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Review

Endoscopic ultrasonography-guided tissue acquisition: How to achieve excellence

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Endoscopic ultrasound (EUS)-guided tissue acquisition is a basic forte of an endosonographer. The multiple skills required to accomplish successful results include not only the puncture itself, but also proper lesion identification, correct puncture sequence, collaboration with the pathologist onsite or remotely, proper handling of the specimens, choosing one or more of cytology, cell-block, and/or tissue core preparation and, last, deciding the immunohistochemistry (IHC) panels and ancillary tests which may be needed for the current case. Error in any of these decisions may lead to incomplete or inconclusive information from the procedure, even if the aspirate is 'adequate.' In the present review, we will describe the technical aspects of EUS-guided tissue acquisition, current needles available and how to choose between them, and how to appropriately handle the specimen. We will also discuss the optimal approach to common targets including lymph nodes, pancreatic masses, pancreatic cysts, and subepithelial lesions.

Key words: cell-block, core biopsy, cytology, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA), immunohistochemistry

INTRODUCTION

EXCELLENCE IN ENDOSCOPIC ultrasound (EUS)-guided tissue acquisition requires an amalgamation of endoscopic and cognitive skills, with knowledge of pathology. It includes thinking beyond mere needle insertion in a suspect lesion. The present review outlines personal tips and tricks to improve the yield of EUS-guided tissue acquisition.

TISSUE ACQUISITION OVERVIEW

THE SITE THAT will allocate the highest stage if positive for malignancy is sampled first. Before puncturing a lesion, the scope is straightened as far as possible. A straight scope position ensures easier needle movement and minimizes deviation of the needle path from the sonographic plane of view. In the second part of the duodenum, scope straightening may compromise stability. An assistant may sometimes need to stabilize the scope position at the mouthpiece to prevent scope recoil and falling back.

The target lesion should be positioned from a 5 to 7 o'clock position in the sonographic field of view. The big-wheel of the scope is used to adjust position of the target lesion in the vertical plane, to position it in the projected needle path. Use of elevator should be minimized, as it significantly increases resistance in needle excursion, and only allows about 15-degree adjustment in needle trajectory. If elevator use is un-avoidable to target the lesion, it should be released before making to-and-fro needle movements inside the target.

Color Doppler is used before puncture to avoid any intervening vessel(s) in the needle path. Beware of compression of venous structures by probe pressure, with loss of color Doppler signal. Never violate obstructed bile ducts to avoid cholangitis, which can be severe. We should also try to avoid intervening ascites and infiltrated gastrointestinal wall in the needle path.

The entire length of the needle must be followed in real time in the ultrasound field (Fig. 1a). A bent needle, curved scope, and side-to-side movement of the scope or operator body may lead to a variable needle length veering off the sonographic vision (Fig. 1b). If the needle tip cannot be visualized clearly, it must be immediately withdrawn.

Attempt is made to move the needle from one end to the other in the lesion, to sample it widely. The trajectory of the needle is changed as it is withdrawn to the most proximal part of the lesion, using the up-down dial and/or the elevator. Aspiration is usually initiated at the left margin of the tumor mass, and then 'fanned' until the right margin of the tumor is

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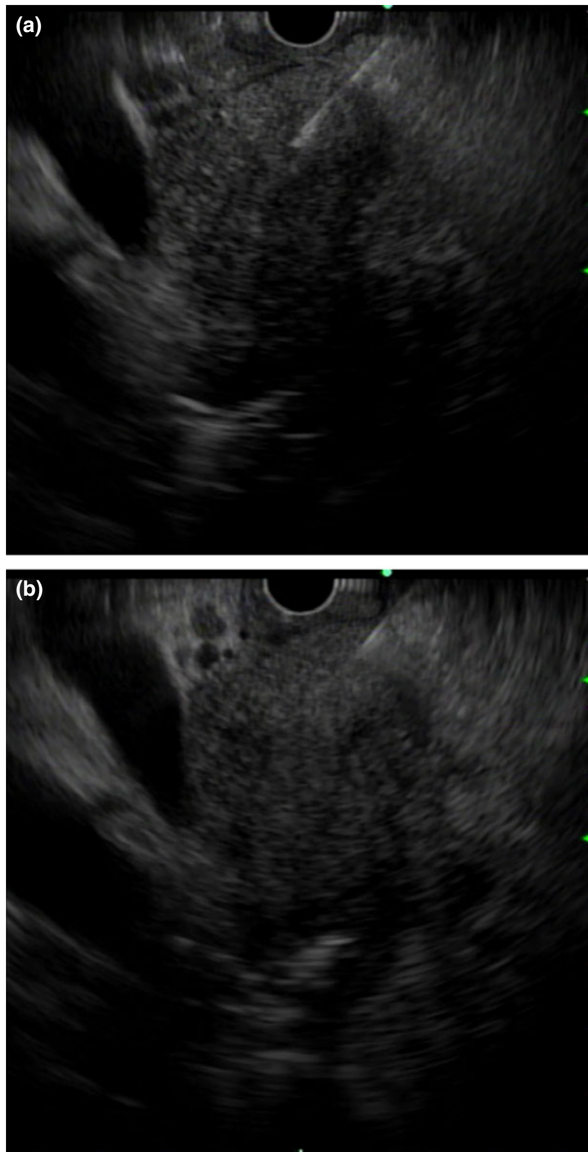

Figure 1 (a) Pancreatic head tumor being aspirated from the duodenal bulb. The entire length of needle is visible in the sonographic field. (b) Slight sideward movement leads to loss of needle tip. The same phenomenon can be seen if the needle is bent, or the scope is torqued. Care must be taken to keep the entire needle length visible in the sonographic view at all times.

sampled. This is called the 'fanning method,' or the 'stroking fan' technique (Fig. 2). For small mobile lesions, such as lymph nodes embedded in loose connective tissue, the 'lock-jam' or 'door-knock' technique of sampling is used. The safety latch of the needle is locked, and rapid inwards needle movements are made so that a knocking

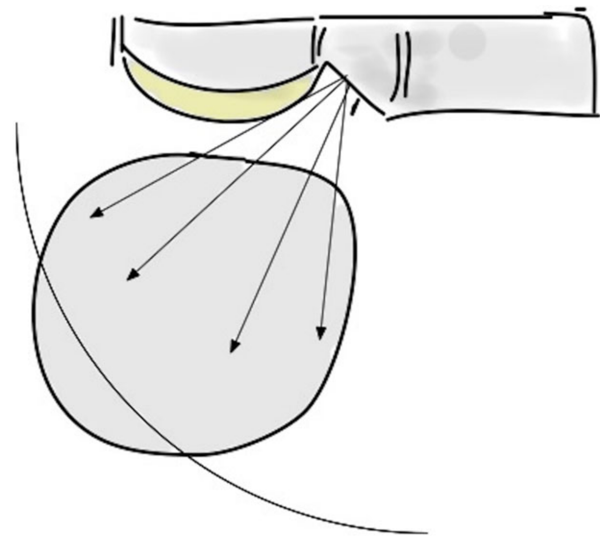

Figure 2 Fanning of the needle in a lesion. The needle is directed by use of the up-down dial, from the left part of the lesion to the right, and then back.

sound of the needle handle against the safety latch is heard. The rationale is to move the needle faster than the target can move. Approximately 10–20 to-and-fro movements are made in the lesion. If suction is applied, it is released before the needle is withdrawn from the lesion.

