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Review article

Tumor pathology

Endoscopic ultrasound-guided fine needle aspiration biopsy — diagnostic principles and workup

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Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) is a well-recognized diagnostic tool of high sensitivity (60–95%) and specificity (85–100%) for diagnosing solid, cystic, and solid-cystic lesions in the gastrointestinal tract (esophagus, stomach, and intestines) or pancreas and biliary tract. The specificity may be lower though for lesions of smaller size or of difficult location.

The quality of received tissue increases with the endoscopist's experience and proper application of the technique and the needles (e.g. vacuum aspiration); the key point for the diagnosis is high level cytomorphologic analysis performed by an experienced pathologist.

Keywords: EUS-FNAB, cytomorphology, diagnostic category, diagnostic workup, immunohistochemistry, EUS-FNAB technique

Introduction

Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) is an ultrasonography-based endoscopic method enabling the assessment of the gastrointestinal wall and surrounding structures with the possibility of harvesting tissue biopsy for histopathological expertise. The aim of this article is to present the diagnostic utility of endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) based on integrated clinicopathological cooperation in terms of the diagnostic of the tumors/lesion in the gastrointestinal tract.

EUS-FNAB technique

The endoscope (echoendoscope) with its terminal ultrasonographic head is used for performing endoscopic ultrasound (EUS). After the echoendoscope's introduction, the EUS_head is placed against the wall of gastrointestinal tract and the surrounding structures are visualized within the range of several centimeters.

These include abnormalities of the esophageal, gastric, duodenal and intestinal walls, lymph nodes of the posterior mediastinum, perigastric and periduodenal regions as well as in the pancreas or biliary tract and perisigmoid and perirectal regions. The most prevalent indications for EUS-FNAB are tumors of the pancreas and biliary tract, submucosal tumors of the esophagus, stomach, and duodenum as well as enlarged lymph nodes of the gastrointestinal tract.

The special needle is introduced via the biopsy channel of the echoendoscope. This needle is visible on the US scan after passing the channel and injecting the lesion, enabling the control of its position (Fig. 1 — needle in the tumor). The tissue is harvested by the movements of the needle as well as creating the sub pressure by connecting the vacuum

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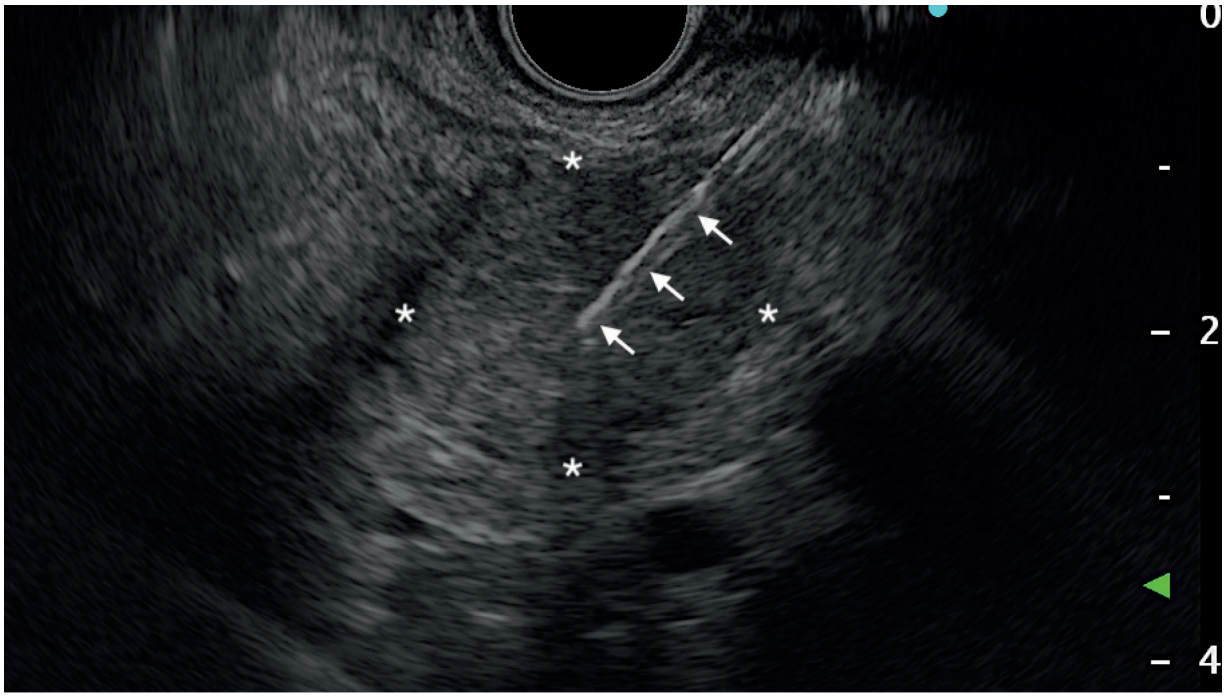

