EUS Guided FNA Cell Block Cytology and Intraoperative Squash Cytology in the Diagnostic Approach of Unfamiliar Malignant Neoplastic Disorders

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ABSTRACT
Endoscopic ultrasound guided fine needle aspiration cytology (EUS-FNAC) with the employment of cell block preparations and intraoperative squash smear cytology upgrade the interpretation accuracy and typing of common malignant lesions. Yet, their capacity in the diagnostic workup of less familiar neoplastic entities is not clearly determined and this analysis was designed towards this direction. We describe four cases of patients with uncommon malignancies and evaluate EUS-FNA cell block cytology and intraoperative squash smear cytology as a necessary (important) step in rendering the diagnosis. All cases enhance the diagnostic role of cytology in a wide variety of neoplastic disorders including lymphoproliferative conditions and rare carcinomas.

KEYWORDS
EUS-FNAC; DLBCL; osteoclast like giant cell undifferentiated pancreatic tumor; squash cytology; ependymoma; glioma

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INTRODUCTION

A number of tumor and tumorlike conditions are considered to present a particular diagnostic hazard in anatomic pathology. EUS-FNA cell block cytology and intraoperative squash smear technique provide multiple and extensive sampling and/or sampling of not neighboring areas of an unfamiliar lesion to determine its heterogeneity.

Endoscopic ultrasound fine needle aspiration (EUS-FNA) permits the examination of the retroperitoneum for lymph nodes as well as pancreatic sampling for neoplastic lesions and the acquisition of cytologic specimens for interpretation (1, 2). Cell blocks contain residual material fragments unsuitable for processing by cytologic techniques but suitable for processing by histologic methods.

Even though architectural structure is missed, cytomorphological features combined with immunocytochemistry and molecular analysis on cell block preparations can render an accurate diagnosis of neoplastic conditions. Specimen sufficiency, tumor morphological appearances, endoscopist's skillfulness and cytopathologist's experience enhance diagnostic capacity.

Intraoperative diagnosis is imperative in neurosurgery and squash cytology provides the neurosurgeon with rapid and accurate results (3). Squash smear cytology is applied upon minimal tissue pieces, permits efficient evaluation of the cellular architecture, detailed morphological features of the cells, provides adequate sampling for immunocytochemical analysis and lacks the ice artifacts of frozen sections. Limitations include failure to manage thickness, crushing artifacts and inappropriate smearing.

EBV related DLBCL arising in the retroperitoneum is infrequent. Traditionally it is diagnosed by histomorphology, immunohistology and flow cytometry of tissue specimens obtained at laparoscopy or open surgery.

Osteoclast like giant cell undifferentiated pancreatic carcinoma is rare. It is also diagnosed by laparoscopically obtained biopsies or at open surgery.

EUS guided FNA cytology combined with immunocytochemical study on cell block preparations provide an accurate diagnosis of retroperitoneal and pancreatic lesions.

Ependymomas in childhood follow astrocytomas and medulloblastomas in frequency of CNS tumors. They originate most commonly in the posterior fossa of the brain and show significant morphological heterogeneity. Astrocytomas compose a large and heterogenous category of CNS tumors. They demonstrate a wide variety in clinical incidence, morphologic features and biologic course.

Both ependymomas and astrocytomas rarely exfoliate neoplastic cells in cerebrospinal fluid specimens. They can be accurately diagnosed in intraoperatively, on demand by the neurosurgeon for rapid diagnosis, prepared squash smears.

Our objective is to highlight the performance of EUS guided FNA cell block cytology in interpretating retroperitoneal and pancreatic lesions as well as to stress the yield of intraoperative squash smear cytology and establish the specific cytological diagnostic criteria in CNS neoplasms.

CASE SERIES

CASE 1. RETROPERITONEAL EBV POSITIVE DLBCL

A 73-year-old female presented with recent-onset artypical abdominal pain and change in bowel habits (alterating conticipation and diarrhea). Her physical examination was normal. Her past medical history included Billroth II surgery without antrum resection, due to peptic ulcer disease 30 years before presentation. She also suffered from rheumatoid arthritis and dyslipidaemia. She received hydrochloroquine, gabapentin, and a statin on a daily basis. An abdominal CT scan revealed multiple retroperitoneal lymph nodes and suspected thickened gastric wall at the level of the antrum. Upper GI tract endoscopy with gastric biopsies as well as ileonoscopy were uneventful. EUS was performed with a curved linear array endoscope (GF-UCT 140 Olympus Medical Europe, Hamburg Germany) under conscious sedation with midazolam and pethidine, in order to examine the gastric wall and obtain cytopathology material from the retroperitoneal lymph nodes.

