

High-Quality Endoscopic Ultrasound-Guided Fine Needle Aspiration Tissue Acquisition

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ABSTRACT

Endoscopic ultrasound-guided fine needle aspiration is a multistep procedure that involves proper clinical indication, correct selection of needles, adapting evidence-based techniques such as the fanning maneuver and not routinely using suction or the stylet for tissue sampling, and establishing reliable cytopathology support. Integrating cytopathology in the training curriculum and developing a more flexible platform of needles and echoendoscopes are likely to further advance the field of endosonography. This review aims to summarize the technical issues that are key to performing high-quality endoscopic ultrasound-guided fine needle aspiration.

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INTRODUCTION

Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) is an indispensable tool for tissue acquisition from within and adjacent to the gastrointestinal tract. EUS-FNA may be considered a disruptive innovation [1] in the practice of pancreatic pathology, such that the National Comprehensive Cancer Network has incorporated EUS-FNA cytology in its diagnostic algorithm for pancreatic cancer [2]. Undoubtedly, the role of EUS-FNA will expand as the accuracy of EUS tissue acquisition increases. For example, the combination of EUS with endobronchial ultrasound-FNA will likely replace surgical mediastinal staging in patients with nonsmall cell lung cancer in the future [3, 4]. Recent studies have also shown the utility of EUS-guided liver biopsy in patients with abnormal liver function tests undergoing EUS [5, 6].

Obtaining a high-quality, diagnostic specimen is the foundation of performing

EUS-FNA. In-room confirmation of diagnostic adequacy is a defining feature that establishes EUS-FNA as a fundamental part of integrated, multidisciplinary patient care. The benefits include procedural efficiency whereby diagnostic adequacy, rather than a predetermined number, governs how many FNA passes are performed. Procedural time and complications are reduced if a diagnosis is rapidly confirmed. If initial samples are nondiagnostic, the sampling location or needle type can be changed in a timely manner, and avoid repeated noncontributory passes. On-site diagnosis limits the need for repeated EUS, with its associated morbidity and cost. Furthermore, the preliminary diagnosis enables collection of additional samples for ancillary testing, such as flow cytometry in a patient with monotonous lymphocytes on in-room cytology assessment. Providing an immediate preliminary diagnosis to patients and their families contributes to quality patient care, and allows timely referral for additional imaging and consultation with surgery or oncology.

There are several factors that may determine the outcomes of EUS-FNA, such as needle selection, maneuvers to procure quality tissue, and the presence of an on-site cytopathologist. This review aims to summarize the technical issues that are key to performing high-quality EUS-FNA. This review is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

TECHNICAL DIFFICULTY AT DIFFERENT LOCATIONS

The degree of technical difficulty of EUS-FNA varies according to the location of the

targeted lesion. Generally, transesophageal and transgastric FNAs are technically easier than transduodenal FNA. The position of the scope is relatively straight when accessing the majority of lesions via the esophagus or stomach. An exception is access from the gastric fundus, where near complete retroflexion may be required. Acute angulation of the tip of the scope, significant torsion through the scope shaft, and maximal elevator use increases the technical difficulty. This is predominantly an issue in the duodenum. The main challenges are passing the needle assemblage through the scope to attach to the working channel, and smoothly advancing the needle into the targeted lesion, while maintaining scope position and needle control and maneuverability. Releasing the up-down control to straighten the tip of the scope, with or without withdrawing the scope into a neutral position enables the needle to be passed and attached to the working channel, and the scope is then repositioned for the FNA. If the needle doesn't advance into the lesion, straightening the scope from a long to a short position often reduces the scope tip angulation and the resistance to passage of the needle. A 25G needle is suitable for sampling the majority of lesions accessed from the second part of the duodenum, and a flexible 19G needle may be beneficial if histology is required [7].