All EUS fine-needle aspiration (FNA) needles have the same basic design, and are currently single use. Details of the currently available FNA needles are given in Data S1 and Supplementary Figure S1. There has been no comprehensive head-to-head comparison of the latest FNA needles available on the market, regarding features such as ease of use, ergonomics, and efficiency of tissue acquisition. Choice between the needles is an individual preference.

Smaller versus larger needles for cytology

Two meta-analyses have shown slight superiority of the 25-G over the 22-G aspiration needles for EUS-FNA of solid pancreatic lesions.[1,2] Passage of the thinner 25-G needle may be easier when the scope is angulated as in transduodenal access to pancreatic head lesions, and into very firm lesions such as pancreatic cancer. As expected, the advantage of 25-G needles appears to be limited to pancreatic lesions, especially within the pancreatic head. For non-pancreatic lesions and lymph nodes, the diagnostic yields of 22-G and 25-G needles are similar.[3,4] Additionally, with the 25-G needles, the quality of cytological smears may be superior with less blood contamination, compared with 22-G needles. Randomized trials suggest that there is no

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incremental diagnostic yield of cytology with the 19-G needles compared with either 22-G or 25-G needles.[5–7]

Individualizing suction and stylet for FNA

Stylet is purported to impart rigidity to the needle, provide additional protection for the scope channel when rounded and protruding, used to unplug gastrointestinal wall contaminants from the needle after puncture, and used to express the aspirated material from the needle after the puncture. A removable stylet is included in all commercially available aspiration needle platforms, and is recommended for use by manufacturers (Fig. 3). However, in trials, no advantage of using a stylet in terms of specimen quality and diagnostic yield has been demonstrated.[8–12] FNA needles may be used with or without stylet as per operator preference.

Similarly, the use of suction should be individualized and not dictated by protocol. Studies have shown that use of suction leads to increased cellularity at the expense of more hemodilution.[13–15] In general, hard fibrotic lesions, suspected pancreatic carcinoma, post-treatment tubercular nodes, and mural mesenchymal tumors may need suction more often to obtain adequate cellularity. The initial pass into lymph nodes and suspected neuroendocrine tumors (NET) may be made without suction. European Society of Gastrointestinal Endoscopy (ESGE) guidelines recommend using suction for EUS-FNA of solid masses/cystic lesions, and not using suction for EUS-FNA of lymph nodes.[16] Depending on the gross appearance of the first aspirate, suction may or may not then be used in the subsequent passes.

Figure 3 The sharp or blunt stylet usually protrudes beyond the needle tip by approximately 2–3 mm, and must be withdrawn by a few millimeters before carrying out the actual penetration through the gut wall.

Suction force increases with the diameter of the FNA needle and with syringe size (aspiration volume). The newly described slow-pull technique generates a weak negative suction pressure of 1.4–4.8% of that generated with a 20-mL syringe, depending on the diameter of the FNA needle.[17] It should be remembered that the purpose of suction is not to directly aspirate material into the needle lumen, but to hold the tissue against the cutting edge of the needle. When the needle is moved through the lesion being sampled, the needle lumen is filled by the cutting action of the needle.

HOW TO HANDLE AND DISTRIBUTE THE ASPIRATED CYTOLOGY SAMPLES

USE OF AN air-filled syringe to expel material may lead to uncontrolled spray of the aspirate. We prefer to slowly reintroduce the stylet to expel the aspirated material in a controlled droplet-by-droplet manner at the frosted end of slides (Fig. 4a). Another slide is then held at a right angle to the lower slide at a 45-degree incline, so that the material spreads along the contacted slide. The upper spreader slide is then lowered over the bottom slide, and spread over it to make an oval smear with a head, body, and tail (Fig. 4b). The larger tissue fragments are seen in the middle of the smear, and single cells dispersed at the periphery. Do not attempt to separate the slides during the smearing process.

Aspirates from a cystic pancreatic lesion are sent for estimation of amylase, lipase, carcinoembryonic antigen (CEA), cyst fluid viscosity, genetic analysis, and cytology (Table 1). Details of cyst fluid analysis are beyond the scope of this review. Salient points to be kept in mind when we aspirate a pancreatic cyst are listed in Table 2, and depicted in Figures 5 and 6.

Air dry or alcohol fix slides?

Alcohol-fixed slides (immersion or spray fixation) are stained with Papanicolaou (Pap), or hematoxylin & eosin stain. For alcohol fixation, the smears must still be wet when

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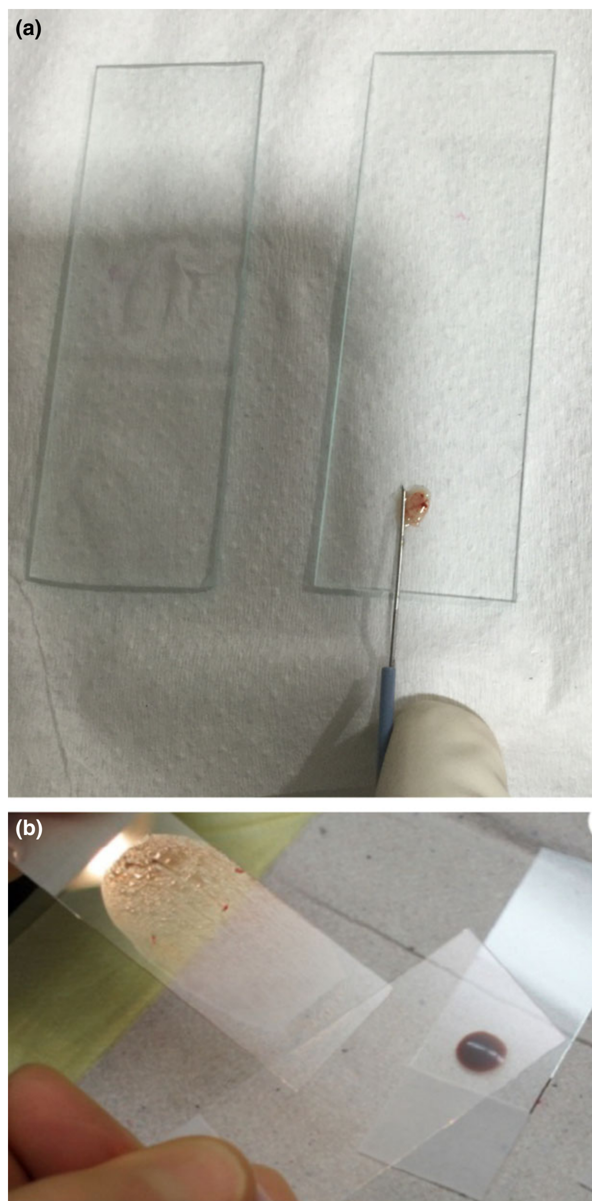

Figure 4 (a) A drop of the aspirate is pushed by stylet onto one end of the slide. (b) A smear is made.

