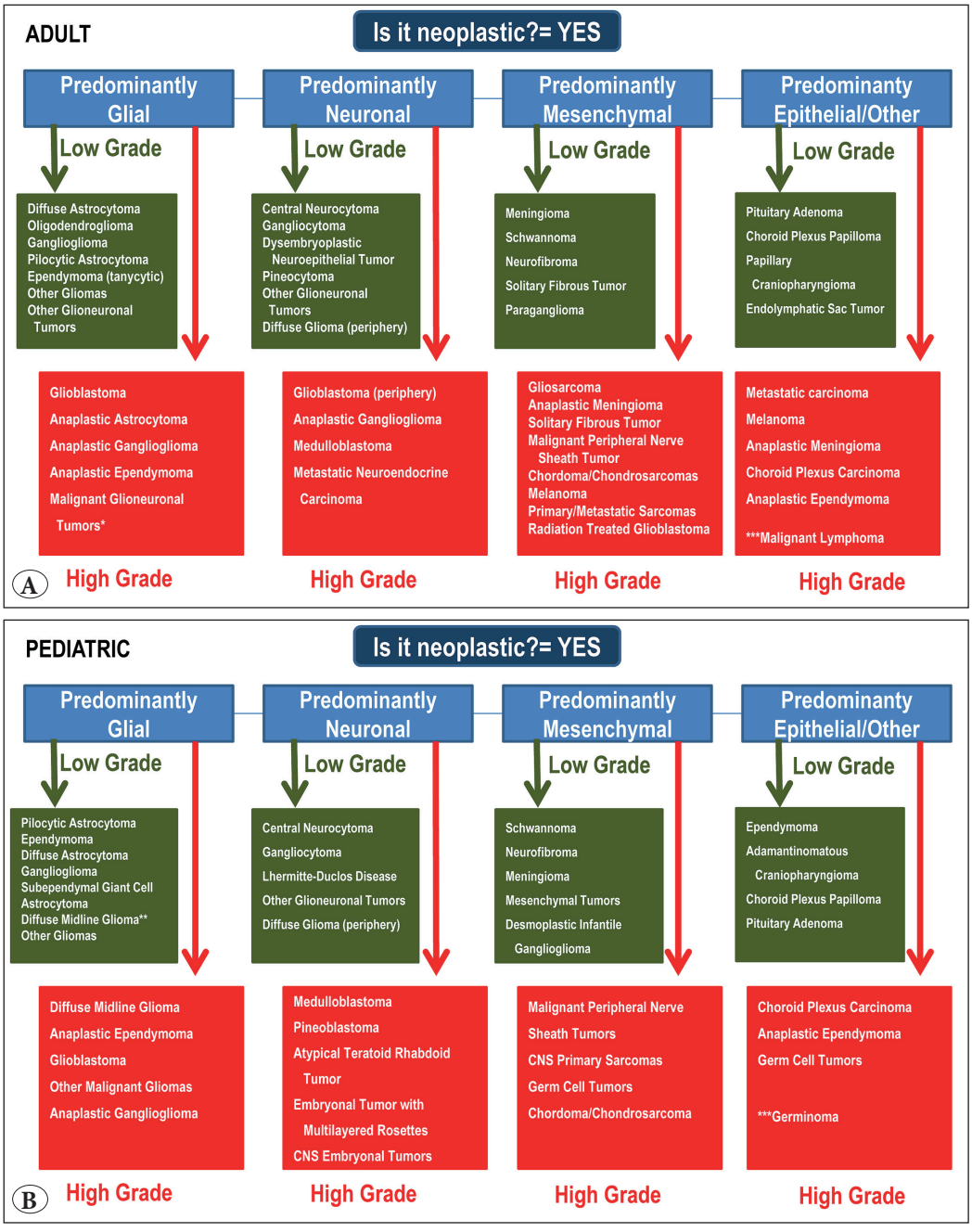

Figure 2: A) An algorithmic approach to adult patients with CNS tumors. **B)** An algorithmic approach to pediatric patients with CNS tumors.

cytological and architectural observations as being indolent or aggressive nature. Histologically benign appearing lesions can be distinguished from histologically and cytologically anaplastic or malignant samples. The cytological and architectural features from both the smear and the tissue section can be used to determine whether the neoplasm is aggressive or more likely to be indolent (**Is it high grade or low grade?).** The next step after the interpretation of the background as glial is the recognition of signs that suggest aggressive biology as well as infiltrative or solid growth pattern. Figure 2A and 2B provide a differential diagnosis of tumors based on information gathered at levels 2-3.