NEEDLE SELECTION

EUS-FNA can be performed using a 25G, 22G, or a 19G needle. The decision to use a specific needle is guided by a number of considerations. The needle should provide an adequate tissue sample to establish a definitive diagnosis, have the degree of flexibility required for lesion

Table 1 Comparison of different needles for EUS-FNA of solid mass lesions: randomized trials and meta-analyses

References	Number of patients	Lesion type	Needle size	Diagnostic accuracy/pooled sensitivity in meta-analysis	Remarks
Camellini et al./RT [8]	127	All lesions	22 vs. 25G	77.8% vs. 78.1%, $P = NS$	25G needle better for uncinate masses and 22G needle better for subepithelial masses
Fabri et al./RT [9]	50	Pancreatic masses	22 vs. 25G	86% vs. 94%, $P = NS$	Trend toward better yield with 25G needle
Siddiqui et al./RT [10]	131	All lesions	22 vs. 25G	87.5% vs. 95.5%, $P = NS$	NA
Song et al./RT [11]	117	Pancreatic/peripancreatic masses	22 vs. 19G	78.9% vs. 94.5%, $P = 0.01$	Technical success for FNA of pancreatic head masses was significantly less with the 19G needle. 19G needle yielded significantly better cellular material
Ramesh et al./RT [12]	72	Pancreatic masses	19 vs. 25G	88.9% vs. 94.4%, $P = 0.69$	19G needle yielded significantly more core biopsy tissue but specimens were bloodier
Vilmann et al./RT [13]	135	Mixed lesions	22 vs. 25G	89% vs. 90%, $P = NS$	25G needle more difficult to visualize
Lee et al./RT [14]	188	Pancreatic masses	22 vs. 25G	89.4% vs. 88.3%, $P = NS$	25G needle had lower complication rate
Madhoun et al./meta-analysis [15]	1,292	Pancreatic masses	22 vs. 25G	85% (95% CI: 82–88%) vs. 93% (95% CI: 91–96%), $P = 0.0003$	NA
Affolter et al./meta-analysis [16]	1,452	Pancreatic/peripancreatic masses	19 vs. 22 vs. 25G	$P = 0.97$	25G needle had higher diagnostic adequacy compared to 22G needle. Sample size for 19G needle too small for analysis

EUS-FNA endoscopic ultrasound (EUS)-guided fine needle aspiration, *NA* not applicable, *NS* not significant, *RT* randomized trial

access, a low risk of complications, and the ability to obtain core tissue when necessary.

Seven randomized trials and two meta-analyses [8–16] have evaluated different needles for EUS-FNA (Table 1). Five randomized trials comparing 22G and 25G needles [8–10, 13, 14] found the needles had a similar overall diagnostic accuracy and a trend in favor of the 25G needle for transduodenal sampling. Two meta-analyses have compared the 25G and 22G needles for EUS-FNA of solid pancreatic masses, and found a higher diagnostic sensitivity [15] and accuracy [16] with the 25G needle.

A randomized trial compared the 19G and 22G needles in 117 patients with pancreatic and peripancreatic masses [11]. Superior diagnostic accuracy and tissue acquisition was obtained with the 19G needle; however, there was a high rate of technical failure for lesions in the head of pancreas. The role of the standard 19G FNA needle for yielding histological samples was prospectively assessed in a single-arm study by Larghi et al. [17]. Of the 120 patients who underwent EUS-FNA, the procedure was technically successful in 119 patients (99.2%) and adequate histological sample was obtained in 116 (97.5%) of these patients. A major limitation of the study was that patients with pancreatic head or uncinate masses were excluded. A recent multicenter randomized trial compared a flexible 19G needle made of nitinol (Flex 19; Boston Scientific, Natick, MA, USA) to a 25G needle for FNA of solid pancreatic masses [12]. While diagnostic accuracy and technical failure was similar between needles, the 19G needle yielded histological core tissue in significantly more patients (86.1% vs. 33.3%, $P < 0.001$).