Table 1 Cyst fluid analysis

Test	Significance
Amylase	<250 U/L excludes pseudocyst >5000 U/L suggestive of pseudocyst
CEA	Useful to differentiate between mucinous and non-mucinous cysts, with variable cut-offs between 180 ng/mL to >800 ng/mL CEA levels not predictive of malignancy In pseudocyst typically <5 ng/mL
Cytology	High specificity (88–97%) Low sensitivity (51–65%)

From references[18–24].
CEA, carcinoembryonic antigen.

Table 2 Tips for safe pancreatic cyst aspiration

1	Single pass
2	Shorter path to access lesion
3	Go through the pancreas
4	Avoid transgressing the main pancreatic duct
5	May use either 22-G or 19-G needle
6	Avoid going through the distal wall (to avoid seeding/through-and-through implants)
7	Evacuate a cyst in one pass if possible
8	Antibiotic prophylaxis (for 48–72 h)

they are immersed in 95% ethanol fixative. If the slides dry out before alcohol fixation, there will be poor staining of the cells, as well as artefactual nuclear enlargement, making distinction between reactive lymphoid tissue and lymphoma difficult. Lots of blood in the aspirated material also interferes with optimal evaluation by the Pap stain.

Slides can be air-dried by briskly waving the smeared slides, or using a hair-dryer or a wall-mounted hand dryer. Staining of incompletely dried smears will lead to uneven staining and loss of crisp cytomorphological detail. Air-dried slides are stained with Romanowsky-type stains (Diff-Quick, May-Gruenwald-Giemsa, Hemacolor). The Romanowsky-type staining kits consist of a fixative (typically methanol), an acidophilic dye for cytoplasmic staining, and a basophilic dye for nuclear staining.[25] These stains are commonly used for blood smears and bone marrow aspirates and are, hence, particularly suited for evaluating lymph node aspirates. Air-dried specimens but not wet-fixed specimens are suitable for further immunocytological (ICC) staining. Air-drying of slides also allows rapid on-site evaluation (ROSE).

Pap smear highlights the nuclear details, chromatin quality, and 3-D cellular clusters. Romanowsky stains highlight the intracytoplasmic material and extracellular substances. Hence, alcohol-fixed and air-dried smears provide complementary information.

Rapid on-site evaluation

Presence of on-site cytopathologist leads to a 10–15% increase in diagnostic yield.[26,27] There is an approximate 20% rate of non-diagnostic aspirates in the absence of ROSE.[28] In our experience, on-site reporting with EUS-

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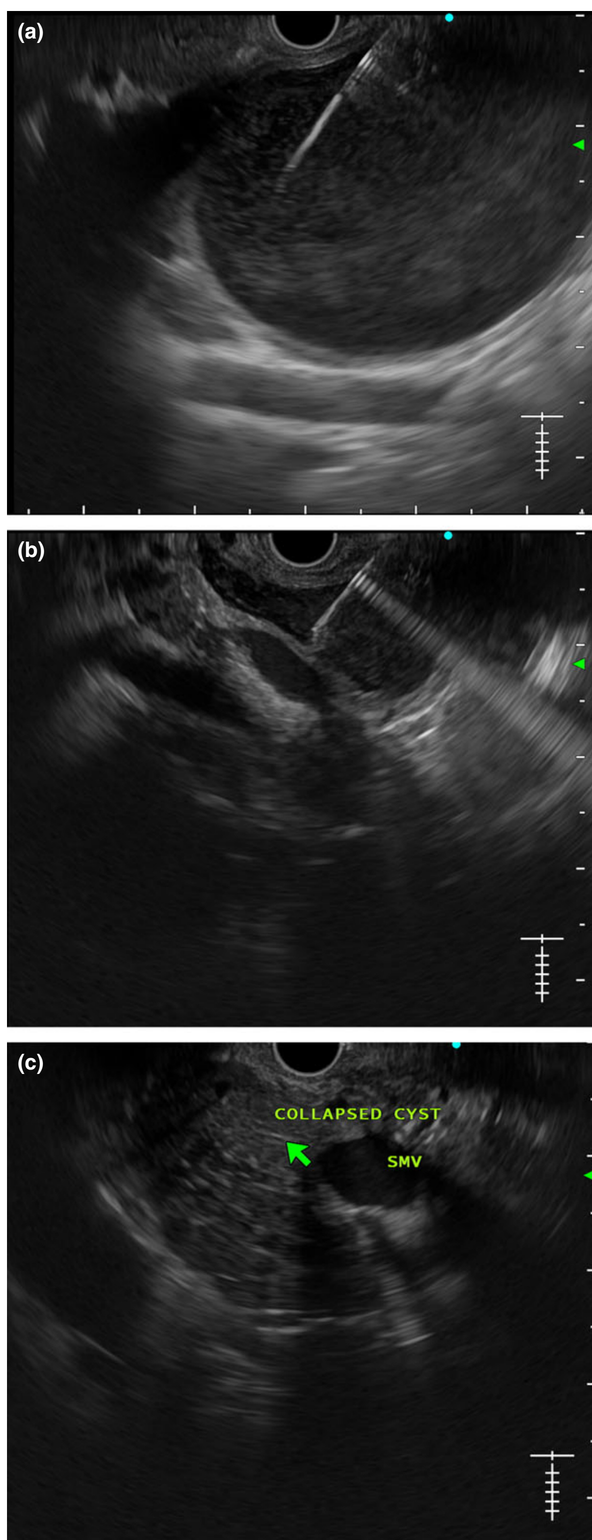

Figure 5 (a) A large cyst in the head of the pancreas being aspirated with a 22-G fine-needle aspiration needle. Although most cysts can be aspirated with a standard 22-G needle, viscous contents may need to be aspirated through a 19-G needle. (b) Around half of the cyst has collapsed. The needle must be adjusted to avoid impaling the receding cyst wall or adjacent vasculature. (c) The cystic lesion has completely collapsed after aspiration. The area must be watched for some time to detect post-aspiration bleeding. Minimizing needle passes into cysts, near complete aspiration, and peri-procedural antibiotics are key to avoid infection.

FNA is more difficult than with percutaneous FNA because of frequently encountered esophageal, duodenal, and gastric contaminants.

Recent data suggest that an on-site cytopathologist may have a role during the learning phase of EUS-FNA only, and in centers with a low specimen adequacy rate (<90%).[29] However, on-site evaluation has benefits beyond ensuring specimen adequacy. It helps to limit the number of passes and hence improves procedural efficiency and reduces patient risk. It also allows a real-time decision on whether additional material needs to be obtained for special analysis, such as cultures or flow cytometry.

