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CLINICAL RESEARCH / KLİNİK ARAŞTIRMA

Diagnostic Value of Smears and Frozen Sections in Neuropathology Practice: Institutional Experience

Nöropatoloji Pratiğinde Smear ve Frozen Kesitlerin Tanısal Değeri: Tek Merkez Deneyimi

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Abstract

Objective: The intraoperative diagnosis of neurosurgical biopsy material is usually tricky due to small size, artifacts of freezed neural tissue. When we are using rapid techniques, we should be aware of the limitations. In this study, we evaluate the diagnostic value of smears, frozen sections in neuropathology practice.

Methods: We evaluated 65 neurosurgical biopsy materials sent for intraoperative diagnosis. All of them were interpreted with both smear and frozen section preparations. Results were grouped as inadequate (normal tissue, full of necrosis, technical artifacts), inflammatory/reactive or neoplastic. Difficulties, limitations of rapid methods were discussed.

Results: Frozen and paraffine diagnoses were concordant in 55 cases. Three were inflammatory/reactive and fifty-two were tumor [low grade glial tumor (11), ependymoma (1), glioblastoma (12), metastasis (5), meningioma (8), craniopharyngioma (2), pituitary adenoma (2), epidermoid cyst (1), central neurocytoma (1), medulloblastoma (2), schwannoma/neurofibroma (6), diffuse large B cell lymphoma (1)]. In two cases, the amount of the material was sufficient but we could not achieve a definitive diagnosis with squash and frozen sections. The paraffine result of these cases was giant cell ependymoma and vasculitis. In eight cases, the material was insufficient for diagnosis. The frozen material contained only necrosis in six cases [glioblastoma (4) and metastasis (2)]; normal parenchyma in a DNET case, cauterized tissue in a fibrous meningioma case.

Conclusion: The accuracy of intraoperative diagnosis of neurosurgical material is very high. Also, the results of smear preparation and frozen section are concordant in most entities. The main role of rapid techniques in neuropathology is to determine whether the tissue is representative and adequate for ancillary testing such as immunohistochemistry and molecular. We should always be keep in touch with neurosurgeon and complemented morphological findings with radiological and clinical characteristics for the most reliable diagnostic interpretation.

Keywords: Squash, frozen section, neuropathology, intraoperative consultation

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Öz

Amaç: Beyin ve sinir sistemi cerrahisine ait biyopsi materyallerinin intraoperatif tanısı, materyallerin genellikle küçük boyutlu olması ve nöral dokunun yoğun donma artefaktı göstermesi nedeniyle oldukça zordur. Bu çalışmada nöropatoloji pratiğinde smear/squash ve frozen kesitlerin tanısal değerini araştırdık.

Yöntem: İntraoperatif tanı için gönderilen, 65 beyin cerrahisi biyopsi materyalini değerlendirdik. Tüm olgularda squash ve frozen kesit preparatları birlikte yorumladık. Squash ve frozen kesitleri yetersiz (normal doku, nekroz, teknik artefakt), enflamatuvar/reaktif veya tümör olarak gruplandırdık. Hızlı metodların zorlukları ve sınırlılıklarını tartıştık.

Bulgular: Frozen ve parafin tanılar 55 olguda uyumluydu. Üçü enflamatuvar/reaktif ve 52'si tümöral [düşük dereceli glial (11), ependimom (1), glioblastom (12), metastaz (5), meningiom (8), kranyofaringiom (2), hipofiz adenomu (2), epidermoid kist (1), santral nörositom (1), medulloblastom (2), schwannom/nörofibrom (6), diffüz büyük B hücreli lenfoma (1)] idi. İki olguda materyal miktarı yeterliydi ancak squash ve frozen kesitlerde tanı verilemedi. Bu iki olgunun parafin sonucu dev hücreli ependimom ve vaskülit idi. 8 olguda materyal tanı için yetersizdi. Bunlardan 6'sında [glioblastom (4), metastaz (2)] squash/frozen kesit sadece nekrozdan oluşmaktaydı; bir DNET olgusunda normal kortikal parankim, bir fibröz meningiom olgusunda ise yoğun koterize doku izlendi.