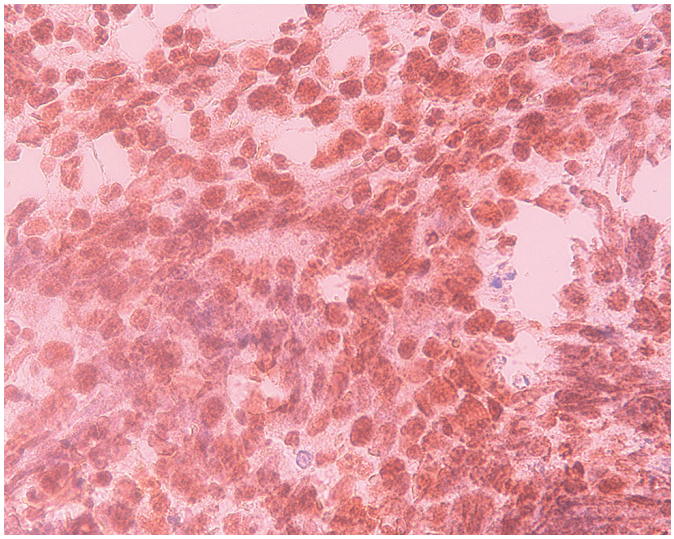

Fig. 1 EBV-DLBCL Cell block preparation. LMP-1 X 400.

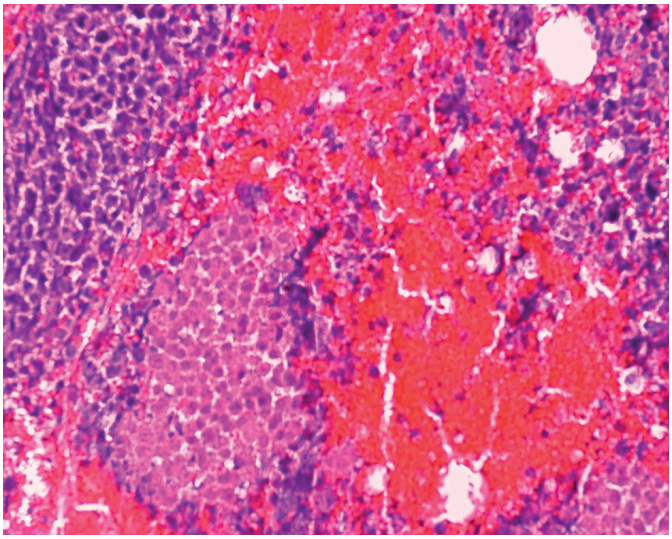

Fig. 2 EBV-DLBCL Cell block preparation. H&E X 200.

On EUS the gastric wall was found of normal thickness with preserved architecture Several enlarged lymph nodes with suspicious EUS features were seen in the retroperitoneum. An EUS guided FNA of a suspicious node was performed through the gastric wall using a 22-gauge needle (Expect Slimline, Boston Scientific, MA) and three passes were made. Suction was applied (10ml vacuum) in the way, and the sample was expelled in normal saline. Soon afterwards it was transferred to the cytopathology lab. The aspirated material was set on smears and air dried, and alcohol fixed slides were prepared. and stained by the Giemsa and Papanicolaou methods respectively A cell block was made from the residual tissue fragments, using the fibrin clot method and it was formalin fixed, and paraffin embedded. Subsequently 5μm thick sections were obtained and stained with hematoxylin and eosin. Additional sections from the cell block were prepared for immunocytochemical analysis.

