REVIEW ARTICLE

Interactive role of the cytopathologist in EUS-guided fine needle aspiration: an efficient approach

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Fine needle aspiration (FNA) is a minimally invasive method for cytologic sampling of peripheral and deep-seated mass lesions. The clinical utility of FNA continues to improve in direct proportion to the increasing sophistication of imaging techniques and the growing experience of radiologists, cytopathologists,[1] and endoscopists. Image-guided FNA can yield near–tissue-equivalent diagnostic material that can form the basis for therapeutic decisions. Minimal morbidity, low cost, and a rapid turnaround time that reduces patient anxiety make FNA a preferred method in the evaluation of neoplasia.[2]

EUS demonstrates the transmural features of the GI tract and can thereby establish a presumptive diagnosis with respect to tumors arising in many anatomic sites. However, appropriate therapy can only be instituted on the basis of a pathologic diagnosis. In 1991, Caletti et al.[3] described the use of a guillotine needle under EUS guidance to obtain biopsy diagnoses of gastric submucosal tumors. EUS-guided FNA (EUS-FNA) for cytologic diagnosis of pancreatic and upper and lower GI tract tumors was described in 1992 in separate studies by Wiersema et al.[4] and Vilmann et al.[5] It has been shown to be both highly accurate and cost-effective in the diagnosis and/or staging of pancreatic and other GI tumors.[6] EUS-FNA is performed with linear array, fiberoptic, or video echoendoscopes. A retractable needle introduced by means of an accessory channel in the echoendoscope is guided ultrasonographically in real time into the target mass. EUS-FNA is particularly useful for sampling mass lesions that cause external compression of the pancreatic ducts without involvement of the GI tract mucosa.[7] It is also useful in the evaluation of mediastinal, celiac, and retroperitoneal lymph nodes for diagnosis and tumor staging.

This article describes techniques for the handling and processing of cytologic specimens and for the efficient use of cytopathology services during EUS-FNA.

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EUS-FNA TECHNIQUE

Identification of an optimal biopsy site is the first step in EUS-guided FNA. When evaluating a malignant process, it is preferable to target the site that will potentially result in the most advanced stage (e.g., a celiac rather than a periesophageal lymph node in esophageal cancer). The target lesion should be positioned as close as possible to the echoendoscope transducer. In this discussion, the North American convention in which the patient's head is at the right of the screen will be assumed. With commercially available convex linear array endoscopes (EG series, Pentax Precision Instrument Corp., Orangeburg, N.Y.; UC 30 series, Olympus America, Inc., Melville, N.Y.), the needle appears at the upper right side of the US display at the base of the transducer and crosses from this point toward the lower left at a 45 degree angle as it enters the lesion. When possible, the lesion should be positioned immediately below the apex of the transducer. The echoendoscopes used for EUS-FNA have an elevator mechanism similar to that of duodenoscopes; to facilitate passage of the needle the elevator should be released or should be in a down position. Once the needle sheath has cleared the elevator, the needle, when advanced, will be visible on the US display. The handle of the needle device should be secured to the Luer-lock at the proximal port of the accessory channel.

Maintaining control of both the endoscope and the position of the target is paramount. Occasionally, as the sheath of the needle enters the GI lumen it will displace the wall thereby creating a pocket of air with loss of the ultrasonographic image; this can be minimized by gentle suction. To maintain control and optimize target acquisition, 3 endoscopic maneuvers are used at this point: torque, push/pull, and upward tip deflection. Occasionally, for EUS-FNA of pancreatic head masses, a long echoendoscope position, analogous to that used for ERCP, may be the only option for optimal positioning. Once an adequate stable position is acquired, the needle is gradually advanced through its sheath until it is seen on the US display. A swift jabbing motion is then necessary to traverse the muscularis propria. When dealing with solid masses, for example in the pancreas, withdrawing the stylet within the needle a couple of millimeters may facilitate puncture. If this technique is used, it is necessary, once the nee-