Figure 1. Endoscopic ultrasound (EUS) — biopsy needle (arrows) in 20 mm pancreatic lesion (asterisks)

syringe. The material is expelled from the needle by delivering a stream of air or saline from the syringe. This material is assessed macroscopically in terms of the presence of fragments macroscopic on-site evaluation (MOSE) and fixed for further histological and/or cytological examinations (Fig. 2 — macroscopic evaluation). Several (2 to 4) passings are performed according to the MOSE.

The EUS needles are usually 0,5 mm to 1,1 mm in diameter (25 to 19 gauge), with 22 gauge needles mostly used (0,7 mm). Nowadays the standard of use are fine needle biopsy (FNB) type needles which replaced the previously used fine needle aspiration (FNA) type. The FNB needles have a special cut blade which allows the harvesting of tissue fragments for histological assessment. (Fig. 3 — the needle tip in the lesion). The biopsies performed with FNB needles are bigger, richer in tumor tissue, preserved histopathological pattern of the lesion and are of greater histological assessment value than those made with FNA needles. This increases the diagnostic yield as well as the sensitivity and specificity of the biopsies [1–6].

The endoscopic ultrasound-guided fine needle aspiration biopsy technique results in diagnostic tissue acquisition in 95% of pancreatic tumors, submucosal tumors, and enlarged lymph nodes, with 90% of the cases containing the tissue fragment. Sensitivity surpasses 90% and the specificity reaches 100% [7, 8]. The complications of the procedure are rare and include acute pancreatitis of mostly indolent course (< 1%), hemorrhage (< 0.7%), infection (< 1%) or endoscopy related perforation (< 0.1%) [9].

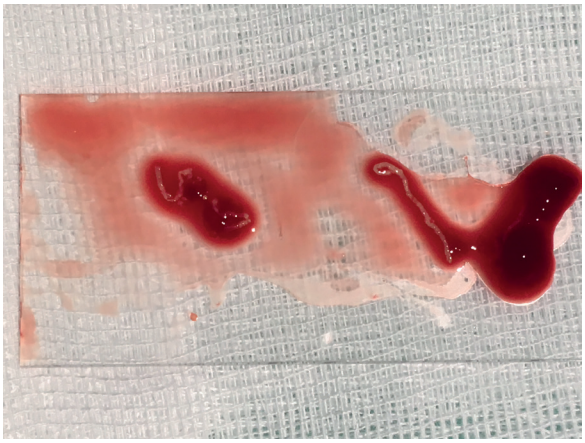

Figure 2. Macroscopic on-site evaluation (MOSE). Tissue of pancreatic lesion, blood, and clots are visible. A 22G biopsy needle was used

The differential diagnosis of endosonography-detected lesions requires clinical data correlation, biochemical analysis of cystic lesion content, and meticulous cytohistological assessment of the acquired tissue with immunohistochemical and/or histochemical stainings. This complex strategy increases the sensitivity and specificity of histopathological studies, reflecting in high quality diagnosis and proper therapeutical choice (Tab. I).