EUS-FNA conventional and cell block smears showed numerous isolated or clustered large atypical cells with high nuclear cytoplasmic ratio, unevenly distributed chromatin (coarse, granular) and visible nucleoli. The differential diagnosis included metastatic carcinoma, metastatic melanoma, DLBCL, Hodgkin's lymphoma, and anaplastic large cell (ALK-1 positive or negative) lymphoma. Immunocytochemical analysis with the employment of cytokeratin AE1/AE3, HMB45, LCA, CD20, CD3, PAX5, CD30, BCL2, BCL6, MUM-1, CD10, ALK-1, LMP-1 and MIB-1, showed a strong positivity for LCA, CD20, PAX5, BCL2, BCL6, MUM-1, CD10 and LMP-1 and a negative reaction with cytokeratin AE1/AE3, HMB45, CD3, ALK-1,and CD30 of the neoplastic cells. MIB-1 index was 90% positive. A cytological diagnosis of EBV positive DLBCL was established.

CASE 2. OSTEOCLAST LIKE GIANT CELL UNDIFFERENTIATED PANCREATIC TUMOR

Undifferentiated pancreatic carcinoma is an uncommon and aggressive variety of ductal adenocarcinoma. It is categorized in 2 types according to WHO 2019 classification: Undifferentiated carcinoma (with 3 variants: anaplastic, sarcomatoid, and carcinosarcoma) and Undifferentiated carcinoma with osteoclast-like giant cells (OGCT). EUS provides high resolution images and FNA sampling of the pancreatic lesions, which gains a high level of diagnostic accuracy nowadays. OGCTs are large and circumscribed and are defined by non-neoplastic phagocytic cells, large pleomorphic mononuclear cells and small spindled or histiocytoid tumor cells that are usually overlooked in the background. Giant cells are CD68 and Vimentin positive, Cytokeratin and p53 negative, and represent benign histiocytic cells. Pleomorphic mononuclear cells and small spindled cells are strongly positive by Vimentin, variably positive by Cytokeratin and p53, negative by INI-1 (which is strongly positive in Undifferentiated carcinoma lacking giant cells), negative by S-100, and exhibit a high ki-67 proliferation index. The progression from dysplastic epithelium to invasive pancreatic carcinoma is well described because of genetics. KRAS, CDKN2, TP53, and SMAD4 genes are involved in the classical ductal adenocarcinoma while KRAS genetic alterations are frequent in OGCT. Pancreatic mucinous tumor (PaMCT), intraductal papillary mucinous tumor (IPMT), pancreatic cystic neuroendocrine tumor (PaNET), solid pseudopapillary neoplasm (SPN), undifferentiated pancreatic carcinoma not otherwise specified (UOC-NOS), gastrointestinal stromal tumor (GIST), undifferentiated rhabdoid pancreatic tumor (URhT), metastatic melanoma, metastatic sarcoma and chronic pancreatitis with granulation tissue formation are considered in the differentials. PaMCT, IPMT may coexist with OGCC. Cystic degeneration may be present in OGCC, and this can cause a radiologic misinterpretation or may limit sampling from the solid tumor component, so it is critical to ensure that mucinous cystic lesions are extensively sampled. OGCCs may protrude as polyps into the pancreatic or bile duct, duodenum or ampulla and may be also misdiagnosed radiologically. UOC-NOS, URhT do not contain giant cells,.PaNET shows plasmacytoid cells with salt and pepper chromatin, SPN shows open chromatin and nuclear grooves. GIST is c-kit positive. Melanoma stains positive for Melan A, S-100, and HMB45.Sarcoma does not express epithelial markers. Pancreatitis with

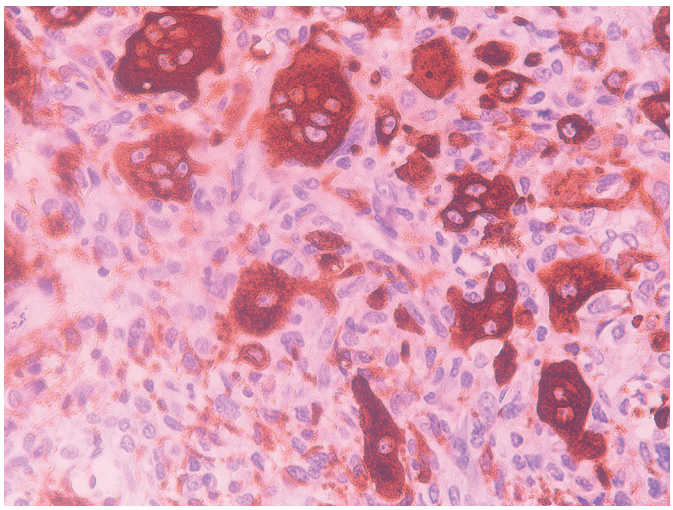

Fig. 3 Osteoclast like giant cell undifferentiated pancreatic tumor. CD68 immunostain X 400.