Table 1. Preparation of smears at the endoscopy suite

1	Appropriately label the frosted end of slides in pencil. Complete the requisition form in ink. Repeat if sampling more than one anatomic site.
2	Place previously labeled slides on working tray before initiating procedure.
3	Cytology technician (or other trained personnel) handling the specimen must always wear protective gear (gloves and face shield).
4	After each aspirate is performed, the needle is handed to the cytology technician by the endoscopist assistant, who can then assist the cytotechnician prepare slides by forcing air through an attached syringe. When handling needles caution must be exercised to avoid accidental spraying or needle punctures.
5	While being helped by an assistant, the cytology technician can express 1 drop of aspirate onto the center of a previously labeled glass slide with the frosted end up. If applicable, the bevel of the needle should be against the slide surface.
6	Place another previously labeled slide, with frosted end down, on top of the drop of aspirate. As the drop spreads, pull the two slides apart—gently but quickly—in a longitudinal axis.
7	One smear from each pass can be air-dried (1 minute) for Diff-Quik staining and rapid on-site assessment of specimen adequacy. The remaining smears are immediately fixed in 95% ethyl alcohol, either by immersion or spray fixation.
8	Needles are rinsed in saline or in Hank's balanced salt solution for concentration procedures and further cell recovery. Alternative methods of cell recovery from needle rinses include rinsing the needle in formaldehyde for paraffin-embedding and cell-block preparation, or in brand-specific liquid-based solutions for thin-layer technologies.
9	Repeat previous steps with each additional needle pass performed, as required.

dle has entered the mass, to reposition the stylet to avoid plugging or contamination of the sample with cells from the wall. The stylet is then removed and negative pressure is applied to the needle with a syringe attached to the needle, either directly or by means of a short extension tube. The degree of negative pressure remains controversial. For lymph nodes or vascular tumors, some authorities advocate minimal or no pressure to avoid clogging the needle with a blood clot, a situation that usually results in an inadequate specimen. Between 5 and 10 gradual jabbing motions are recommended while maintaining the needle within the lesion at all times to avoid inclusion of cells from the GI wall or surrounding structures. Before removing the needle, the negative pressure is released and the needle is retracted within its sheath. The needle handle is then unscrewed from the echoendoscope and the needle is brought out to a work surface for sample preparation.[8,9]

SPECIMEN COLLECTION AND HANDLING

The diagnostic accuracy and cost-effectiveness of EUS-FNA is significantly enhanced by an interdisciplinary approach that includes the active, on-site participation of a cytology team. If feasible, a cytopathologist and associated technical personnel should be present during the aspiration to ensure that the specimens collected are adequate for cytologic evaluation. Optimal slide preparation is fundamental to accurate cytologic diagnoses. Preferably, the smears should be prepared by cytology personnel or by an endoscopy staff member thoroughly trained in the proper techniques for preparing satisfactory slide material.[10] Therefore arrangements should be made in advance of the procedure to ensure that the cytology team will be available at the time of aspiration.

The activities of the cytology team are facilitated by use of mobile carts that carry all materials required for specimen preparation, including a microscope. For practical purposes, this cart serves as a mobile laboratory unit. On arrival in the endoscopy suite, the endoscopist should provide the cytopathologist with all relevant clinical data. In particular, the cytopathologist must be aware of the anatomic location, size, and relationship of the mass to adjacent structures, as well as the clinical impression and pertinent history.[10]

The aspirates collected by the endoscopist are handed to a cytology technician (or other trained GI personnel) for preparation of smears and other diagnostic material (Table 1). A representative smear from each needle pass can be air-dried and rapidly stained with Diff-Quik (Baxter, McGaw Park, Ill.), and handed to the cytopathologist for immediate assessment of specimen adequacy. The cytopathologist is usually able to provide feedback regarding adequacy of the specimen within 5 to 10 minutes. Additionally, the cytopathologist is often able to render a preliminary diagnosis or, at least, provide a differential diagnosis.

The remaining prepared smears are immediately fixed (by immersion or spray fixation) with 95% ethyl alcohol by the cytology technician. The fixed smears are processed at a later time in the cytology laboratory for Papanicolaou staining and/or for additional ancillary studies such as immunochemistry, as required. After preparing smears, the

cytotechnician then rinses the needle in saline solution for concentration procedures and further cell recovery. Endoscopy personnel can assist the cytology technician in making the cytologic preparations as needed (Table 1). In most cases a final report will be available within 24 hours.

During EUS-FNA the cytology team must be informed of any change in the selection of the anatomic site of aspiration, particularly if more than 1 target organ is to be sampled. Specimens from different anatomic locations should be processed separately and corresponding needle rinses collected in separate containers, properly labeled as to site, to avoid cross-contamination.