Sonuç: Beyin cerrahisi materyallerinde intraoperatif tanı doğruluğu oldukça yüksektir. Ayrıca squash preparatlar ve frozen kesitlerin sonuçları çoğu tanı kategorisinde uyumludur. Hızlı tekniklerin nöropatolojideki ana rolü, dokunun moleküler ve immünohistokimyasal inceleme gibi yardımcı testler için temsili ve yeterli olup olmadığını belirlemektir. Eğer sonuçlar enfeksiyöz süreç olduğunu gösteriyorsa, mikrobiyolojik analiz için doku örnekleri alınmalıdır. Son büyük avantajı ise lenfoma ve küçük hücreli karsinom gibi total rezeksiyon gerektirmeyen tümör tiplerini belirlemektir. Nöropatolojide en güvenli intraoperatif tanısal inceleme için her zaman cerrah ile iletişim halinde olmalı ve morfolojik bulgularla radyolojik ve klinik özellikleri bütün halinde ele almalıyız.

Anahtar Kelimeler: Squash, frozen kesit, nöropatoloji, intraoperatif konsültasyon

Introduction

The intraoperative diagnosis of neurosurgical biopsy material is usually tricky due to small size and several artifacts of freezed neural tissue. Although the diagnostic accuracy of frozen sections is 90%[1] and of smears ranges from 83% to 95%[2,3], sometimes we can't achieve the definitive diagnosis with smears and frozen sections because of tissue characteristics, technical problems and pathologist experience. When we are using rapid techniques, we should be aware of advantages and limitations. We should keep in touch with the neurosurgeon and always complement morphological findings with radiological and clinical characteristics for most reliable diagnostic interpretation.

Stereotactic biopsies, open biopsies, partial resection, gross total resection, lobectomy can be done according to the aim of the surgery. So the specimen size varies due to the surgical procedure. In our routine practice, the spectrum of neurosurgical materials consists of open biopsies, partial resections and gros total resections. Our neurosurgeons usually send a small piece of material for intraoperative evaluation. We did freeze all of the tissue. We prepared both smears and frozen sections. Such combining these methods improves the accuracy of peroperative consultation. Sometimes we see only normal cortex/parenchyma, necrosis, or hematoma. So we request more material for definitive diagnosis, grading, immunohistochemical/molecular analysis. If possible, they sent more material for paraffine embedded permanent sections.

Intraoperative smear/squash technique was introduced in 1920-1930s[4-6]. Most CNS lesions are suitable for this technique because of soft texture. But it is inappropriate for tough and rubbery (nerve sheat tumor, craniopharyngioma, desmoplastic tumors) tissues. For the smear preparation, a small piece of tissue fragment (0.1 cm) is placed on a glass, crushed between two glass slides, stained with hematoxylin and eosin[7,8]. We should lightly and rapidly smear the tissue along the slide. Too much pressure causes crush artifact, whereas too little pressure causes thick smears. The slides were immediately fixed with alcohol. For firm pieces of tissues, a little air drying period will help the adhesion of the tissue to the slide. In this study, we evaluate the diagnostic value of squash and frozen sections in neuropathology practice. Difficulties, limitations of these techniques are discussed.

Materials and Methods

We evaluated 65 neurosurgical biopsy materials sent for intraoperative diagnosis. All of them were interpreted with both smear and frozen section preparations. Smear preparation was done with a small piece of biopsy, fixed in alcohol, and stained with hematoxylin and eosin. Frozen sections were also performed. Smears and frozen sections were grouped as inadequate (normal tissue, full of necrosis, technical artifacts), inflammatory/reactive or neoplastic. If we decide that the smear was lesional, mainly we try differentiating it as inflammatory/reactive or neoplastic (glial, metastatic, embryonal, mesenchymal, lymphoid) according to morphological characteristics, age, localization

and radiological data. In glial tumors, the most important issue was if it is diffuse glial or not. In diffuse glial tumors, grading as low and high was the main subject. So we searched for the degree of cellularity (hypocellular/hypercellular), background characteristics (fibrillary, mxyoid, cystic), cell type (glial, meningeal, schwannian, epithelial, neuroendocrine, primitive, lymphoid, melonocytic), nuclear features, necrosis, mitosis, microvascular proliferation, rosenthal fibers, inflammatory cell components (macrophages, lymphocytes, acute inflammatory cells), microorganisms.