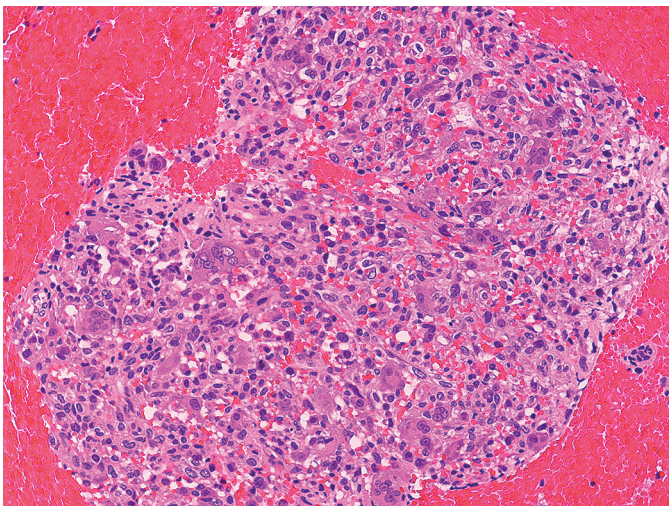

Fig. 4 Osteoclast like giant cell undifferentiated pancreatic tumor. Tissue section X 200.

granulation tissue formation does not express neither epithelial nor vascular (CD31), nor lymphoid markers (ALK), nor S-100, nor c-kit, for neoplastic cells.

An 80 aged female was admitted due to jaundice and epigastralgia. Her past medical. history included arterial hypertension, cardiac arrhythmia, asthma, and chronic frenal failure. CT scan showed a 12mm tumorlike lesion in the head of pancreas which obstructed the common bile duct causing dilatation. EUS revealed a 3,5 hypoechoic heterogeneous poorly defined mass in the pancreatic head. EUS-FNA conventional and cell block slides demonstrated atypical pleomorphic mononuclear cells admixed with multinucleated osteoclast like giant cells with multiple nuclei and ill defined nucleoli. The mononuclear cells were positive by cytokeratin AE1/ AE3, EMA, and CEA but negative by S-100 and c-kit. The multinucleated cells were CD68 positive. A cytological diagnosis of osteoclast like giant cell pancreatic tumor was rendered.

CASE 3. CEREBROSPINAL FLUID AND INTRAOPERATIVE SQUASH CYTOLOGY OF CHILDHOOD EPENDYMOMA

A 19 months old female presented at The University hospital of Heraklion, Crete, Greece in a hemicoma and was intubated. MRI disclosed a tumor in the posterior fossa. The haematological and biochemical work-up was within normal counts. A diagnostic paracentensis was determined and CSF sample was obtained for cytological evaluation. Cytological smears were prepared after cytocentrifuging for 5 minutes. Microscopic interpretation of slides demonstrated isolated neoplastic cells with medium sized oval nuclei and basophilic cytoplasm. Nucleoli or mitotic figures were absent. Dysgerminoma, medulloblastoma, glioma, ependymoma and lymphoma were encountered in the differentials. Neoplastic cells were positive by GFAP and S-100 immunostain but negative by AFP, β-HCG, synaptophysin and LCA. The diagnosis was of a glial tumor favoring ependymoma, based on the age and the anatomical site of the mass. Intraoperative squash preparations were obtained as follows: 1–2 mm^3 of fresh tissue from the suspicious area after gross examination was crushed between two slides to fix smears (4, 5). In squash preparations atypical cells were abundant, organized in papillary formations or rosettes or pseudorosettes with oval medium or large sized nuclei and scanty cytoplasm stained basophilic. GFAP and S-100 positive enhancing the CSF diagnosis of ependymoma. Histological slides showed numerous neuroepithelial neoplastic cells in a rosette-pseudorosette architecture with oval basophilic nuclei and mild atypia. Focally high cellularity, severe nuclear atypia and mitoses were found. Atypical cells were reactive with GFAP, S-100, CD36 and negative for synaptophysin, CD99, CK AE1/AE3. The Ki-1 proliferation index was 10%. Histological diagnosis was of ependymoma WHO grade II and focally grade III (anaplastic) (WHO 2016 earliest classification). The patient was administered with chemotherapy (first course) VEC (vincristine, etoposide and cyclophosphamide).