In the absence of ROSE, we advise making 4–5 passes in solid pancreatic lesions, and 2–3 passes in lymph nodes, liver, and adrenal lesions.[30]

Liquid-based cytology

Liquid-based cytology (LBC) allows automated slide preparation (e.g. ThinPrep, SurePath), with uniform, mono-layered dispersion of cells. Liquid-based fixatives eliminate red blood cells, background mucus, and protein precipitates. Removal of extracellular background may lead to loss of potentially relevant information. There is cell disaggregation with loss of tissue architecture. Liquid-based fixatives contain methanol, which is a coagulative fixative (unlike formalin which is a protein cross-linking fixative). This may lead to suboptimal fixation for immunohistochemistry (IHC). Data on use of LBC for EUS-FNA is contradictory and limited.[31–34] A randomized trial by Lee *et al.* suggested that LBC may serve as a complementary preparation technique, especially if blood contamination of smears is abundant.[33]

WHEN AND HOW TO MAKE CELL-BLOCKS

CYTOLOGY ALONE MAY be insufficient with the following differentials: non-ductal carcinoma

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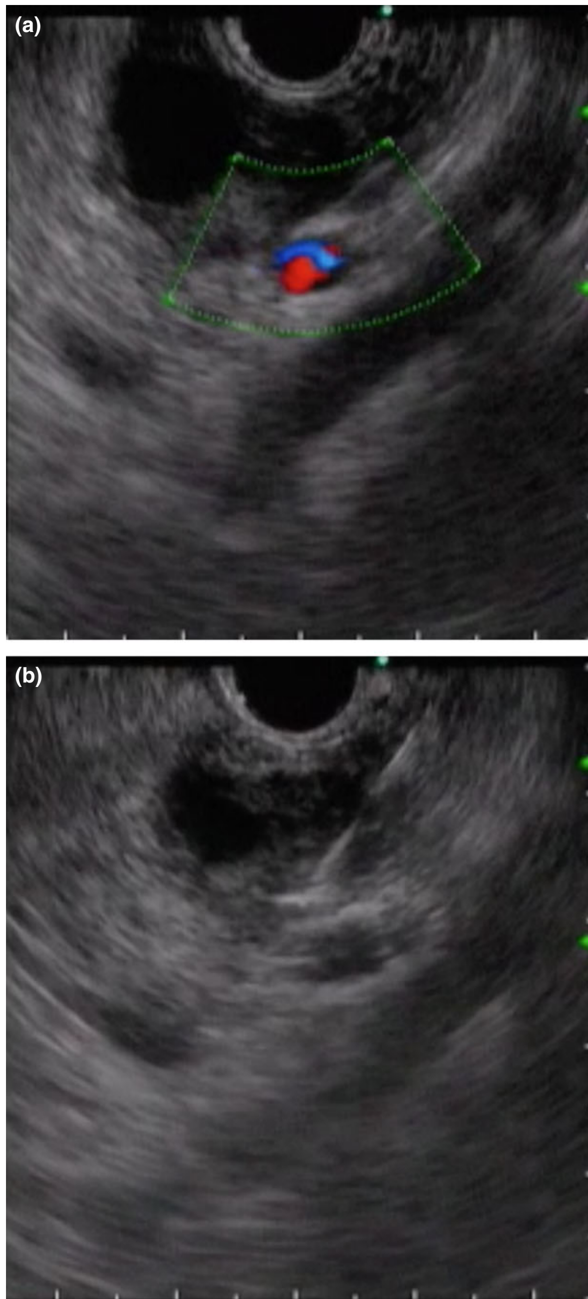

Figure 6 (a) A pancreatic body, predominantly cystic lesion with a solid component at its periphery. In pancreatic cysts with a significant solid component, we should also consider the differential of cystic degeneration of a solid tumor such as neuroendocrine tumor and invasive malignancy arising in a cystic pancreatic tumor. In such cases, we may target the solid component alone for cytology. (b) The needle is placed initially to aspirate the solid part of the lesion.

pancreatic tumors (such as acinar cell carcinoma, solid-pseudopapillary tumor, metastasis), autoimmune pancreatitis, lymphoma, mesenchymal tumors such as gastrointestinal stromal tumor (GIST), leiomyoma, or schwannoma, NET (Fig. 7), non-small-cell lung cancer (NSCLC), and some pancreatic cystic tumors. Molecular profiling of many solid tumors has become important for personalized oncological treatment. In these and many other clinical settings, cellular features alone may be insufficient, and tissue architecture, IHC, and molecular analysis may be essential for accurate pathological assessment. Cell-blocks are required when special stains need to be carried out and/or some tissue architecture information is needed for diagnosis (Fig. 8).

We suggest making two committed passes for cell-block preparation. The material is expelled in a cell-preservative solution such as Roswell Park Memorial Institute Medium (RPMI-1640). We can also collect material in isotonic saline. However, the longevity of cells in saline is only about 1h, and hence, samples in saline must be transported to the laboratory immediately.

Traditionally, cells are harvested by centrifuging the collection tube. Although processing of cell-block material follows typical pathology tissue processing, there are additional steps necessary because of the comparatively minute amount and fragmented nature of the specimens. Either an agarose gel or a fibrin clot is used to hold the specimen together as a 'tissue fragment', before dehydration and embedding in paraffin, sectioning, and staining. If the aspirates are mixed with blood, then 5–10 drops of 0.2% glacial acetic acid are added, and the material centrifuged again.

Cell-blocks recapitulate morphology seen on tissue sections. However, the cell-block specimens are usually fragmented and minute, and seldom sufficient for a diagnosis by themselves. The main utility of cell-blocks is as a repertoire of tissue for IHC panels, and for molecular analysis. The IHC panels which need to be requested in commonly encountered scenarios in practice are detailed in Table 3. The cell-blocks must be prepared with care, because if cell fragmentation and necrosis occurs, antigen specificity will be lost leading to poor quality and even non-diagnostic IHC. Immunocytochemistry is, in general, inferior to IHC, and cannot be used as a substitute. Additionally, if direct smears are negative or non-diagnostic, the probability of detecting malignancy in material obtained through cell-blocks is negligible. Cell-blocks should be considered as a complement to, rather than a replacement for, cytological smears.

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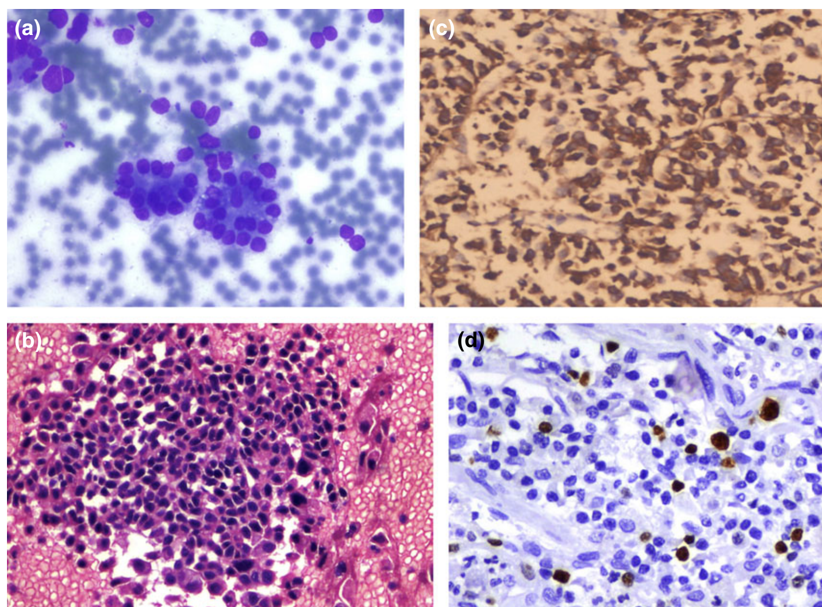