Individual approach is advised in each clinical case based on the principles of good clinical practice (GCP) and classifications/recommendations of scientific societies in endosonography [10].

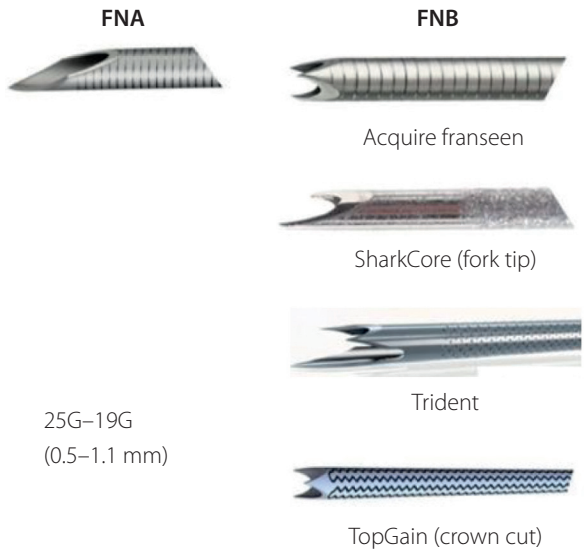

Figure 3. Types of needles; FNA — fine-needle aspiration; FNB — fine--needle biopsy

Cytology (EUS-FNAB) — diagnostic workup

The diagnostic quality of EUS-FNAB materials is influenced by the EUS technique and the endoscopist's experience. The preliminary real-time assessment of the harvested samples may be performed with rapid on-site evaluation (ROSE) during the EUS-FNAB procedure. ROSE improves the quality of samples, decreases the number of passes optimizing diagnostic time slots and qualifying samples for further examinations (histochemistry, immunohistochemistry, flow cytometry or molecular studies) [10, 11]. Based on the type of lesion, the biopsy material can be fixed in 95–96% ethyl alcohol or cytofix for cytology smears or in 10% pH 7,2–7,4 room temperature (20–25°C) buffered formalin for cytohistology. It is of importance that the amount of the fixative should outnumber the amount of the material by at least a factor of 10. The time of fixation should be between 6 and 24 hours. Then the biopsies are routinely proceeded to be embedded in paraffin blocks and cut into 4 µm thick layers to be hematoxylin and eosin (H&E) stained. Endoscopic ultrasound-guided fine needle aspiration biopsy materials enable diagnosis of the type of the neoplasm, the source of the metastasis as well as predictive factors for personalized therapy. The experience of the pathologist and the ability to interpret the results of results of histochemical and immunohistochemical stains and genetic studies in light of the histopathological picture are crucial for making credible diagnosis (Fig. 4).

The principle of immunohistochemistry is to identify specific proteins (antigens) in cells and tissues, which are highlighted by a color reaction and then examined under the microscope in the context of the tissue. There are several hundred markers to establish the differentiation of the neoplasm, stratify the risk or qualify patients for molecular studies or personalized therapies. The immunohistochemistry staining is performed on paraffin embedded tissue using mostly ready to use kits in automatized stainers. The staining is assessed as to its intensity and localization (nuclear, membranous, cytoplasmatic etc.).

Differential diagnosis

Cystic *vs.* solid-cystic lesions: the cystic lesion can be present in different parts of the gastrointestinal tract, including the pancreas. The main objective is to identify the cystic lesion producing mucus. Intraductal papillary mucinous neoplasms (IPMN) are cystic lesions of the main pancreatic ducts that produce mucus and carry a risk of malignancy. In contrast, mucinous cystic neoplasms (MCN) are cystic lesions that also produce mucus but are not connected to the main pancreatic ducts, and they too have malignant potential.

The cytomorphologic features of IPMN and MCN include mucus, proliferating papillary epithelial structures with possible dysplasia, and ovarian-like stroma in MCN. The histochemistry staining is used for mucus detection and high levels of carcinoembryonic antigen (CEA) confirm diagnosis [12, 13].