CASE 4. INTRAOPERATIVE SQUASH CYTOLOGY OF DIFFUSE GLIOMA NOT OTHERWISE SPECIFIED OF THE CEREBELLUM

A 48 aged female was hospitalized at the University hospital of Heraklion, Crete, Greece suffering from headache and unsteadiness. She was soon afterwards diagnosed of a tumor arising in the cerebellum by MRI. Past personal and family medical history was free. Haematological and biochemical values were normal. Intraoperative squash preparations cytological examination demonstrated the presence of isolated elongated epithelial-like neoplastic cells with ovoid nuclei and scanty cytoplasm. Mitoses and necrosis were not observed. Glioma, ependymoma, medulloblastoma and teratoid/rhabdoid tumor were included in the differentials. Immunocytochemical analysis showed a positive cytoplasmic expression of GFAP and a positive expression of S-100 by the tumor cells. Gross total resection of the tumor was performed and histological examination revealed medium sized cells with spindle shaped or oval nuclei, rare mitotic figures and neoangiogenesis. Atypical cells were GFAP podsitive (cytoplasmic positivity), S-100, and vimentin positive,but negative for synaptophysin, NF, EMA,CD34 and p53 antibodies. MIB-1 proliferation index was 5% positive. IDH status and 1p/1q status

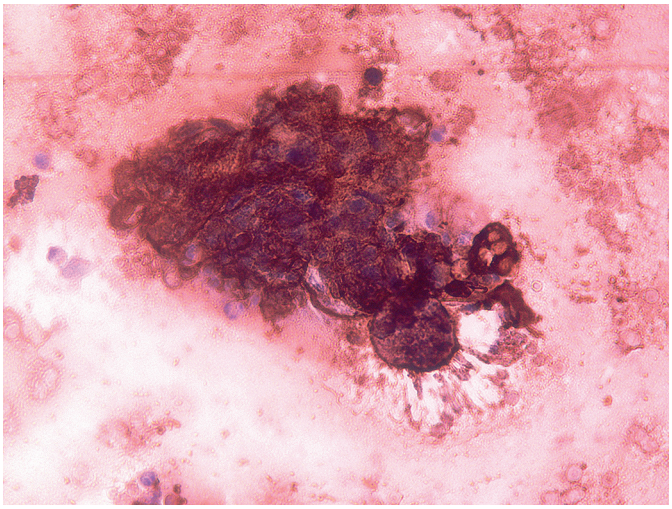

Fig. 5 Ependymoma. Squash smear. GFAP immunostain X 400.

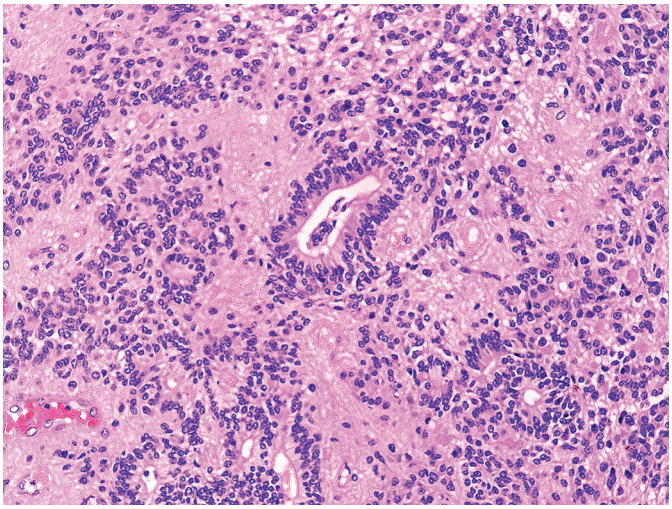

Fig. 6 Ependymoma. Tissue section H$E X 200.