Figure 7 Endoscopic ultrasonography (EUS)-guided sampling of a pancreatic neuroendocrine tumor (pNET). (a) Cytology smear shows uniformly cellular, relatively monotonous population of cells. The cells are predominantly arranged singly, but also in loose clusters and pseudo-rosettes. This appearance is very suggestive of a pNET, but acinar cell tumors can be confused with pNET on cytology, and clear-cell variants of pNET have a cytological appearance similar to renal cell cancer or solid pseudopapillary tumor. (b) Cell-blocks must be prepared when NET is suspected. This serves as a tissue repertoire for confirmatory immunohistochemistry (IHC), and for tumor characterization. (c) IHC shows positive synaptophysin staining. (d) Ki-67 staining characterizes this as a G2 NET. Such staining on samples obtained by EUS has excellent concordance with tumor grading after surgical resection, despite intratumoral heterogeneity of the Ki-67 index. This is useful information for treatment and prognostication for this patient.

WHEN SHOULD WE ATTEMPT CORE-BIOPSIES?

MANY MALIGNANT TUMORS are highly cellular, and diagnosis of malignancy is possible based on cytological features alone. Hence, cytological smears along with cell-blocks for IHC, may suffice in such cases.

Core biopsies should be obtained when tissue architectural details are required to establish a specific diagnosis. These situations include certain well-differentiated tumors, tumors with extensive desmoplasia, and tumors such as GIST when adequately cellular specimens are difficult to obtain (Fig. 9). Lymphomas, especially low-grade varieties, also need histological (architectural) evaluation for a conclusive diagnosis. Unlike malignancies, many benign pathologies such as autoimmune pancreatitis (AIP) are less cellular and, additionally, need tissue architectural details for a diagnosis (Fig. 10). Like cell-blocks, core biopsies also allow extensive IHC panels and molecular analysis to be carried out.

The endosonographer must be aware of the clinical details and the preprocedure imaging features, as well as be cognizant of the sonographic features of the lesions being evaluated to decide when to obtain cell-block, core biopsies, or both.

Recently, core biopsies are being increasingly obtained when an on-site evaluation is not available. This trend is being driven by increasing availability and popularity of the so-called ‘core biopsy needles’. The accuracy of dual sampling (cytology and core biopsies) is superior to either technique alone.

The technical details of the currently available core biopsy needles are given in Data S2 and Figure S2.

HOW TO HANDLE ‘EUS CORE BIOPSY SPECIMENS’

THERE ARE TWO related ways to handle the specimens obtained from core biopsy needles:

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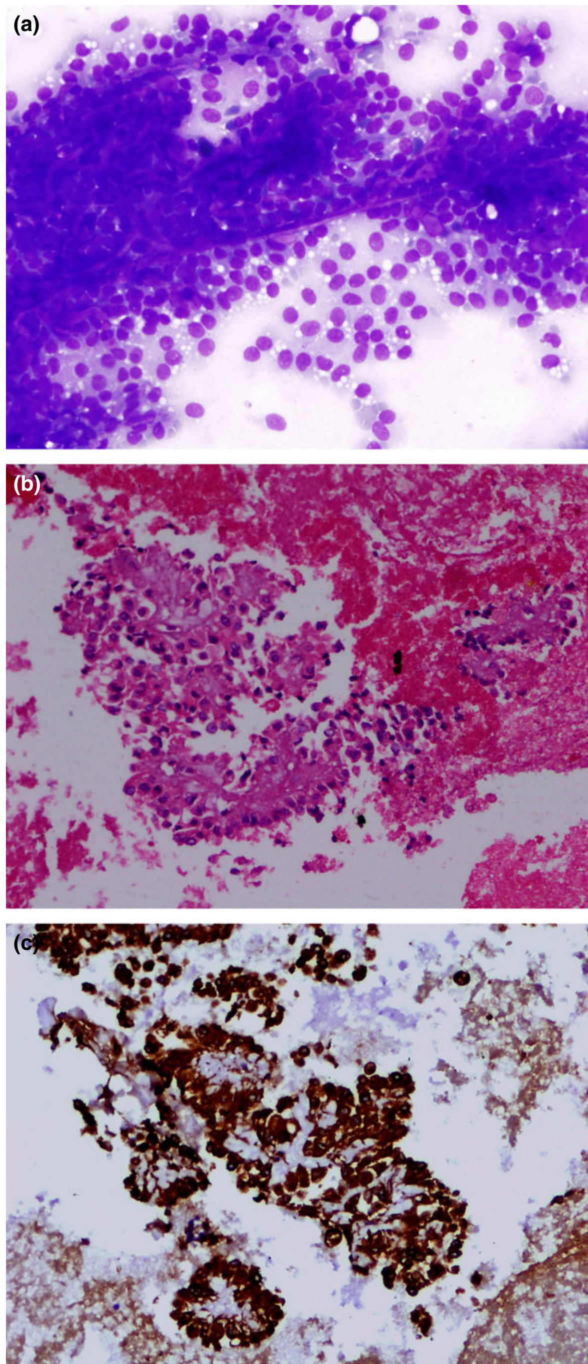

Figure 8 Endoscopic ultrasonography-guided sampling of a pancreatic tumor. (a) Cytology shows small, bland, monomorphic cells along fibrovascular cores. These findings are suggestive of a solid pseudopapillary tumor (SPT). (b) Cell-block preparation. (c) Immunohistochemistry done on the cell-block showing positive staining for vimentin. SPT is the only epithelial tumor of the pancreas which is vimentin positive.

Table 3 Molecular analysis/IHC commonly required in EUS aspirates

Tumor	Basic IHC panel/Molecular analysis
NSCLC	CK7 and CK20† Lung adenocarcinoma markers (TTF-1, Napsin-A)*, †squamous cell carcinoma markers (p63, p40, CK5/6)** common driver mutations (EGFR, KRAS, and EML4-ALK rearrangement)
Pancreatic adenocarcinoma	CK7, CK20, CEA, CA19.9 Basic markers to distinguish metastasis to the pancreas: Vimentin§, TTF-1§, surfactant protein A§
NET	Synaptophysin, chromogranin, PGP 9.5, NCAM/CD56, CK8, CK18, Ki-67
Spindle cell tumors (GIST/leiomyoma/ schwannoma)	CD34, c-kit (CD117), DOG-1¶, S-100, smooth muscle actin (SMA), Ki-67, mitotic count
Lymphoma with small cell morphology††	CD3, CD5, CD10, CD20, CD23, Cyclin D1, Bcl2, Bcl6, Ki-67, SOX-11

From references.[35,36]

†Most lung adenocarcinomas are CK7 positive and CK20 negative.