ROUTINE STAINING OF SLIDE PREPARATIONS

Our preference is to use both wet-fixed and air-dried smears in the evaluation of cytologic specimens. The air-dried smears are stained with a Romanowsky stain (e.g., Diff-Quik, Quik-Dip [Mercedes Medical, Inc., Sarasota, Fla.], modified Wright-Giemsa). The slides fixed with 95% ethanol are stained by the conventional Papanicolaou method. These 2 methods for slide preparation are complementary[10] and highlight different cellular details. An ultrafast Papanicolaou staining technique is also available for rapid on-site assessment of smear adequacy.[11] Diff-Quik is a rapid, 3-step, metachromatic stain that uses methanol as a quick fixative (10 dips), followed by 2 staining solutions: xanthine (eosin) dye (10 dips) and a thiazine dye mixture (Azure A/thyazine/methylene blue) (10 dips), followed by rinse in tap water. Diff-Quik is probably the most widely used stain for rapid on-site assessment of cytologic specimens.

Routine paraffin embedding and further staining with H & E are required for evaluation of cellblock material. Cellblocks are prepared from formaldehyde-fixed needle rinses. Some laboratories prefer to use H & E routinely for staining of smear material. Immunochemical stains can be performed on Cytospin (Shandon, Pittsburgh, Pa.) or cellblock preparations, as well as on direct smears.[10] The immunoperoxidase (avidin-biotin complex) is the most frequently used methodology for immunochemical stains.

ON-SITE ASSESSMENT OF CYTOLOGIC SPECIMENS

On-site assessment of cytologic specimens is useful for quality assurance and has 3 major purposes: (1) The adequacy of the aspirates should be assessed to ensure that the target organ has been appropriately sampled, that is, that cells have been collected in sufficient numbers for reliable cytologic evaluation. However, there are no specific guidelines as to what constitutes an adequate cytologic specimen from a quantitative standpoint. A specimen is adequate for evaluation when, based on the cytologic findings, the cytopathologist is able to formulate a reliable diagnosis as to the nature of the sampled lesion. (2) The nature of the disease process (e.g., infectious, neoplastic) affecting the organ sampled should be assessed to determine the most appropriate immediate course of action, including additional needle passes for ancillary studies such as microbiologic cultures (when aspirates suggest an infectious etiology), flow cytometric studies (when smears suggest lymphoma), and special histochemical and immunochemical stains for identification of microorganisms and for further characterization of malignant neoplasms. Cytology technicians usually have the responsibility for making slide preparations that will be used for ancillary studies (histochemical and immunochemical stains) whenever additional material has been aspirated for such purposes. (3) A preliminary diagnosis (or at least a differential diagnosis) should be rendered in much the same manner, and with similar implications, as an immediate diagnosis based on evaluation of frozen sections obtained at surgery. Appropriate use of a cytology service for these 3 purposes reduces the probability of false-negative and unsatisfactory aspirations. This results in significant improvements in the sensitivity and accuracy of cytology in the diagnosis of carcinoma.[12]

HANDLING OF NEEDLE RINSES

After preparing smears from each aspirate, the needle is rinsed in saline solution, Hank's solution (Gibco BRL, Grand Island, N.Y.), or other suitable solutions for ultracentrifugation and further cell recovery. When aspirates are obtained from different anatomic locations, special care should be taken to ensure that the corresponding needle rinses are placed in separate containers (correctly marked as to site) to avoid cross-contamination. Alternatively, when thin-layer technologies, such as AutoCyte (TriPath Imaging, Burlington N.C.), and ThinPrep (Cytyc Corporation, Boxborough, Mass.), are used, the rinses can be collected in special, brand-specific, liquid-based fixatives. Once in the cytology laboratory, the rinse material can be processed for centrifuged cell preparations by the Cytospin (Shandon/Lipshaw, Pittsburgh, Pa.) technique (Table 2). Other available methods for processing the rinse material include liquid-based thin-layer technologies and cellblock preparations, according to the preference of the laboratory. Histochemical and immunochemical stains, as required, can be performed with either of the above options.