The 19G Trucut biopsy needle (Cook Medical, Winston-Salem, NC, USA) was developed to attain core histologic tissue [18]; however, the needle's rigidity limited its use for

transduodenal sampling [19]. A new 19G fine needle biopsy (FNB) device (ProCore; Cook Medical, Winston-Salem, NC, USA) with reverse bevel technology was subsequently developed. Histological samples were successfully obtained in a majority of patients with a diagnostic accuracy of $>90\%$ [20], however, some technical difficulties were encountered with transduodenal passes. 22G and 25G ProCore needles are also available, which facilitate transduodenal sampling. Three recent randomized trials comparing the 22G or 25G ProCore needles to standard FNA needles in pancreatic masses and peripancreatic lymphadenopathy [21–23] have concluded that there is no significant difference in establishing a correct diagnosis between needles. In a study using the 25G ProCore needle, while a cytological diagnosis was established in 96% of 50 patients, histological core tissue was procured in only 32% of patients [22]. A study in 144 patients using the 22G ProCore needle vs. a standard 22G FNA needle showed similar diagnostic accuracy between needles, and fewer passes were required to obtain sufficient tissue with the ProCore needle (1.2 ± 0.5 passes with ProCore vs. 2.5 ± 0.9 passes with standard needle, $P < 0.001$) [23].

FNA TECHNIQUE

Fanning

The center of a malignant mass is usually more degenerated than the periphery and hence more likely to provide nondiagnostic tissue when sampled [24]. Furthermore, repeated sampling along the same trajectory through a lesion is more likely to result in bloodier specimens. Two studies have explored whether aspirating a lesion at the peripheries or across

multiple trajectories improves the diagnostic yield [25, 26]. In a randomized trial of 54 patients with solid pancreatic masses, the fanning technique resulted in a significantly higher first pass diagnosis compared with the standard FNA technique (85.7% vs. 57.7%, $P = 0.02$) [26]. The fanning technique of FNA involves positioning the needle at four different areas within a mass and then moving the needle back and forth four times in each area to procure tissue. We term this the '4 × 4' rule. Aspiration is usually initiated at a margin of the tumor mass and then 'fanned' until the opposite margin of the tumor is sampled. The trajectory of the needle can be altered using either the 'up/down' endoscope dial or the elevator.

Suction

Applying suction during FNA increases the quantity of the FNA sample; however, the specimen is bloodier thereby diminishing the aspirate quality. A randomized trial on FNA of lymph nodes demonstrated that suction resulted in a bloodier specimen and no difference in diagnostic accuracy [27]. In a randomized trial by Lee et al. [28], 81 patients with pancreatic masses underwent EUS-FNA with and without suction in all lesions. Suction resulted in higher diagnostic samples and cellularity, however, specimen bloodiness was increased. In another randomized trial on pancreatic masses, there was no difference in diagnostic yield, cellularity, or specimen bloodiness, irrespective of whether or not suction was used [29].

Although manufacturer guidelines must be followed when using specially designed biopsy needles, there is no objective data on the best technique to use a 19G needle. In our experience, suction or a stylet should generally

be avoided to minimize bloodiness [30]. The needle should be carefully 'fanned' within the mass, moving back and forth only two to three times (4 × 2 rule), and avoid repeated 'jabbing' at one area. It is usually unnecessary to perform more than three FNB passes in a lesion, as repeated sampling increases the sample bloodiness. In routine clinical practice, suction is used primarily for aspiration of cystic lesions.

Wet Suction and Slow Pull Techniques

Two modified FNA techniques have been recently trialed. In the 'wet suction' technique [31], the FNA needle is flushed with sterile saline prior to puncturing the target lesion, and suction is then applied. The authors randomized 117 lesions to commence with either the wet suction technique or 'dry' FNA with suction, and subsequent passes using alternating techniques. The wet suction technique had significantly higher cellularity and diagnostic yield, with no difference in hemorrhage.

The 'slow pull' technique is performed by gradually withdrawal of the stylet as the needle is repeatedly passed through the target lesion. The rationale is the withdrawing stylet produces a low level of negative pressure within the needle which increases tissue acquisition and limits specimen bloodiness. In a retrospective study of 97 EUS-FNAs performed with either the slow pull technique or suction using a 25G FNA needle [32], the slow pull technique resulted in lower cellularity and bloodiness but higher diagnostic sensitivity.