Statistical Analysis

Statistical Package for Social Sciences 24.0 was used for descriptive and frequency analysis.

Results

We evaluated 65 cases, 37 of which were male, 28 were female. The mean age was 48.1 (minimum: 4, maximum: 81). Smear/frozen section and permanent paraffine section diagnosis were concordant in 55 cases. Three were inflammatory/reactive and fifty-two were tumor [low grade glial tumor (11), ependymoma (1), glioblastoma (12) (Figures 1, 2), metastasis (5), meningioma (8) (Figure 3), craniopharyngioma (2), pituitary adenoma (2), epidermoid cyst (1), central neurocytoma (1), medulloblastoma (2), schwannoma/neurofibroma (6), lymphoma (1)].

In two cases, the amount of the material was sufficient but we could not achieve a definitive diagnosis with smear and frozen sections. The paraffine result of these rare cases was giant cell ependymoma and vasculitis. We could diagnosed them after permanent paraffine section examination and immunohistochemistry. Squash and frozen sections of giant cell ependymoma contain cohesive papillary structures with highly atypical, pleomorphic cells, with hyperchromatic nuclei, coarse chromatin, and a large eosinophilic cytoplasm (Figure 4)[9]. Some of the atypical cells were highly bizarre, like a monster cell. Small and medium-sized mildly atypical cells accompanied the large cells. Although the highly

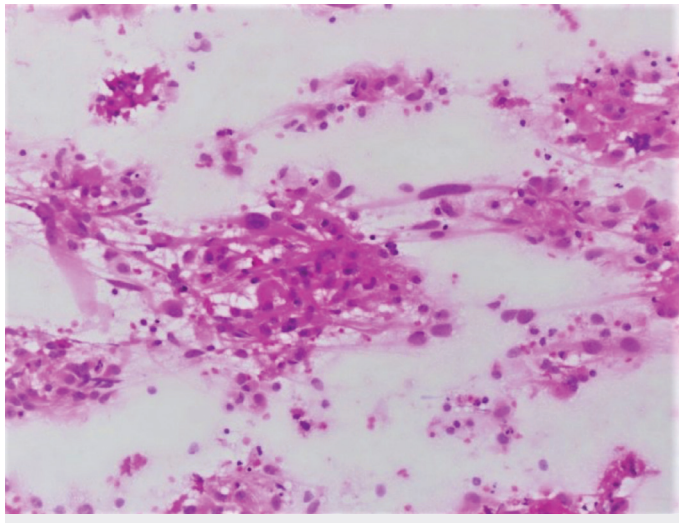

Figure 1. Pleomorphic tumor cells in a fibrillary background in squash preperation (hematoxylin and eosin, 20X)

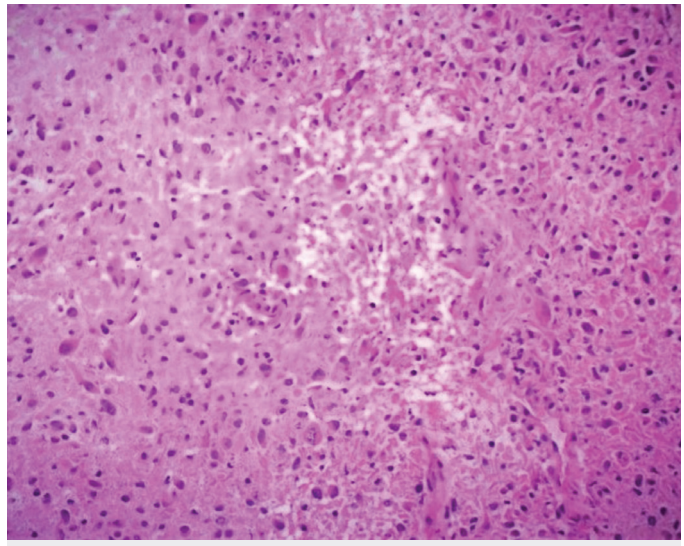

Figure 2. Karyorrhectic tumor cells and palisading necrosis in frozen section of glioblastoma case (hematoxylin and eosin, 10X)