Some typical histological features may be difficult to recognize on FS. For example, the features typical of oligodendrogliomas such as the "chicken-wire" vasculature and "fried-egg" cells are due to paraffin processing and not readily recognizable on FS. Freezing also imparts significant artefactual changes in tumors such as oligodendrogliomas, rendering them difficult to distinguish from other gliomas (Figure 3A-C). While nuclear features and chromatin structure may be helpful in telling them apart, recent molecular studies imply that it may not be possible to make this distinction easily on morphological grounds. With the changes suggested in the WHO 2016 classification, it may be sufficient to recognize the tumor simply as "diffuse glioma", and avoid the problem of the astrocytoma vs. oligodendroglioma distinction during FS altogether. For histologically malignant lesions, it may be sufficient to express the presence of a "high grade glioma", especially if multiple mitotic figures, vascular proliferation or palisading necrosis is present.

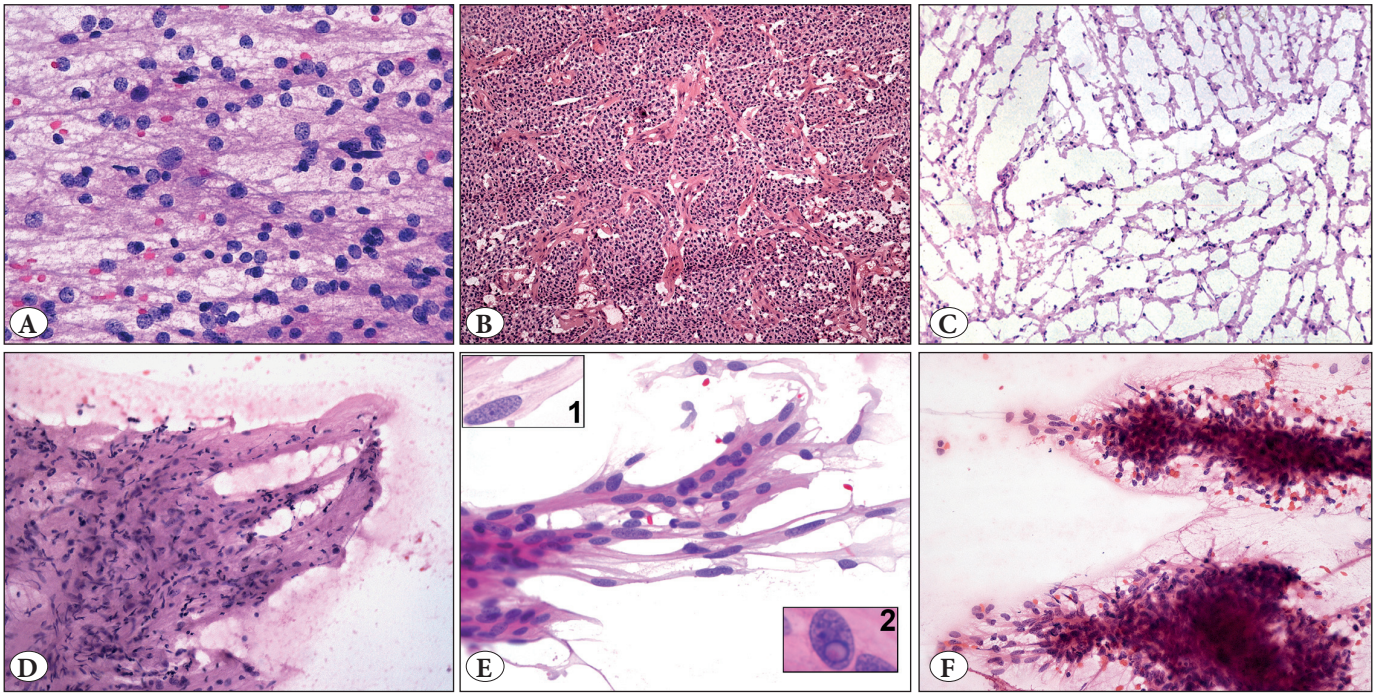

Figure 3: A) Oligodendroglioma, smear – Intraoperative smear preparation of IDH-mutant, 1p/19q co-deleted oligodendroglioma. The smear preparation highlights the glial (fibrillary) background, round nuclei, scant cytoplasm and absence of mitotic activity (H&E; x400). **B)** Oligodendroglioma, frozen - The frozen sections from oligodendrogliomas often do not exhibit the typical chicken-wire vasculature and perinuclear halos that are associated with formalin fixation and paraffin embedding. Nevertheless, the delicate, thin vascular channels that portend the tumor its chicken-wire vasculature can be appreciated (H&E; x200). **C)** Oligodendroglioma, frozen –Frozen sections in low grade oligodendrogliomas may be subjected to significant freezing artifact, rendering assessment of cellularity and nuclear morphology impossible. Without smear preparations, such frozen sections can be ver difficult to interpret (H&E; x200). **D)** Schwannoma, smear – Schwannomas are among tumors that are difficult to smear. Elongated, irregular nuclei, inconspicuous nucleoli and a mesenchymal type background are distinctive features in smear preparations (H&E; x400). **E)** Meningioma, smear – The smear preparations of meningiomas demonstrate the prototypical "mesenchymal background" with abundant cytoplasmic folding creating the so-called "train-track" appearance. The nuclei are often round to oval with intranuclear inclusiong and open chromatin pattern, (H&E; x400)- Inset 1 –Traintracks; Inset 2- Intranuclear inclusion, (original magnification ; x1000). **F)** Solitary fibrous tumor, smear – Similar to meningiomas, solitary fibrous tumors have a typical "mesenchymal" background with prominent fragments of collagen and spindle cells with ovoid or round nuclei. Features such as intranuclear inclusions, train tracks or nuclear grooves are absent (H&E; x400).

Tumors with neuronal background are often in the glioneuronal, neurocytic or embryonal category, with the exception of samples obtained from the periphery of diffuse gliomas containing substantial amount of neuropil. While the background in such samples of diffuse gliomas may appear neuronal, the glial characteristics of the tumor are almost always prominent in smears and tissue sections, allowing the surgical pathologist to recognize diffuse glioma. The differential diagnosis of smears with neuronal background should also include embryonal tumors, especially in pediatric patients. Level 2 and 3 observations will identify malignant or "high grade" features in these tumors.

Distinguishing spindle cell neoplasms such as meningioma, schwannoma or solitary fibrous tumor can be challenging on FS. These spindle cell tumors are very similar on clinical and radiological grounds. On level 1 all such lesions have a mesenchymal background, yet cytological and architectural clues may help to distinguish them from one another (11). Finding isometric, round or oval nuclei with the "train track sign" is suggestive of a meningioma, whereas a tumor with markedly irregular, pleomorphic nuclei and highly cohesive tissue clusters may suggest schwannoma. Most often, however, the differential diagnosis of benign mesenchymal tumors is not of practical significance. The FS diagnosis can simply be "spindle cell neoplasm, no high grade features" and the specific diagnosis may be deferred to permanent sections, especially for difficult lesions that appear fibroblastic (Figure 3D-F).