‡Any one marker each for adenocarcinoma and squamous cell carcinoma may be used initially.

§To distinguish pancreatic ductal carcinoma from metastasis to the pancreas. Most common primary sites are melanoma, lung, kidney, breast, and colon.

¶Especially useful in c-kit-negative GIST.

††Small-cell morphology in NHL is seen in small lymphocytic lymphoma/chronic lymphocytic lymphoma (SLL/CLL), mantle cell lymphoma, follicular lymphoma, and Burkitt lymphoma. Reactive lymph nodes also come into the differential when a mixed lymphoid cell population is seen.

Bcl, B-cell lymphoma; CA19.9, cancer antigen 19.9; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CK, cytokeratin; EGFR, epidermal growth factor receptor; EML4-ALK, echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase; EUS, endoscopic ultrasonography; GIST, gastrointestinal stromal tumor; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma viral oncogene homolog; NCAM, neural cell adhesion molecule; NET, neuroendocrine tumor; NSCLC, non-small-cell lung cancer; PGP, protein gene product; SOX, Sry-related HMG box; TTF, thyroid transcription factor.

1. Expel the entire material in 10% formalin, and process as tissue cores.
2. Expel the material on a glass slide or Petri dish, and microdissect out the tissue cores with a small tweezer or a needle.

The expelled material from cutting needles is seen as elongated red, or red and whitish pieces (Fig. 11). The whitish or discolored fragments are tissue pieces, and red cylinders are coagulated blood. However, the red coagulum

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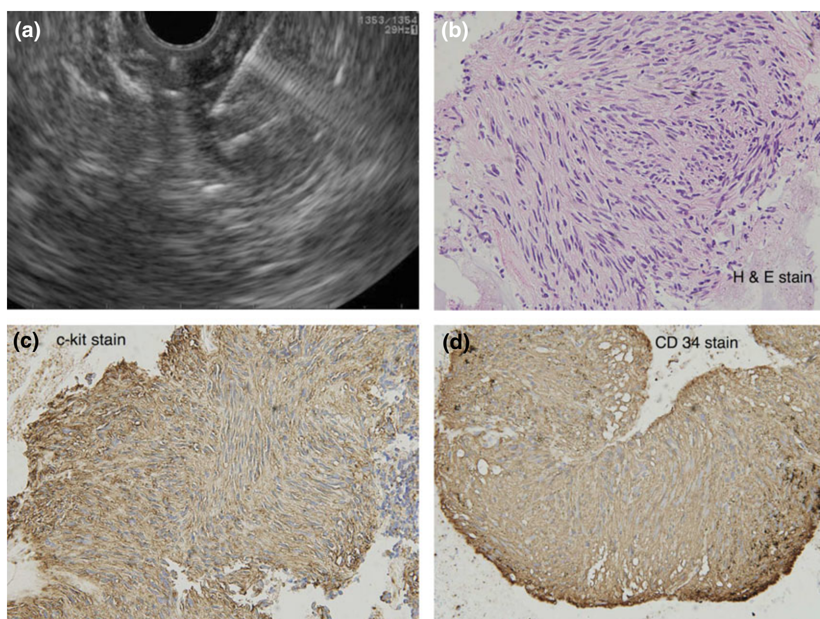

Figure 9 (a) Tissue acquisition from a hypoechoic mural lesion arising from the fourth layer (muscularis propria) in the stomach using a 19-G hollow needle. The lesion is to be diagnosed as a gastrointestinal stromal tumor (GIST), but endoscopic ultrasonography (EUS) appearance alone is not diagnostic. (b) Tissue core showing a spindle cell tumor, with a differential of GIST, leiomyoma, schwannoma, or even gastrointestinal tract muscle cells. Cytology alone would not have been sufficient to give a conclusive diagnosis in such a case, and procuring a tissue core is mandatory for a conclusive diagnosis. (c) Immunohistochemistry helps in a conclusive diagnosis. This tumor is c-kit positive, and (d) CD34 positive. The final diagnosis is GIST. Remember that the biological behavior of this tumor cannot be predicted by fine-needle aspiration biopsy results, but tumor size, morphology, and contrast-enhanced EUS may suggest a higher risk for aggressive behavior.

may also contain tumor tissue.[37] Often, the blood clots far exceed the tissue cores in quantity. However, once paraffin embedded, the tissue micro-cores can usually be identified. These distinctive features of the EUS-acquired tissue cores must be conveyed to the pathologist for proper sample processing.

Although gross visual inspection may suggest that the specimen is adequate for histology, false-positive misinterpretation occurs in about 13.5–33% of cases.[38,39] Collecting tissue fragments for histology still allows further cytopathological evaluation of the remaining specimen.[39]

FALSE-NEGATIVE AND FALSE-POSITIVE RESULTS OF EUS-FNA

THE RATES OF false-negative EUS-FNA results (with adequate material) are 8–9% for lymph nodes, 0–25% for biliary strictures, 4–25% for solid pancreatic lesions, and 54–74% for pancreatic cancer in the setting of chronic pancreatitis.[31] Therefore, in the presence of clinical suspicion, it may be worthwhile to repeat the FNA procedure, and keep surgical options open.

Cells of the gastric mucosa can mimic mucinous epithelium, and duodenal mucosa is similar to pancreatic-ductal epithelium.[40] Well-differentiated adenocarcinomas may be extremely difficult to distinguish from mucosal epithelial cell contamination. Yet, false-positive interpretative errors are rare. The specificity and positive predictive values of EUS-FNA for malignancy have traditionally been estimated to be 100%. However, in studies with good reference standards and long follow up, false-positive EUS-FNA cytology has been reported in 1.1–5.3% when cases with positive cytopathological results only were considered, and in 7.8% if suspicious cytopathology results were included.[41] Most false-positive cases resulted from malignant cell contamination when carrying out EUS-FNA of lymph nodes in patients with luminal cancer. False-positive rates seem to be higher in luminal compared with extraluminal (e.g. pancreatic) primary cancer.[42,43]

Gleeson *et al.* reported 377 patients with EUS-FNA cytology interpreted as positive or suspicious for malignancy, with a final diagnosis based on direct surgical resection without prior neoadjuvant therapy. The false-positive rate was 20/377 (5.3%) and increased to 27/377