Table 2. Ultracentrifugation of needle rinse material by the Cytospin technique

	Procedure for cellular specimens	Procedure for hypocellular specimens
1	Collect needle rinse material in 1 mL of saline solution (proceed to step 4)	
2	If aspirated material is fluid, spin down in standard centrifuge at 1500 rpm	
3	Re-suspend the pellet by aspirating material in and out of a pipette into a clean cup for cytocentrifuge preparation	If no pellet is visible, use the bottom part of the material (approximately 1-2 mL) for cytocentrifuge preparation
4	Add 1 drop of Shandon solution to chamber	
5	Deliver 1-3 drops of centrifuged material into each ultracentrifuge chamber	Deliver 3-6 drops of centrifuged material into each ultracentrifuge chamber
6	Spin for 2 minutes at 1500 rpm	
7	Immediately immerse slides into 95% ethyl alcohol and fix for 15 minutes	
8	Perform routine staining	

REPORTING OF CYTOLOGIC DIAGNOSES

Cytologic diagnoses on specimens obtained by means of EUS-FNA are reported using the conventional diagnostic nomenclature for nongynecologic cytologic specimens.[10] Malignant cytologic findings that correlate with clinical and radiologic findings indicative or suggestive of malignancy are considered "conclusive" in most instances. When benign cytologic findings are found to correlate with benign clinical and radiologic findings consistent with benign disease, no further diagnostic evaluation is necessary. However, additional diagnostic studies are required if the cytologic findings are "inconclusive" and reported as "atypical/indeterminate" or "suspicious for malignancy," or the specimen is reported to be "unsatisfactory" (Table 3).

DIAGNOSTIC PITFALLS AND HOW TO AVOID THEM

A false-negative diagnosis occurs when pathologic examination (cytology or histology) of a specimen from a lesion is reported as benign (within normal limits) but the lesion is later proven to be malignant. False-negative diagnoses are usually caused by sampling error, interpretive error, and/or technical problems during performance of EUS-FNA.

Sampling error is the most common cause of false-negative diagnoses in relation to cytologic samples. The aspirate may contain rare benign cells (hypocellular) or apparently adequate numbers of benign cells (normocellular), otherwise native to the target organ; however, no malignant cell is present on the slide preparations. Sampling error can be caused by any of the following: (1) small tumor masses (usually less than 1 cm) that are technically difficult to approach, (2) metastatic deposits in an organ (e.g., lymph node) that are too small to accurately target, (3) an anatomic location that renders a lesion difficult to target, and (4) medical conditions that significantly limit the performance of EUS-FNA. The most effective measure for avoiding sampling error is the immediate assessment of the adequacy of samples by a cytopathologist on-site during EUS-FNA.

Interpretive error occurs when, upon initial microscopic examination of the specimen, the cytopathologist is unable to recognize malignant or dysplastic cells present on the slide preparations. Interpretive errors can lead to false-negative diagnoses. However, another type of interpretive error is the "overcalling" of a specimen as malignant in cases in which a lesion is later proven to be nonmalignant (false-positive diagnosis). Although infrequent, interpretive errors are more likely to occur with inexperience and/or when evaluating specimens and slide preparations that are hypocellular or of suboptimal quality. The latter is also referred to as "technical error." The most effective measure for preventing interpretive and/or technical errors is preparation of the slides by cytology technicians or by thoroughly trained endoscopy assistants; this results in slide material of better quality. The avoidance of air-drying artifact or smears that are too thick or bloody, among other technical artifacts, is a prime requirement to ensure that the material will be adequate for diagnosis. Again, it is essential to verify that diagnostic cells have been collected in sufficient numbers to enable the cytopathologist to render a reliable, confident diagnosis, a stipulation best addressed by a cytopathologist on-site during EUS-FNA.

As with FNA cytology of breast lesions, any deep-seated mass lesion should be evaluated with a triple test-like strategy[13] in which the cytologic results are correlated with the clinical and imaging findings. Diagnostic accuracy is maximized when all components of this diagnostic triplet are in agreement. However, additional studies are required for any inconclusive, non-negative cytologic results. Further investigation is also warranted when negative cytologic results are inconsistent with the clinical and imaging findings.

Technical pitfalls may be encountered during EUS-FNA that can also lead to false-negative or