If the background of the smear is not appropriately recognized, the interpretation of a small blue round cell tumor can be quite challenging on FS. Such tumors can be embryonal neoplasms, lymphomas, malignant gliomas, anaplastic ependymomas, or metastatic carcinomas, neuroendocrine tumors or small cell sarcomas. A careful review should identify the nature of the background and allow recognition of ependymal, glial, or embryonal tumors. Metastatic carcinomas would yield a dirty epithelial background, while melanomas or sarcomas have partial mesenchymal background characteristics.

Stage 3- Post-Analytical Phase

The critical function of the surgical pathologist during the post-analytical phase is the accurate and timely reporting of the FS diagnosis. It is not appropriate to argue about the necessity of any intraoperative consultation during surgery, and the surgical pathologist could convey the ambiguity of making certain diagnoses on FS to the neurosurgeon at a less stressful time.

In the past, it has often been the custom to report whether a diffuse glioma was astrocytic or oligodendroglial, as well as mention the presumed WHO Grade. Today, understanding the genetic diversity and the importance of IDH, ATRX, PT53 mutations as well as TERT, EGFR and PTEN alterations forces us to be less definitive on the type and grade of diffuse gliomas during FS. The presence of nuclear atypia without aggressive histological features is often little comfort, especially if the patient is older (typically >65 years) or if there are worrisome radiological features. Reporting of diffuse gliomas that appear low grade on histology should always be made with caution since a subsequent sample may show higher grade features (20). Studies highlight the caveat of undergrading tumors in small samples or during FS procedures (3,11).

Tumors with clearly malignant or anaplastic features such as numerous mitoses, vascular endothelial proliferation (microvascular proliferation) or necrosis with or without palisading are important clues for the recognition of a "high grade glioma". All these features must also consider the importance of establishing an integrated final diagnosis consistent with WHO 2016 criteria (see below).

Recognition of the mesenchymal or spindle cell features may allow the pathologist to suggest a spindle cell tumor without high grade features, which may be sufficient for the patient management intraoperatively. While it may be helpful to distinguish meningioma from schwannoma or from a solitary fibrous tumor later, such distinctions can easily be deferred to permanent sections. Grading of spindle cell neoplasms such as meningiomas should be avoided on FS.

In case of highly malignant neoplasm with a primitive appearance, i.e. small-blue-round cell tumors, there is a good reason to be able to sort out what type of primitive tumor has been sampled. Primary neuroepithelial tumors should be distinguished from malignant lymphomas or metastatic tumors. If this distinction is not clear, the diagnosis is essentially a deferral to permanent sections, and will most certainly require special studies. Clinical and radiological features must be evaluated with caution, and the age of the patient should be taken into consideration when reporting these lesions. It is also critical to directly communicate with the neurosurgeon, because it may be sufficient to render a diagnosis of "neoplasm" for practical purposes, and the neurosurgeon may not need the pathologist to agonize over a difficult, and often an impossible differential diagnosis.

What has changed with WHO 2016?

Many modifications of the WHO 2016 CNS tumor classification and the subsequent clarifications have changed the way FS diagnosis can be reported, and the two major groups affected by these modifications are glial and

embryonal tumors. A few points need to be made about some diagnostic statements that can be used in compliance with this classification:

- Diffuse glioma, no high grade features: Once the tumor is identified as infiltrating or diffuse glioma, it may not be necessary to subclassify tumors as either astrocytoma or oligodendroglioma. Especially if there is no radiological or histological evidence of a high grade tumor, the FS diagnosis could be "diffuse glioma, no high grade features". Since there is always the possibility of finding a higher grade component on permanent sections, the statement of "no high grade features on FS" may be used instead of "low grade diffuse glioma" to avoid giving the impression that the FS diagnosis suggests a final grade. It is more prudent to be remain in a more general diagnostic category for the cases that will typically require further genetic/molecular characterization.
- Diffuse glioma, high grade: For tumors with glial background, radiological and clinical features are critical to determine their aggressive potential. Most diffuse gliomas in the elderly, diffuse gliomas with substantial (or ring) enhancement on MRI, histological features such as vascular proliferation and necrosis are most often high grade tumors. Histological evidence of malignancy should prompt "Diffuse glioma, high grade" designation. However, even in the absence of histological evidence, the clinical and radiological evidence may allow the pathologists to report the tumor as "Diffuse glioma, favor (or suspect) high grade tumor". Diffuse gliomas in the midline with H3K27M mutations are also considered WHO grade IV lesions, so the possibility of this type of tumor may also lead the pathologist to "favor or suspect" high grade diffuse glioma.
- Low grade glioma, NOS: This nonspecific diagnosis would imply a number of diagnostic possibilities and is not appropriate as a final diagnosis. However, solid tumors with no sign of aggressive features radiologically and histologically may be reported as "Low grade glioma" and a discussion with the surgeon about the non-infiltrative nature of the tumor should be made. This designation is best avoided for diffuse gliomas, regardless of their FS appearance. For cases when a diffuse glioma cannot be entirely excluded, it is best to DEFER the diagnosis, since the designation of diffuse versus non-infiltrative/solid tumor is an important one.
- Embryonal Tumor, NOS: The term embryonal tumor has replaced the old "primitive neuroectodermal tumor" category, and there is a larger list of tumor entities in this group. Most embryonal tumors in the cerebellum are diagnosed as medulloblastoma. However, before one can clearly use this diagnosis, it is imperative to be certain that it cannot be one of the recently described tumor entities such as the embryonal tumor with multilayered rosettes (ETMR), or atypical teratoid/rhabdoid tumor (AT/RT). All of these tumors have a neuronal-like background with small-blue-round cells, but each has somewhat unique feature that may not be readily apparent in FS. For tumors in the posterior fossa, "embryonal tumor, favor/suggest medulloblastoma" may be a better option than a simple "medulloblastoma" since there are overlaps with the entities mentioned above. In the rare supratentorial example, the differential diagnosis includes even more entities, so "embryonal tumor" or "embryonal tumor, NOS" could be the diagnosis of choice until a better classification is made on permanent sections. One important issue in the differential diagnosis is recognizing pediatric ependymal tumors within the posterior fossa that could easily be confused with medulloblastoma.
- Tissue for Molecular studies: Ever increasing number of tumors require molecular characterization for accurate typing and grading, and providing sufficient tissue for these analyses is very important. It is imperative to be aware of these tumors, secure enough material for the appropriate studies, and alert the surgeon to the need of extra tumor tissue for these studies during the FS procedure. Tumors in this category may include all diffuse gliomas including glioblastoma, all embryonal tumors in the pediatric population, tumors with both glial and neuronal elements, and any tumor that the pathologist finds problematic in characterizing with a high degree of confidence. A minimalistic list of molecular studies would include *IDH, ATRX, TP53, H3K27M, H3G34R/V, SHH, WNT, BRAF, CTNNB1, SMARCB1* mutations *MYC, C19MC, RELA, BRAF, BCOR*, *MN1* rearrangements and chromosomes 1, 7, 10, 19 alterations. This list could be expanded and is growing with every passing day.

Testing The Algorithm: Practical Utility

Method: To determine its practical utility, the algorithm was tested by three of the authors without formal neuropathology training on a group of cases selected from 3288 FS procedures performed in our institution between 2013 and 2017. We reviewed all the FS procedures during this period to acquire a sense of the frequency and distribution of diagnoses and types of cases in our