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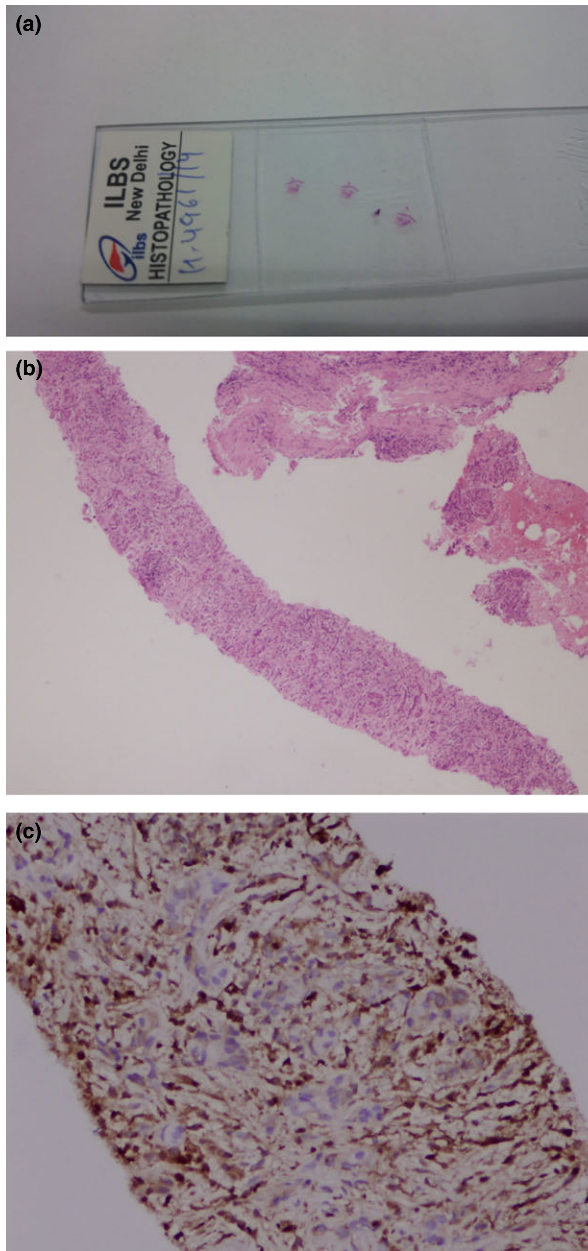

Figure 10 (a) Transgastric biopsy from pancreatic body using a 22-G ProCore needle (Cook, Bloomington, USA.), in a patient with autoimmune pancreatitis. (b) Low-power view of the tissue core (×4) shows distorted lobular architecture, with fibrous replacement of acini/parenchyma. (c) Positive cytoplasmic staining of plasma cells for immunoglobulin (Ig)G4. Remnant acinar cells are seen unstained as faint purple cells (×40). The definition of significant IgG4-positive cells is >10/ high-power field (hpf) on biopsy specimens, in contrast to resection specimens where positive staining is traditionally defined as >100/hpf.

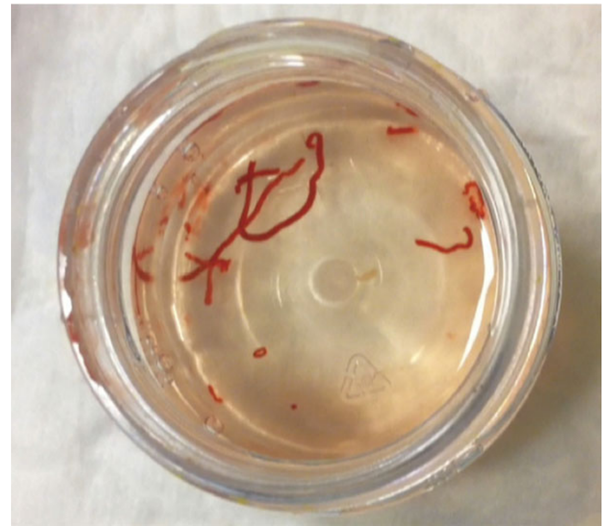

Figure 11 The 'micro-cores' from a core needle can be expelled directly into 10% formalin. Much of the 'cores' would be blood clot, and the tissue pieces are often embedded as whitish-pale fragments in the long worm-like piece.

(7.2%) when false-suspicious cases were included. Most of the false-positive results occurred in non-pancreatic samples, including FNA of peri-esophageal or peri-rectal lymph nodes in the setting of luminal malignancy. The authors suggest that contamination of the luminal fluid by tumor cells from the primary site contributed to the higher false-positive rates in the setting of luminal cancers.[44]

This hypothesis was supported by Levy *et al.* who collected luminal fluid present within the suction channel of the scope. Luminal fluid-positive cytology from these specimens was found in 48% of patients with luminal cancer (not influenced by FNA), 10% of patients after EUS-FNA of pancreatic tumors, and no patient with non-malignant disease.[45]

ESGE suggest that flushing the working channel of the scope before every needle pass, and collection of tissue cores may reduce this risk of false-positive results.[39]

ANCILLARY TESTS

COMMONLY USED ANCILLARY tests in EUS-guided aspirates include polymerase chain reaction (PCR) and culture for tuberculosis, flow cytometry (FC), and mutational analysis. For tubercular culture, the material can be submitted in saline to the microbiological lab, or liquid media can be inoculated in the endoscopy room (Fig. 12a,c). If tuberculosis is suspected later, PCR for

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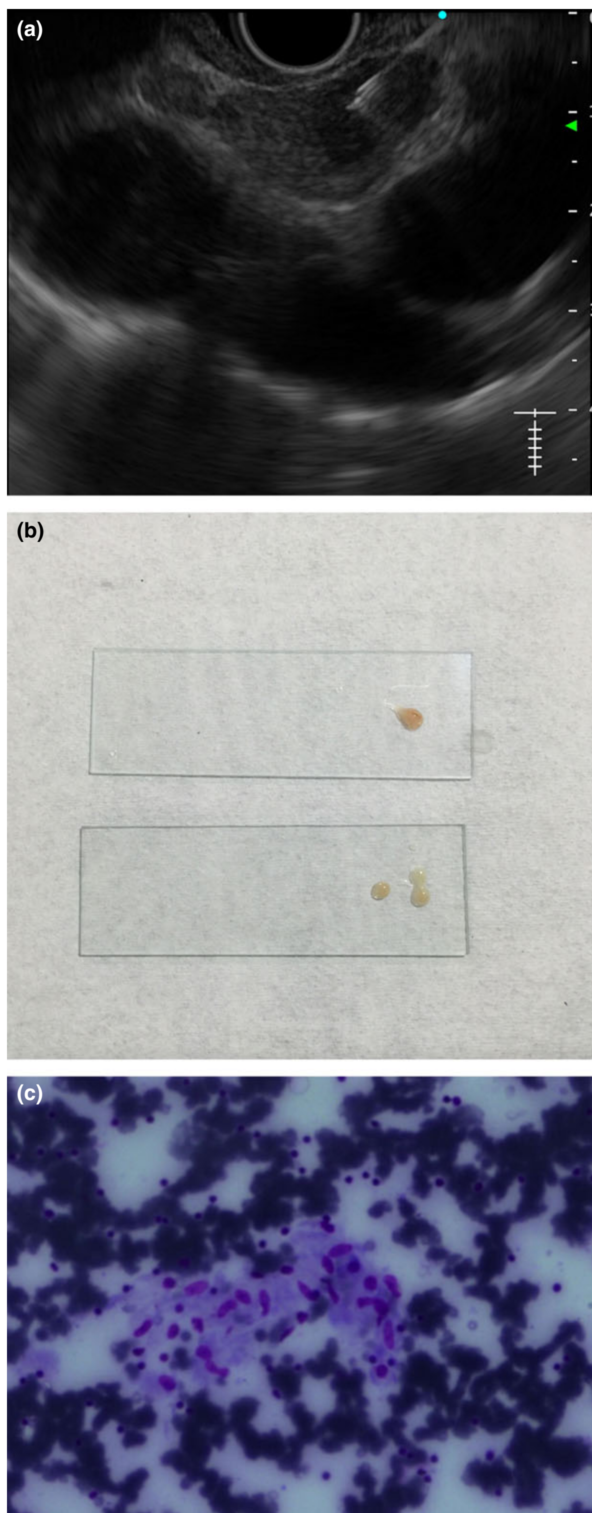