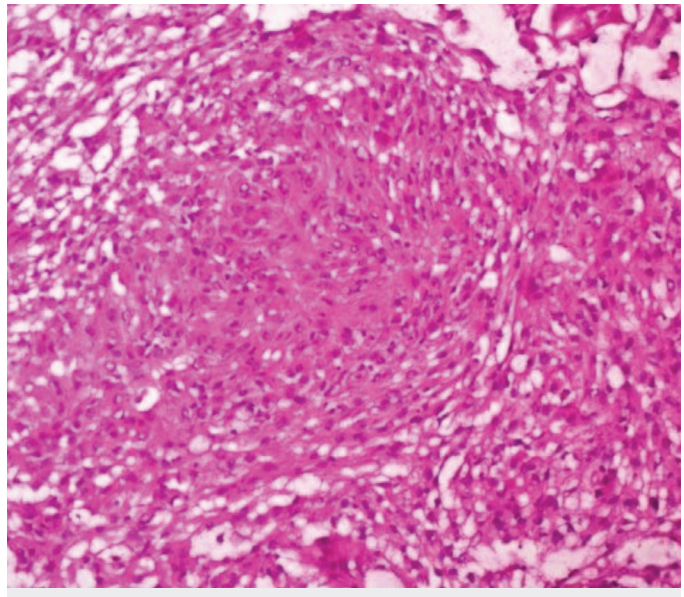

Figure 3. Frozen section revealing meningothelial whorl formation (hematoxylin and eosin, 20X)

atypical tumor cell component, necrosis, mitosis was not identified. It was a conflicting finding for a high-grade glial tumor. The other case was 12 years old boy who presented with acute epileptic seizure. Radiological examination showed pathologic signals in the right frontal lobe. This focus showed heterogeneous contrast enhancement. The radiological preliminary diagnosis was glial tumor. Frozen sections revealed intense perivascular lymphocytic infiltrate (Figure 5). Such infiltrate can be seen in lymphoma, viral and protozoal encephalitis, demyelinating disease. There were no tachyzoites of toxoplasma gondii in squash preparations of the case. Our intraoperative opinion was preferentially

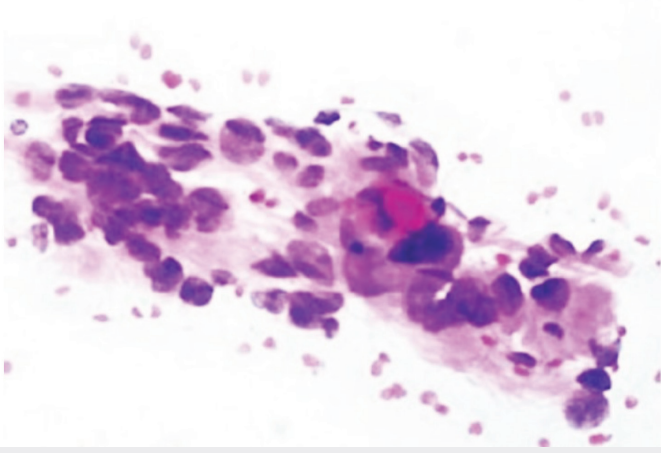

Figure 4. Atypical, pleomorphic cells with hyperchromatic nuclei, large eosinophilic cytoplasm in a squash preparation of giant cell ependymoma (hematoxylin and eosin, 20X)

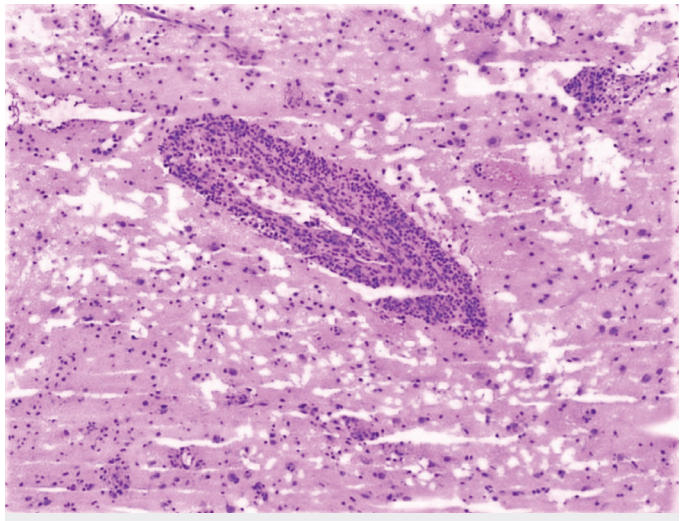

Figure 5. Perivascular dense lymphocytic infiltrate in frozen section of vasculopathic lesion (hematoxylin and eosin, 10X)

vasculitis or lymphoma. A paraffine section examination revealed neurofilament protein negative, coin like infarct areas and dense, T cell rich perivascular lymphohistiocytic aggregates. Immunohistochemistry results were not concordant with lymphoid neoplasia.