institution. Clinical and radiological information, original FS diagnoses, and final pathology diagnoses for all cases were reviewed by two of the authors (EC, TT). A subset of 160 cases was selected by one of the authors (GEY) for testing the algorithm by three of the authors (EC, GO, CD). Each diagnosis was rendered by one of the authors. The subset of cases for the study were identified among cases with sufficient clinical and radiological information. Original FS diagnoses were recorded for all cases, and the final diagnoses were confirmed through additional special studies and clinical follow-up information. The final diagnoses for each case was considered the gold standard (i.e. correct diagnosis). Three of the authors reviewed the smears and tissue sections as well as the clinical information that was available at the time of the original FS. In addition, 30 of the cases were used in an interobserver variability study. Each pathologist was asked to use a checklist composed of the algorithmic steps (Figures 1, 2A,B) and to provide the diagnosis for which they felt confident enough to report to the neurosurgeon. The use of the algorithm required selecting options from a decision tree and to identify a diagnostic category. The diagnoses were then compared to the original FS diagnoses as well as the final diagnoses. Concordance and interobserver variability were evaluated to determine the effectiveness of the algorithm. Discrepancies were defined as either major or minor. Major discrepancy was defined as a difference between two diagnoses (original FS versus final diagnosis; algorithmic FS versus original FS diagnosis; algorithmic FS versus final diagnosis) that would significantly alter patient care, a change in the major diagnostic groups in the WHO classification, or a change in WHO grade of more than 1 level (e.g. WHO grade I to grade III or II to IV, or vice versa). Minor discrepancy was defined as a change within major diagnostic groups or a change in the WHO grade of one (1) level AND no potential adverse effect on patient care.

Statistical analyses were performed using the SPSS Version 23 with Advanced Statistics Package. The McNemar Test was used in the analysis of the differences between the ratios of categorical variables in independent groups. The Kappa Test was used to evaluate interobserver agreement by the Online Kappa Calculator (21). The concordance between authors' diagnosis, original frozen diagnosis and final diagnosis were tested by Intraclass Correlation Test and presented with 95% confidence intervals. A p value less than 0.05 was considered statistically significant. This study was approved by the UCSF Committee for Human Research (CHR 10-01252).

RESULTS and DISCUSSION

The distribution of all FS cases between 2013 and 2017 is presented in Figure 4. The most common tumors were Glioblastoma (702 cases; 21.3%), meningiomas (541 cases; 16.5%), and diffuse astrocytic tumors (348 cases; 10.5%). Among those, 160 cases were randomly selected by one of the authors and the other authors were blinded to the selection process.

There were 9 cases with major and 12 cases with minor diagnostic discrepancies between the algorithm-based diagnosis (author's diagnoses) and the final diagnosis

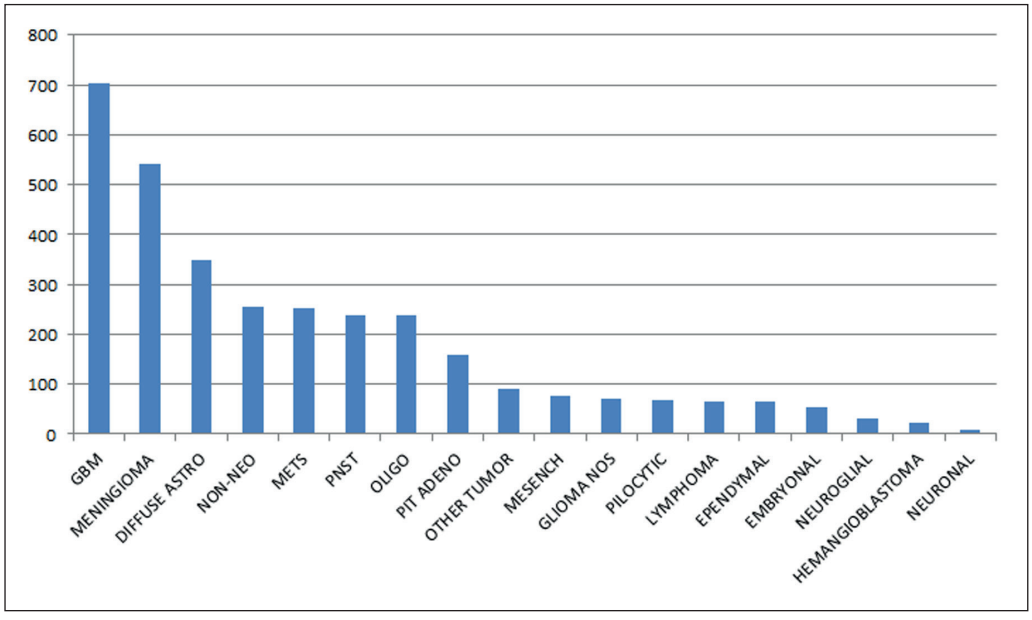

Figure 4: The frequency of each tumor entity among all FS cases between 2013 and 2017.