Stylet

The role of the stylet in the FNA needle assemblage is to prevent the hollow tip of the needle from filling with esophageal, gastric, or

duodenal wall tissue before it enters the target lesion. However, three randomized studies have shown the use of a stylet does not improve the diagnostic yield for malignancy, and increases the bloodiness of the tissue specimen [33–35]. A fourth study [28] found the diagnostic yield was higher using the stylet, and specimen bloodiness was decreased if the needle was flushed with air rather than using the stylet to express the specimen. Reinserting the stylet for each FNA pass is time consuming and may increase the risk of needle-stick injury. The weight of evidence suggests limited advantage to reinserting the stylet between passes, and it should be kept for controlled expression of aspirates onto the slides.

Number of Passes

The aim of performing FNA is to obtain a diagnostic specimen using the fewest number of passes possible. An on-site cytopathology team, to assess specimen adequacy, establish an on-site diagnosis, and advise on whether additional sampling is needed, improves the yield and decreases the number of passes required [36]. In the absence of an on-site cytopathologist, adequate passes should be performed to avoid the need for repeat procedures. Studies have shown that with solid pancreatic mass lesions, 7 passes provided a sensitivity and specificity of 83% and 100%, respectively, and 5 passes on lymph nodes yielded sensitivity and specificity of 77% and 100%, respectively [37]. Another study [38] recommended 5–6 passes for pancreatic mass lesions and 2–3 passes for lymph nodes. In a study of 209 consecutive EUS-FNA at multiple locations, 90% of adequate samples were obtained within 6 passes, after which there was only a marginal increment in obtaining adequate samples [39]. When on-site cytopathology support is not available, a

general recommendation would be to perform at least four to five FNA passes for cell block or three FNB passes for histopathological analysis.

SAFETY

The safety profile of EUS-FNA is excellent [40, 41]. The majority of complications are encountered during aspiration of cystic lesions, and prophylactic antibiotics are recommended to reduce the risk of sepsis [40]. Clinically significant intracystic hemorrhage is rare and bleeding usually resolves spontaneously. Low-dose aspirin can be safely continued, but clopidogrel must be stopped 7 days prior to EUS-FNA. Low-molecular weight heparin and unfractionated heparin must be discontinued 12–24 and 6 h prior to FNA, respectively. Warfarin should be withheld 5 days before in low-risk patients, and bridged with heparin in patients at high risk for thrombotic events [42]. A systematic review concluded that there is a low risk of pancreatitis following pancreatic EUS-FNA, at 0.44% [43]. A recent meta-analysis comparing rates of complications between standard 19G and 22G/25G needles for pancreatic EUS-FNA [44] showed no significant difference in rates of pancreatitis, bleeding, infection, perforation, or abdominal pain. Experience with the 19G needle for FNA is limited, and its safety profile will become more defined with increased use.

ENDOSONOGRAPHER EXPERIENCE

There is a learning curve for performing EUS-FNA. While recommendations of the number of supervised EUS-FNAs vary [24, 45–47], competence in linear EUS is essential before commencing FNA. Two learning curve studies have been performed, both in pancreatic

masses. In a study of 57 patients who underwent EUS-FNA by a self-taught endosonographer, the diagnostic sensitivities for malignancy from the first to the last 10 quintiles were 30%, 40%, 70%, 90%, and 80%, respectively [48]. In second study of 300 consecutive patients who underwent EUS-FNA by a trained endosonographer, the median number of passes required to establish a diagnosis decreased significantly with operator experience but without any difference in diagnostic accuracy [49]. This suggests that while the diagnostic accuracy may plateau over time, the procedural expertise continues to improve with experience.