Figure 12 (a) Lymph node (LN) showing necrotic area, identified as a demarcated more hypoechoic area. This is a very suggestive feature of tuberculosis, and is rare in sarcoidosis. However, metastatic nodes, especially from gallbladder cancer or squamous cell cancer, can also have areas of necrosis. Sampling of all areas of the LN by fanning is recommended to avoid false-negative results in focal malignant infiltration. (b) Cheesy, pus-like aspirate from the necrotic area. Although granulomas would be difficult to identify, Ziehl-Neelsen staining would often demonstrate acid-fast bacilli in such aspirates. (c) The non-necrotic area of the lymph node should also be targeted for cytological demonstration of granulomas (Giemsa; ×40). In a necrotic lymph node, selective sampling of different areas is usually possible optimizing the results.

Mycobacterium tuberculosis can be done on aspirates in cell media, alcohol or formalin-containing solutions, and even on paraffin-embedded material.

For FC, three additional passes should be made, and the material collected in heparinized, phosphate-buffered saline, or tissue culture transport medium with calf serum such as Hanks or RPMI medium. Use of heparinized collection solutions prevents clotting of the EUS-FNA sample with entrapment of cells in clot material, which will get filtered before FC. Use of FC aids in the diagnosis of non-Hodgkin's lymphoma (NHL). FC is not useful for Hodgkin's lymphoma.

Genomics-driven oncology is rapidly developing, and soon we may see expansion of personalized cancer therapeutics to the routine care of patients. Tumor biopsies and tissue sampling are the necessary first steps in the genomic profiling of patients who have cancer. Material for such genetic studies can be either the fresh specimens or paraffin-embedded sections of the cell-blocks obtained by EUS-FNA. Micro-cores obtained from pancreatic tumors, and fixed directly in formalin are also suitable for molecular studies. RNA can be extracted from fixed material and mutational analysis carried out by reverse transcriptase PCR with or without direct sequencing methods.

For pancreatic ductal carcinoma, K-ras mutation analysis and telomerase activity estimation have been reported as useful. Ogura *et al.* showed that addition of K-ras mutation analysis increased sensitivity by 6%, and overall accuracy by 5%.[46] K-ras mutation analysis may be useful when pancreatic ductal adenocarcinoma is suspected, yet EUS-FNA results are inconclusive (Fig. 13). K-ras mutations are extremely rare in pancreatic inflammation and other pancreatic tumors. Mishra *et al.* found positive telomerase activity in 6/7 patients of pancreatic ductal adenocarcinoma with negative cytology.[47]

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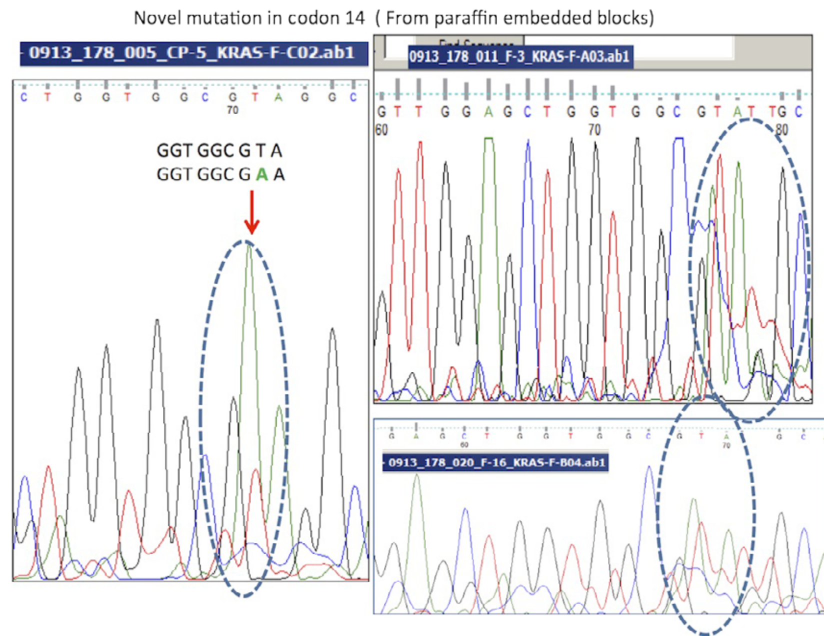

Figure 13 K-ras mutation analysis by reverse transcriptase polymerase chain reaction on an aspirate from a ductal adenocarcinoma of pancreas.

SAFETY OF EUS-GUIDED TISSUE ACQUISITION

USUALLY, A PLATELET count <50 000/mL and an international normalized ratio (INR) >1.5 are regarded as contraindications to EUS-guided sampling. EUS-guided sampling should not be done in patients on oral anticoagulants, heparin, and low molecular weight heparin (LMWH). Aspirin use and non-steroidal anti-inflammatory drugs (NSAIDs) do not increase the risk of bleeding after EUS-FNA.[17,48] Aspirin is stopped only before aspiration of pancreatic cysts. There are no data on safety of EUS-guided sampling in patients on thienopyridine drugs such as clopidogrel.

EUS-FNA procedures have a good safety profile. Complications such as bleeding or bacteremia occur in <1% of all patients.[49] In a systematic review, the overall complication rate of EUS-FNA was 0.98%, with rates of severe bleeding of 0.13%, and procedure-related mortality in 0.02%.[50] Perforation rates with EUS procedures are 0.03–0.15%, with the risk of duodenal perforation being higher than for esophageal perforation.[21] Infection rates are very low after EUS-guided aspiration, except in mediastinal cysts where this procedure is considered contraindicated. Bacteremia is rare after EUS-FNA, including peri-rectal lesions. Antibiotic prophylaxis is used after aspiration of pancreatic cysts, duplication cysts, and may be indicated in immunocompromised patients. Antibiotics are not indicated after aspiration of solid lesions, or as infective endocarditis prophylaxis.

CONCLUSION

WE HAVE OUTLINED the current line-up of tissue acquisition needles, and cytology, cell-block, and micro-core preparation techniques. The operators must individualize the tissue-acquisition process and handling of the aspirated specimen for each patient, as per the presumptive diagnosis.

CONFLICTS OF INTEREST

AUTHORS DECLARE NO conflicts of interest for this article.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article at the publisher's web site.

Data S1 Technical details of currently available fine-needle aspiration (FNA) needles from different manufacturers.

Data S2 Technical details of currently available core biopsy needles from different manufacturers.

Figure S1 (a–g) Current endoscopic ultrasonography (EUS) needles for obtaining cytology smears.

Figure S2 (a–e) Current endoscopic ultrasonography (EUS) needles for obtaining tissue cores for histology.

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