Solid-cystic and solid epithelioid mass in gastrointestinal tract (GI) and pancreatobiliary tract: include cytokeratins (pan CK) positive and vimentin negative neoplasms of various histopathology and differentiation. Endoscopic ultrasound-guided

Table I. Morphology of endoscopic ultrasound (EUS) detected lesions — diagnostic techniques

EUS	EUS-FNAB	Fluid analysis	Technique IHC, HC	Histopathology/results
Solid lesions, epithelial	Required	No indication	Valuable, required (nen, nec)	Epithelioid tumors of GI/adenoma, LG-IEN or HG-IEN, NEN, NEC, carcinoma
Solid lesion, mesenchymal	Required	No indication	Required	Mesenchymal tumors of GI/benign, border-line or malignant
Solid cystic lesions	Required	Valuable	Required	IPMN, malignant mucinous cystic neoplasm, cystic ductal carcinoma, NEN, SPN
Cystic lesion (micro-macrocystic)	Required	Valuable	Valuable	Brunch duct IPMN, serous cystic neoplasm, mucinous cystic neoplasm, pseudocyst
Unilocular cyst	No indication	Required	----	Pseudocyst

EUS — endoscopic ultrasound; EUS-FNAB — endoscopic ultrasound-guided fine needle aspiration biopsy; GI — gastrointestinal tract; HC — histochemistry; HG-IEN — high grade intraepithelial neoplasia; IHC — immunohistochemistry; IPMN — intraductal papillary mucinous neoplasm; LG-IEN — low grade intraepithelial neoplasia; NEC — neuroendocrine carcinoma; NEN — neuroendocrine neoplasm; SPN — solid pseudopapillary tumor