In eight cases, the material was insufficient for the diagnosis. The frozen material contained only necrosis in six cases; calcification and cortical parenchyma in one case, cauterized/fibrotic tissue in one case. Two of six necrotic samples belong to glioblastoma, 2 of them were radionecrosis due to glioblastoma therapy, two of them were metastatic cases (lung, colorectal), one of them was the normal cortex of a dysembryoplastic neuroepithelial tumor (DNET) case and the last one was fibrous meningioma.

We also categorized the normal tissues as inadequate material. DNETs are usually cortical multinodular lesions. So the frozen material may reflect normal or dysplastic cortical tissue. In our DNET case, the frozen biopsy contained normal cortical parenchyma and calcification. Cytologic preparations from DNET shows mucin rich background, floating neurons, oligodendroglioma like cells and eosinophilic granular bodies[10]. The adjacent cortex may show cortical dysplasia with disturbed lamination and architectural disarray.

Craniopharyngiomas show different microscopic findings due to adamantinomatous or papillary types In our craniopharyngioma cases, smears contained only amorphous debris but the frozen sections included all of the diagnostic features. They are usually resistant to smearing. According to cystic, degenerative changes in amorphous debris, calcifications, xanthogranulomatous reactions with giant cells may occur in the background of the cytologic preparations. Frozen sections are diagnostic in craniopharyngiomas. Compact sheets of well-differentiated squamous epithelium, peripheral cells showing nuclear palisading, stellate reticulum, anucleate squames ("ghost" cells) and "wet" keratin, also piloid gliosis and rosenthal fibers in the adjacent brain are the main findings. When we see only gliotic parenchyma with rosenthal fibers, we should be aware of the localization to exclude pilocytic astrocytoma.

An epidermoid cyst was truly diagnosed during intraoperative consultation due of keratin content.

Our case serial also contain pituitary adenomas. Besides localization and clinical data; monotonous loosely cohesive, hypercellular appearance, lack of marked atypia/mitosis, neuroendocrine features of the nuclear chromatin assisted

our diagnosis. The loss of fibrovascular septa and expanded lobules is discriminative finding from the acinar pattern of normal adenohypophyseal tissue. Squash preperation is more useful for nuclear characteristics but should be complemented with frozen sections in terms of architectural features to differentiate from normal/hyperplastic hypophysis.

Discussion

Intraoperative consultation of the neurosurgical material can be challenging according to various factors. So most of the neuropathologist prefer both smear preparations and frozen sections to achieve the most reliable diagnosis. Smears are more useful for the background analysis also nuclear/cytoplasmic features. As we use a small portion of the biopsy material for cytologic preparation, we may encounter a sampling error. Making multiple smears will reduce this disadvantage, but evaluating the entire smear will be time consuming. According to our experience, squash preparations alone are not enough for the diagnosis of craniopharyngioma, pituitary adenomas, ancient schwannomas and grading of gliomas.

Frozen section appearance and interpretation is similar to paraffine sections and demonstrate the entire surface of the tissue. Frozen sections provide better data about architectural details. Degree of cellularity, infiltrative pattern, endothelial proliferation, pseudorosette formation, heterogeneous areas are better differentiated in frozen sections so they are more useful in grading and subtyping.

When the biopsy material is minute, we must rapidly decide how to manage it. If we have enough material, we can select and freeze a small tissue for molecular analysis. For minute stereotactic biopsies revealing a tumor, additional cores for immunohistochemistry and molecular analysis are necessary as ancillary testing has diagnostic, prognostic, also predictive value due to recent molecular developments.