(major discrepancy rate (5.6%). These discrepancies are presented on Table III. In addition, 12 cases showed major discrepancies between the original and the algorithm-based FS diagnoses. In addition, there were a total of 6 discrepancies between the original frozen diagnosis and final diagnosis (discrepancy rate 3.8%). Two of these diagnoses were rendered by surgical pathologists (neuropathologist discrepancy rate %2.5). Two of the original frozen section diagnoses were deferred to permanent sections (deferral rate 1.25%). Others were associated with grading or inadequate sampling. The concordance analysis is presented on Table IV.

Seven of the 9 major discrepancies fell into a particular type where the algorithm diagnosis and final diagnosis differed in neoplastic versus non-neoplastic diagnoses. The interobserver concordance was high among the authors, and the differences often emerged at level 4, when the pathologists were asked to select a specific diagnosis from the final list. Nevertheless, interobserver variability was quite low with high concordance in most tumor categories (Table V).

Using the algorithm during the mock FS procedures helped identify general categories of tumors more easily and provided a standard checklist to remember. While this was difficult to quantify, specific questions and in the algorithm allowed pathologists to consider the options not considered originally in their differential diagnosis. Recognizing the smear background also allowed to start with a narrower list of differential diagnosis. The algorithm also led the pathologists to better define low grade and high grade features within the same categories. This was particularly helpful since in many occasions, the surgeons may be satisfied with a descriptive diagnosis or a category rather than a specific entity.

Table III: Major discordance between authors' diagnosis and the final diagnosis.

Case #	Authors' diagnosis	Original frozen diagnosis	Final diagnosis
Case 20	Non-neoplastic	Diffuse Astrocytoma	Anaplastic Astrocytoma
Case 40	Non-neoplastic	Possible Lymphoma	Glioblastoma
Case 50	Non-neoplastic	Diffuse Astrocytoma	Diffuse Astrocytoma
Case 59	Benign mesenchymal tumor, NOS	Non-neoplastic, acute inflammation	Osteomyelitis with epidural abscess
Case 75	Anaplastic Astrocytoma	Recurrent Oligodendroglioma	Recurrent Oligodendroglioma WHO Grade II
Case 76	Glioma, NOS	Non-neoplastic	Radiation necrosis
Case 123	Malignant neoplasm, NOS	Malignant neoplasm, NOS	Central Neurocytoma
Case 126	Low grade glioma	Non-neoplastic	Focal Cortical Dysplasia
Case 131	Non-neoplastic	Suspicious for low grade Chondrosarcoma	Low Grade Chondrosarcoma

Table IV: Concordance between authors' diagnoses and original frozen and final diagnoses.

	Diagnostic Category Based on the Algorithm (authors' diagnoses)	Agreement with frozen diagnosis %	Agreement with final diagnosis %
Cases (n=160)	Non-neoplastic lesions	77.8	88.0
	Pituitary adenomas	100.0	100.0
	Benign mesenchymal tumors	88.5	95.8
	Metastatic tumors	90.0	90.0
	High grade neuroepithelial tumors	90.4	90.7
	Low grade neuroepithelial tumors	85.2	85.2
	Others	88.9	100.0
	Intraclass Correlation (95% C.I., p)	**89.93 (86.2-92.7), p<0.001**	**89.90 (86.2-92.7), p<0.001**

Table V: Interobsever variability.