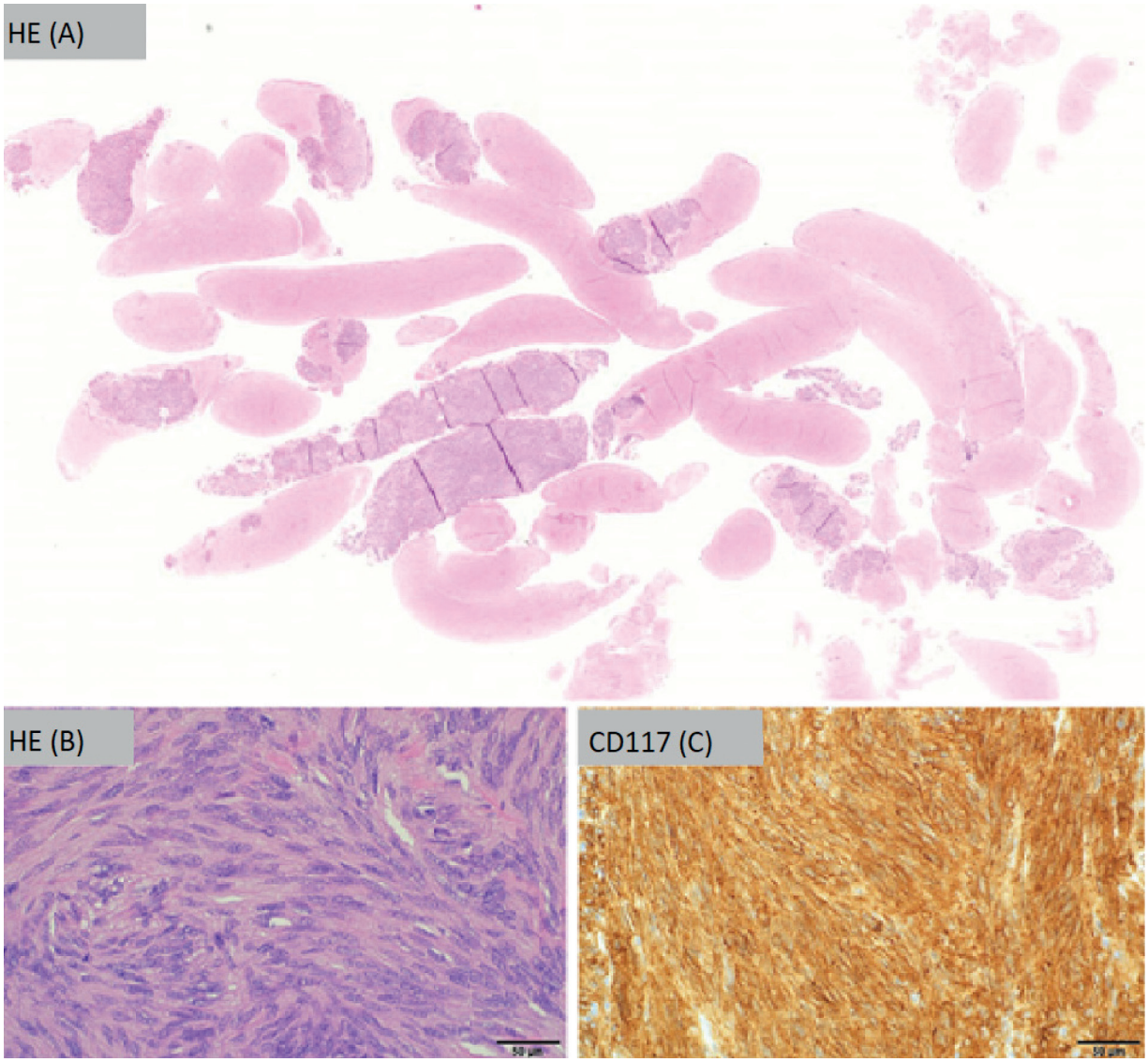

Figure 4. Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) derived material from a gastric tumor diagnosed as gastrointestinal stromal tumor (GIST); **A.** Hematoxylin and eosin (H & E) staining, magnification 20×; **B.** H & E staining, magnification 400×, showing spindle cell morphology; **C.** CD117 immunohistochemistry, positive staining (brown deposits localized in the plasma membrane or cytoplasm of GIST cells), magnification 400×

fine needle aspiration biopsies are utilized to assess invasiveness of glandular proliferations [intraepthelial neoplasia (IEN), IEN/dysplasia *vs.* carcinoma] or establish the source of metastases.

Adenocarcinoma in situ (AIS) cells are moderately polymorphic with nucleoli, mucus filled cytoplasm arranged in glands or small papillary formations. It is of most importance to differentiate IEN form reactive atypia usually present in the healing phase of inflammatory bowel diseases (IBD), pancreatitis or after endoscopic retrograde cholangiopancreatography (ERCP).

Endoscopic ultrasound-guided fine needle aspiration biopsy adenocarcinoma cells are highly polymorphic with hyperchromatic, enlarged nuclei, and mitotic activity. The cells form glandular structures or are dispersed, the background can be necrotic and inflamed. In histopathology, atypical glands are found in desmoplastic stroma. Immunohistochemistry helps to establish the origin of the cancer e.g. CK7+, CK20– for stomach, CK7–, CK20+ for large bowel or MUC4+ for pancreas [12, 13].

Neuroendocrine tumors (GEP-NET) derive from neuroendocrine cells in different parts of the gastrointestinal tract (jejunum, ileum, pancreas, stomach, large bowel, and appendix). They are classified and staged according to localization and histological grade. Endoscopic ultrasound-guided fine needle aspiration biopsy is employed for the diagnosis and monitoring of patients with neuroendocrine tumors. The neuroendocrine cells obtained through EUS-FNAB typically exhibit a uniform appearance, with round or oval nuclei containing "salt-and-pepper" chromatin. The cytoplasm of the cells may be eosinophilic, the cells are arranged in nests, stripes or rosettes. Immunohistochemistry demonstrates the expression of neuroendocrine markers such as chromogranin A, synaptophysin,

INSM1, and the Ki67 proliferation index, all of which are essential for the diagnosis and classification of GEP-NET (gastroenteropancreatic neuroendocrine tumor) family neoplasms [13, 14].