Although the material appears adequate, it may not be sufficient due to extensive necrosis. In our study, the frozen material contained only necrosis in six cases. Two of six necrotic samples belong to glioblastoma, 2 of them were radionecrosis due to glioblastoma therapy, two of them were metastatic cases (lung, colorectal cancer). Necrosis may be a future of high-grade glial tumor, metastasis, lymphoma, infections. Also, radiotherapy leads to radionecrosis in treated glioblastoma cases. Sampling through the central portion of the high-grade glial and metastatic tumors may result with insufficient sampling. So the surgeons should prefer sampling from the peripheral portion. Also, drag preparation of multiple necrotic fragments on the same slide will maximize the detection of viable cells.

Nuclear pleomorphism, mitotic figures and vascular endothelial proliferation accompanying necrosis is compatible with glioblastoma. Some low-grade entities like pleomorphic xanthoastrocytomas, giant cell ependymoma, subependymal giant cell astrocytoma may show pleomorphic cells, but they do not consist high mitosis, necrosis and microvascular proliferation and high-grade tumor imaging features. In our study giant cell ependymoma case was a surprising entity for us. Its a very rare tumor and seems like a high grade because of nuclear pleomorphism during intraoperative evaluation. We should always keep in mind this rare, high grade looking but low-grade entities.

Sometimes we come across abscesses during intraoperative examination because of ring-enhancing lesions, especially in immunocompromised patients. The characteristic features of abscess in cytology are necrosis, acute inflammatory cells[11]. Hyphae and granulomatous reaction with giant cells may be observed in fungal etiology. Frozen sections show fibrous capsules in developed brain abscess.

Squash/smears are also helpful in metastatic lesions. They consist of cohesive nests, glandular structures of highly atypical cells with prominent nucleoli. A mucinous background and squamous features may be present. In our study squash preparations were mostly diagnostic in metastatic lesions.

Perivascular lymphocytic cuffing can be associated with neoplastic and non-neoplastic lesions. Coexisting macrophages strongly suggests nonneoplastic processes such as infection, infarction, or demyelination but the exception is macrophage response in treated tumors[4,12]. We observed dense perivascular lymphocytic infiltration in a 12-year-old boy who was considered low-grade glial tumor by radiological examination. Peroperative diagnosis was difficult in this case. Because we can only exclude neoplastic infiltration by immunohistochemical and clonality analysis. The final diagnosis was vasculitis after a broad immunohistochemistry panel.

Fibrotic, desmoplastic and cauterized tissues are also problematic issues. They do not well smear/squash so other cytologic techniques are suggested. Scrape and drag preparations can be used for rapid cytologic examination of

the tough, rubbery biopsy materials. When the outer surface of the tissue is extensively cauterized, bisecting the tissue and making the cytologic drag preparation with the fresh cut surface is advised. Cytologic drag preparation is also suitable for bony tissue fragments. For fibrous and desmoplastic tissues, collecting cellular debris by scraping the tissue gives better results. We did not try these two methods in our study so we could not achieve diagnosis in a cauterized, fibrotic meningioma case.

The accuracy of intraoperative diagnosis of meningiomas is usually high, except fibrous and extensively cauterized ones. Meningothelial whorls, cohesive sheets of cells with intranuclear cytoplasmic pseudoinclusions and psammomatous calcifications are diagnostic features[13].

In schwannomas showing degenerative changes may not observe typical morphological findings. Frozen sections may only show fibrotic, hemorrhagic tissues with hemosiderin pigment. You can observe spindle schwannian cells in limited areas. In our study most helpful findings in ancient schwannoma cases were spindle cells with degenerative atypia in focal areas and hyalinized vascular structures.

Squash/smears are helpful for low-grade diffuse glial tumors. Especially, the glial processes and fibrillary background, nuclear/cytoplasmic details are well demonstrated. But the cellularity may be overestimated in cytologic materials so we must combine cytologic features with frozen sections. Cytologic preparations and frozen sections are demonstrative for pilocytic astrocytomas. A fibrillary background, bipolar neoplastic cells with elongated hair-like processes, brightly eosinophilic hyaline rosenthal fibers, eosinophilic granular bodies are important cytologic features. Frozen sections indicate a biphasic pattern with varying proportions of piloid areas alternating with spongy areas. Misdiagnosing and overgrazing may occur in pilocytic astrocytomas due to vascular proliferation, degenerative atypical cells, oligodendroglioma like areas. Rosenthal fibers may be observed at the periphery of chronic lesions, hemangioblastoma, superior sagittal sinus vascular malformations, craniopharyngiomas. So we should be alert about age, localization and radiological findings.