Percent Agreement of Algorithm Based Frozen Diagnosis to determine interobserver variations	Categories	% within frozen diagnosis	Agreement with Author 1	Agreement with Author 2	Agreement with Author 3
	Non-neoplastic		100.0	83.3	83.3
	Pituitary adenomas		100.0	100.0	100.0
	Benign mesenchymal tumors		100.0	100.0	100.0
	Metastatic tumors		100.0	100.0	100.0
	High grade neuroepithelial tumors		86.7	93.3	100.0
	Low grade neuroepithelial tumors		100.0	80.0	80.0
	Other tumors		100.0	100.0	100.0
	Intraclass correlation (95% C.I., p)		**96.0 (91.7-98.1) p<0.001**	**95.3 (90.1-97.7) p<0.001**	**95.5 (90.5-97.8) p<0.001**
Concordance between authors' diagnoses (95% C.I.) = 93.1(87.4-96.5), [p<0.001]					
Concordance between all authors' and frozen diagnoses (95% C.I.)= 96.3(93.6-98.1), [p<0.001]					

In the present study, the pathologists using the algorithm most often misdiagnosed non-neoplastic lesions, since the algorithm had focused on identifying tumors and the non-neoplastic possibilities were often overlooked (7 of 9 major discrepancies). This was interpreted as a typical cueing error, since the participants were focused on identifying the type of tumors in the slides. This was particularly important because once the surgical pathologists committed themselves to either a neoplastic process, it was very difficult to change course. Therefore, the most critical stage prior to the application of the algorithm was recognized as the first stage when the question "is it neoplastic or non-neoplastic?" is being determined. This was also due to the fact that the number of non-neoplastic lesions is often very low in surgical pathology practice, and the recognition of the non-neoplastic lesions often requires keeping them in mind and being familiar with their frozen section appearance.

The two other major discrepancies were related to the grade of the neoplasm (is it high grade or low grade?). However, one of the critical issues in this exercise was the limited amount of information, and in both cases, the pertinent clinical information not immediately available during frozen section (prior diagnosis of oligodendroglioma in one case and radiological diagnosis of central neurocytoma in the other). In real life, it would have been easier to correctly diagnose these cases.

While there were other minor discrepancies related to individual factors, the authors have reached a differential list containing the correct diagnosis in the overwhelming majority of the cases. The algorithm was not fool-proof, and additional information and scrutiny would have certainly helped in the discrepancies. Due to the nature of the study, there was no opportunity to contact the clinician or review radiological findings or further scrutinize the cases, which makes us believe that the discrepancies could have been minimized if the suggestions above for the pre-analytical phase could be followed.

One limitation in this study is the potential cuing or bias that may be present among the observers. While each pathologist independently reviewed the slides and used the algorithm, they communicated freely and routinely during their daily practice while conducting the study. This interaction could have introduced a certain level of bias in the use of the algorithm, leading to a high level of interobserver concordance. Further studies will be needed to determine the interobserver variability and utility of the algorithm among pathologists working in different settings and independent of each other.

One of the other limitations of this validation study is the lack of comparison with other algorithms or attempt at diagnosis without an algorithm. However, the participants in this study preferred to use guidance for frozen section diagnosis, since they had already begun analysis with the algorithm. Therefore, they could not be used as unbiased observers. Currently, studies are being designed to evaluate this algorithm in different settings, larger samples and the actual practice environment to better define the contribution of the algorithm to the confidence of pathologists for CSN frozen sections.

CONCLUSION

During the FS process, the errors made in deciding tumor versus no tumor may lead to erroneous diagnoses, so this step should be made very carefully, and any algorithm is of little use once the pathologists chooses the wrong option at this stage. Discrepancies can also occur due to sampling and inadequate information, and the algorithm would be of no use in these circumstances either. Once these hurdles are overcome, an algorithm can be a good learning tool and may help the pathologist to consider all the realistic options. While our initial "test" of the algorithm is quite limited and in no way should reflect the practicability of the algorithm on a larger scale, it does provide us with a helpful tool. We also felt that it was critical to reiterate the changes imposed by the new WHO classification and further revisions by C-IMPACT NOW publications (20,22,23) and to use this review to bring these changes to attention. We hope that our review will help the practicing surgical pathologists when they encounter a CNS tumor for FS in their everyday practice.

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