Solid mesenchymal mass in GI and pancreatobiliary tract: there are a wide range of various benign and malignant neoplasms which are vimentin positive and cytokeratins (pan CK) negative on immunohistochemistry. The most prevalent tumor in this group is gastrointestinal stromal tumor (GIST), others include leiomyoma, leiomyosarcoma, schwannoma, lipoma, desmoid tumor. Endoscopic ultrasound-guided fine needle aspiration biopsy diagnosis is relies on cytology, where the cells are typically elongated, spindle-shaped, and arranged in small nests, and primarily on histopathology, which provides the histopathological features and variants (including spindle cell, epithelioid, myxoid, or pleomorphic patterns) can be correlated with additional IHC stains like DOG1 and CD117 for GISTs, SMA and desmin of myomatous tumors, S100 for schwannoma and STAT6 for solitary fibrous tumor [15, 16].

Summary

Classifications of malignancy for gastrointestinal tumors diagnosed on EUS-FNAB are complicated and require the proper application of several factors. The are several systems like the World Health Organization (WHO) for epithelioid lesions (cancer), Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) for mesenchymal tumors, Fletcher's Risk Stratification System for GIST or Papanicolaou for pancreatobilliary mass are the bases for risk factor stratification and administration of optimal therapies [17, 18].

Cytological diagnosis based on EUS-FNAB for GI and pancreatobilliary tumors can be grouped in several categories:

- positive for malignancy: unequivocal cytomorphological features of malignancy. This category includes primary and secondary cancers, solid pseudopapillary neoplasm of the pancreas, neuroendocrine carcinomas, lymphomas, sarcomas;
- suspicious for malignancy: features suggestive of malignancy both in cytological and histopathological aspects but no definitive diagnosis can be made due to the paucity of the tissue or concomitant inflammatory-reactive changes. These cases should be consulted by an expert gastropathologist and if still nonconclusive, the biopsy should be repeated with the ROSE technique for a prompt assessment of the quality of the sample;
- atypia of uncertain significance (AUS): atypical cells, identify for malignancy, usually due to paucity of the cells or tissue with concomitant inflammation and necrosis (inflammatory bowel diseases, primary sclerosing cholangitis, stents, stones). It is required to make another attempt of obtaining representative material (preferably with the ROSE technique);
- benign the samples are adequate as to the cellularity and the representativeness, no atypia or dysplasia found. This category includes: nonneoplastic lesions (heterotopic lesions, accessory spleen, foci of endometriosis, inflammatory pseudotumors) and benign tumors (serous cystic neoplasm, leiomyomas, lymphangiomas, schwannomas, lipomas;
- nondiagnostic: aspirates with a paucity of cells or non-representative samples containing only normal gastric or intestinal epithelium. There is no consensus on the minimum number of cells required for a definitive diagnosis; acellular aspirates or those containing only mucus do not fall into this category, as they may still provide diagnostic value and should be correlated with radiological and clinical data (e.g., IPMN).

Conclusions

Endoscopic ultrasound-guided fine needle aspiration biopsy is a safe, minimally invasive method for cytological and histopathological diagnosis of GI and pancreatobilliary lesions. This technique requires close cooperation between endoscopists, radiologists, and pathologists. In many cases precise diagnosis can be made; prognostic and predictive factors can also be established.

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Małgorzata Lenarcik — conceptualization, methodology, formal analysis, resources, writing — original draft preparation, writing — review & editing.
Magdalena Misiak-Gałązka — visualization, writing — review & editing.
Andrzej Mróz — formal analysis, visualization, writing — review & editing.

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Conflict of interest

The authors have no conflict of interest in reference to this article.

Supplementary material

None.

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