Cytologic imprint/touch preparations should be preferred in lymphoid neoplasia. Numerous large, atypical discohesive cells in a cellular background strongly suggest lymphoma in the presence of concordant clinical and radiological findings.

We also deal with technical artifacts during intraoperative diagnosis. Vasogenic edema increases the water content of the tumor tissues and leads to freeze artifacts. Freeze artifacts distort nuclear morphology. Especially in oligodendroglioma cases, freezing causes nuclear irregularity and misdiagnosed as astrocytoma. We examined this artifact in frozen sections of oligodendroglioma cases but smears were more helpful as they preserve nuclear morphology better. Discrimination of the tumor type may not be essential during intraoperative examination. But well-preserved cytologic material may be used for further examination of small tumor biopsies. Frozen technique, temperature, freezing time optimization by more practice and experimentation may reduce freeze artifacts.

The cavitron ultrasonic surgical aspirator (CUSA) is a sensitive tool preferred for the safe excision of brain tumors without damaging the surrounding normal tissues. The CUSA performs cavitation by ultrasonic waves and vibrations that destroy the hydrogen bonds resulting in the denaturation of the protein contents of tissues[14]. The morphology of the tissue removed by the CUSA may be slightly inferior compared to that removed by conventional biopsy, especially fragile tissues such as gliomas. Artefactual microcyst formation and more edema can be observed. Meningiomas, schwannomas and metastatic carcinomas can be better preserved in the CUSA. If your neurosurgeon is performing the CUSA method, the pathologist should know the negative effects of this method on the morphology during intraoperative and paraffine section examination.

From the viewpoint of neurosurgeons view, technologically advanced radiological examinations provide great convenience to the surgeon during surgery. Surgeons make their preoperative surgical plan with the help of these examinations. Although some entities are predicted preoperatively, surprising pathologies are common. In such cases, surgeons can use rapid pathological examinations to decide the extent of the excision. Additionally, although almost all approaches made with microsurgical methods, sometimes it is difficult to distinguish microscopic images whether it is pathological or normal tissue. Frozen examinations can guide the surgeon in determining the surgical margins of low-grade glial tumors. As another example, when a posterior fossa pilocytic astrocytoma reported, the surgeon will struggle for not to leave residual mass. Consequently, rapid pathological examination is always an adjunct that guide the surgeon positively and has the benefit of surgery as much as radiological examinations.

The accuracy of intraoperative diagnosis of neurosurgical material is very high due to the adequacy of the material. Studies recommend a minimum of 3-4 samples[15,16]. The accuracy of single biopsy is 76% whereas it is improved to 88.2% with 3 bits[16]. Tilgner et al.[17] found an overall accuracy of 90.3% when both methods were used together. Firlik et al.[18] reported 90% correlation between cytology and final diagnosis. In our study, the results of smear preparation and frozen section were concordant in most entities. Correct diagnosis by combined histology and cytology in adequate materials was 96%.

Study Limitations

We used a small piece of tissue sample for cytologic preparation so it may not represent the whole tissue. In fully necrotic specimens, both cytology and frozen sections were insufficient.

Conclusion

The main role of rapid techniques in neuropathology is to determine whether the tissue is representative and adequate for ancillary testing such as immunohistochemistry and molecular testing. If results suggest an infectious process, tissue samples should be taken for microbiological analysis. The last major advantage is to determine tumor types which are not required gross total resection like lymphoma and small cell carcinoma. So we should always keep in touch with neurosurgeon and complemented morphological findings with radiological and clinical characteristics for most reliable diagnostic interpretation.

Ethics

Ethics Committee Approval: The approval of the Non-Invasive Ethics Committee of the University of Health Sciences Turkey, İzmir Tepecik Health Practice and Research Center was obtained (decision no: 2020/8-15, date: 08.07.2020).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.E.P., E.D., S.E., B.G.Ö., M.Ç., Design: E.E.P., E.D., S.E., B.G.Ö., M.Ç., Data Collection or Processing: E.E.P., E.D., S.E., B.G.Ö., Analysis or Interpretation: E.E.P., E.D., S.E., B.G.Ö., Literature Search: E.E.P., E.D., Writing: E.E.P., E.D.

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