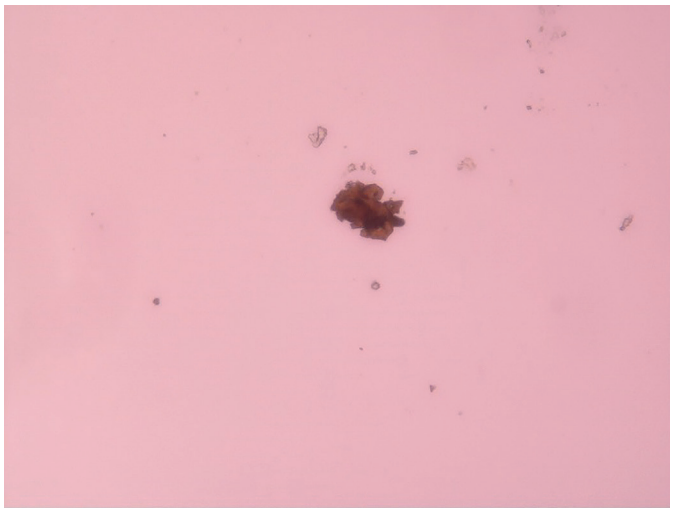

Fig. 7 Low grade glioma of the Cerebellum. Cytological squash smear. GFAP immunostain X 400.

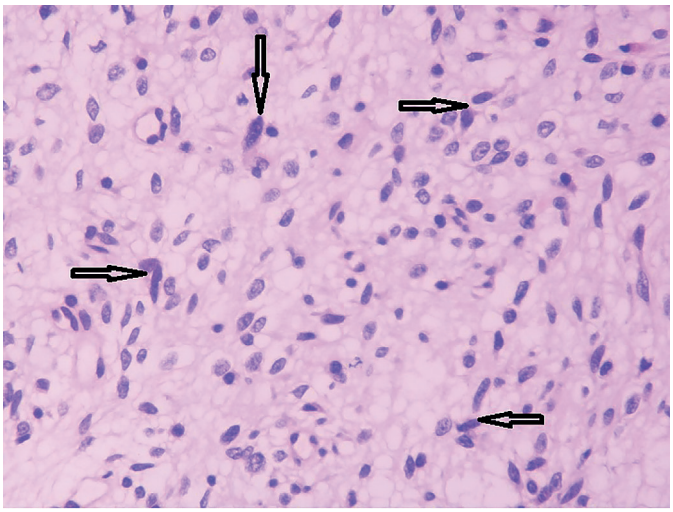

Fig. 8 Low grade glioma of the Cerebellum. Tissue section H&E X 400.

were determined by molecular study and the patient received RT (54 Gy/39 fractions (fr)) followed by 6 courses of PCV (procarbazine, CCNU = lomustine, vincristine) chemotherapy. She is disease free 5 months after surgery.

DISCUSSION

EUS-FNA is a minimally invasive method winning more and more popularity in the nonsurgical appraisal of retroperitoneal lymphoproliferative lesions because of increased accuracy and low morbidity and mortality compared to surgery (6–13).

Echo-findings are important for the evaluation of lymph nodes during EUS, but criteria for malignancy are yet to be established (10, 13). Catalano et al (14) have suggested a number of features such as hypoechoic, sharp borders, rounded contour and size over 10 mm, suggesting malignancy, however this is not always the case as reported by others. In a study by Wang et al (6) enlarged nodes due to lymphoma, were featured by lesion fusion and homogeneous echogenecity. The acquisition and preparation of EUS-FNA samples are critical in the procedure performance because even the most excellent performed aspiration may be wasted if the handling of the specimen is incorrect (15). Three aspirations (passes) of the needle are adequate for the diagnostic yield, a 22 gauge needle is preferred for cytological sampling, the use of forceps enhances the diagnostic yield in lymphoproliferative disorders, the use of suction does not affect the diagnostic yield, neither the use of general anesthesia or sedation nor the number of needle movements from the proximal to distal side of the node (15). The cell block method employs the process of small tissue fragments from aspirated material to form a paraffin block. Cell block slides represent the best material for immunocytochemical and molecular analysis.