Table 3. Diagnostic nomenclature for reporting of cytological diagnoses

Diagnostic category	Definition	Suggested course of action
Unsatisfactory	Non-diagnostic aspirates: acellular or extremely hypocellular material. Also applies to too thick smears, or specimens significantly obscured by inflammation, blood, or air-drying artifact.	Repeat specimen collection or perform additional diagnostic studies.
Negative for malignancy	Applies to adequately cellular specimens containing benign cellular elements consistent with normal components of the target organ (e.g., lymphoid tissue from lymph nodes, benign acinar/ductal epithelium from pancreas). No evidence of cellular abnormality.	No additional studies indicated, unless the benign cytologic findings are not consistent with abnormal clinical and radiographic findings. This requires a repeat specimen or further diagnostic study to resolve the diagnostic dilemma.
Positive for malignancy	Reserved for aspirates exhibiting unquestionable cellular features of malignancy, which must be present on sufficient numbers of cells to enable a confident diagnosis.	No confirmatory studies are usually indicated.
Atypical/ indeterminate	Used to report minimally abnormal cellular findings, which most likely represent reactice/ reparative and inflammatory-type changes. Malignancy is unlikely, although it cannot be totally excluded on the basis of cytology.	Clinical and radiologic correlation required. Further diagnostic studies may be performed depending on the degree of clinical suspicion for malignancy.
Suspicious for malignancy	Used to report cytologic specimens exhibiting abnormal cellular findings, which display some, but not all, the features of malignancy. Also used for specimens containing highly abnormal cells in very limited numbers.	Further diagnostic studies are indicated to confirm or exclude malignancy.

nondiagnostic results. Depending on the nature of the target lesion, it may be difficult to penetrate with the needle due to hardness or excessive fibrosis. On passage toward the target mass, the needle may become clogged with GI tract mucosa, which necessitates unclogging. Occasionally, the endoscopist can encounter problems in keeping the target lesion properly positioned for EUS-FNA. The cytopathologist should be advised of any technical problems that arise during EUS-FNA.

EFFECTIVENESS OF EUS-FNA

Several case series in which EUS-FNA has been used in the diagnosis and management of cancer have been published over the last decade. The diagnostic accuracy of EUS-FNA is reported as greater than 90% in the evaluation of mediastinal lymph nodes or masses in patients with lung cancer.[14,15] When applied to GI masses, extrinsic or submucosal, the sensitivity and specificity of EUS-FNA in published reports has ranged, respectively, from 76% to 94% and from 50% to 100%.[16,17] In one study, the overall diagnostic accuracy was 86%.[18] In the large case series of Giovannini et al.,[19] which included submucosal lesions, pancreatic tumors, and mediastinal or celiac lymph nodes, the sensitivity and specificity of EUS-FNA were, respectively, 77% and 100%; in 10.6% of cases EUS-FNA provided unsatisfactory results. By anatomic site, the sensitivity in this study was 60% for submucosal tumors, 75% for neoplasms of the pancreas, and 80% to 88% for mediastinal and celiac mass lesions. Bentz et al.,[7] in a study that included 60 patients, reported a diagnostic accuracy of 94% for pancreatic tumors and 100% for GI-associated lymph nodes and for mediastinal and retroperitoneal masses. Overall, the sensitivity and specificity for the diagnosis of malignancy were 90% and 100%, respectively. The use of EUS-FNA has also been described in the diagnosis of rare pancreatic neoplasms such as microcystic adenoma,[20] insulinoma of the pancreas,[21] and malignant carcinoid tumor of the duodenum.[22] The cytologic diagnosis of unusual neoplasms like these often requires supportive ancillary studies such as special histochemical and immunochemical stains. Thus, the accumulated clinical data prove that EUS-FNA is an

effective technique for the diagnosis and staging of cancer. It is reasonable to expect that, with increasing experience on the part of endoscopists and cytopathologists, further enhancements are likely with respect to the diagnostic accuracy of EUS-FNA.

CONCLUSION

A high degree of accuracy is achieved in the cytologic diagnosis when deep-seated mass lesions are evaluated in an interactive team approach involving clinicians and cytopathologists.[1] Immediate examination of aspirates by a cytopathologist for adequacy during EUS-FNA is therefore highly recommended. This reduces the number of inadequate samples, and it also decreases the number of needle passes required or identifies the need for additional passes and ancillary studies.[10] Although all medical centers may not have the luxury of having a cytology team, cytopathologist, and cytology technician on-site during EUS-FNA, there are alternatives. Cytology technicians or properly trained endoscopy personnel can handle the aspirates on-site by making smears in the endoscopy suite and then transporting them to the pathology laboratory for staining and immediate evaluation by a cytopathologist. By telephonic conversation or via intercom with the endoscopy suite, the cytopathologist can then provide immediate feedback to the endoscopist.

The present health care environment is marked by stringent constraints on the use of technologic procedures in the interest of cost-efficiency. It is therefore essential, and of greatest benefit to patients, that methods such as EUS-FNA achieve the best possible results in terms of diagnostic accuracy. With respect to cytologic diagnosis, close and immediate collaboration between endoscopist, cytopathologist, and cytology technicians is the best approach to the attainment of this goal.

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