There are several drawbacks in the technique for the interpretation of lymphoma: The diagnosis of Hodgkin's lymphoma amends integration of cytomorphology, immunophenotypically, and clinical characteristics so it continues to favor open biopsies for interpretation and classification. T cell lymphoma when occurring (usually rare) also needs open biopsy for the diagnosis. There is need for more than 3 passes from several angles of the lymph node to obtain adequate sampling. Additionally, avoiding necrotic tissue and blood contamination is an important issue (11–13).

Obtaining tissue specimens from pancreatic lesions is considered difficult with CT/ultrasound guided FNA and diagnostic laparoscopy (2). The proximity (accessibility) of the echo-endoscope within the gastrointestinal lumen to the pancreas allows for accurate imaging of the pancreas from the duodenum and the stomach. Sensitivity and specificity of the method is higher (2). EUS-FNA is also effective in patients with inconclusive ERCP brushing cytology (2). The presence of an onsite cytopathologist for rapid onsite evaluation (ROSE) is tremendously improving the diagnostic yield of this technique (2, 15–19). Rapid onsite evaluation (ROSE) with cytology preparations is important in minimally invasive procedures. ROSE activity upgrades patient care by reducing the number of repeat procedures. This reduction saves from potential side effects, such as infection, hemorrhage, and pneumothorax in lung biopsies. ROSE with cytology preparations is also useful in core biopsies (CB) as it minimizes the loss of material that could have been wasted during frozen section analysis. Yet, it decreases the patient anxiety when a procedure does not yield the adequate material or the hazard on the patient by avoiding unnecessary admissions to the clinic. Also, complex oncologic specimens often require fast feedback for clinical management and ancillary studies. ROSE with cytological preparations is performed by cytologists that have to go to the site where the procedure is being performed. The personnel stay on-site until the diagnostic material is obtained or the specimen is considered adequate. The time spent performing ROSE can extend to hours if the lesion is in a location not easy to access and these procedures are managing depended. The standby of the performer cannot be repaid, and it may influence the proceedings of cytology services (25). In our department we do not employ ROSE due to the lack of personnel.

Moreover, ancillary studies and other testing including flow cytometry or culture can be employed (2, 11–13).

Intraoperative diagnosis of brain tumors routinely employs frozen sections. However, they produce artifacts including ice crystals and water logging in the tissue resulting in a smudgy, foggy and shattered tissue appearance (3, 19). Additionally frozen brain tissue displays artifacts in paraffin sections (3, 5, 19). Cytology may sample much wider areas, different areas, and different depths are examined (3, 5, 19).

Definition of brain tumors requires clinical information (patient's age and gender, location of the lesion), medical history, and CT scan or MRI findings. A good cytological squash preparation produces high cellularity, crisp nuclear and cytoplasmic details and occassionaly tissue architectural pattern. In our case of ependymoma, rosette or pseudorosette formation was a critical tip. Limitations include difficulties in preparing smears when lots of intracellular collagen or fibers cannot be spread into slides and this is the case with meningiomas, schwannomas and low grade gliomas (astrocytomas) (5, 19–22). Immunocytochemical and molecular analysis is easy to perform and enhances the diagnostic yield (5, 19–22). Again, the presence of an onsite cytopathologist for ROSE is imperative for improving accuracy. Intraoperative smears of neurosurgical specimens permit rapid and accurate diagnosis (5, 19–22). Cytological assessment should always be followed by histological confirmation (23). Cytological intraoperative report must provide a preliminary accurate diagnosis and assure the neurosurgeon that representative pathological tissue has been obtained for histological definite diagnosis (23, 24).

Our cases facilitate the diagnostic capabilities of EUS-FNA cell block cytology and intraoperative squash cytology over a wide spectrum of neoplasms including unfamiliar lymphoproliferative disorders and carcinomas.

In conclusion the employment of EUS-FNA cell block cytology reinforces an accurate approach of retroperitoneal lyhmphoproliferations and pancreatic lesions utilizing immunocytoclemical and molecular analysis and intraoperative squash cytology can be reliable as the combined skills and flexibilities the management group including the neurosurgeon, the radiologist, and the cytopathologist in the interpretation of brain tumors.

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