CYTOPATHOLOGY

The success of EUS-FNA is largely dependent upon the experience and technique of the endosonographer, as well as the proficiency

and diagnostic experience of the pathologist (Fig. 1) [50]. The presence of on-site cytopathology increases the diagnostic accuracy of the procedure while decreasing the number of inadequate and suboptimal specimens [51]. The nondiagnostic rate has been reported as low as 1% for FNAs with rapid on-site evaluation (ROSE) as compared with 20% without ROSE [52]. This is particularly important, as specimens obtained by EUS-FNA are often the only histologic proof of malignancy, permitting tissue-based (immunohistochemical, molecular, and genomic) testing for optimal patient management. A recent meta-analysis showed that ROSE was associated with a 10% higher tissue adequacy rates on average, but had no impact on diagnostic accuracy [53].

ROSE is not available at many centers secondary to the considerable time commitment, cost, and inadequate

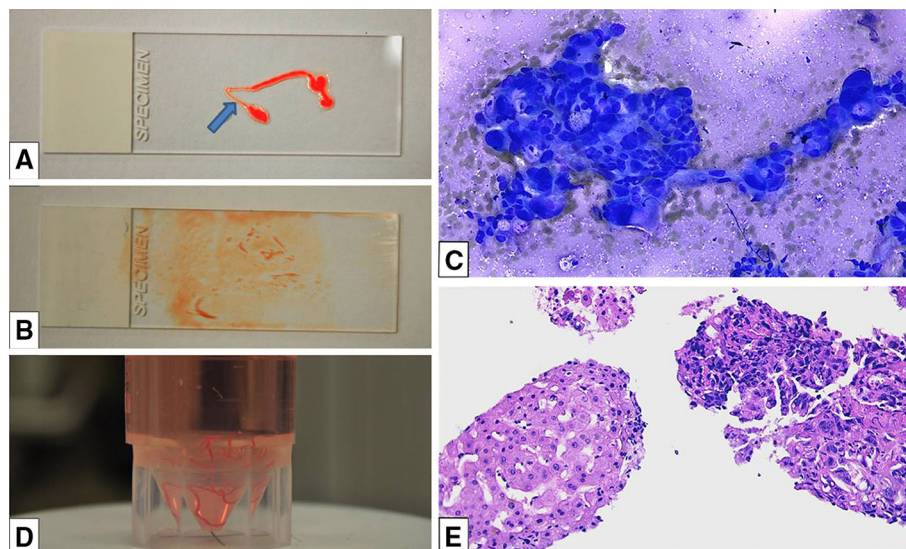


Fig. 1 EUS-FNA cytology preparation and assessment. **a** Core-like material from a 19G needle expressed onto a slide shows a tan-pink (*arrow*) section of tissue distinctly different from the hemorrhagic tissue. **b** A portion of the tan-pink tissue is smeared and the granular tissue fragments are easily discernible. **c** ROSE shows a pleomorphic mucin-

producing carcinoma. **d** Multiple nonhemorrhagic tan-pink cores were collected in CytoLyt[®] (Cytoc Corporation; Boxborough, MA, USA) for cell block. **e** The cell block showed normal liver (*left*) and adjacent cholangiocarcinoma (*right*). Samples such as this are excellent for molecular and genomic studies

reimbursement or compensation by insurance or medicare. This has led to practices relying upon either endoscopy nurses, technologists, and/or endosonographers to independently handle, collect, and evaluate aspirated material. Many practices have attempted to avoid the need of on-site evaluation by performing FNB and/or core needle biopsy. However, regardless of the modality used, the sample obtained will be limited in size and cellular yield. Therefore, the independent endosonographer may benefit from knowing the basic cytopathologic techniques for handling and processing tissue to optimize the specimens sent for off-site evaluation [54].

The most important factor in handling, processing, and evaluating the procured tissue is consistency. Having one dedicated person handling the aspirated material establishes a protocol for preparation and minimizes the artifacts of improper tissue handling and collection. In addition, the level of independence must be defined. Cytopathology assistance can be on-site, at a close proximal location, or remote (telepathology) assistance. Therefore, the independent endosonographer should be aware of the operational structure of their EUS center and thereby the supplies and equipment required for correct specimen handling.

While most endosonographers do not routinely practice in-room basic cytomorphology analysis, a study has shown that microscopic evaluation of smears by independent practitioners (with basic cytomorphology training) without access to on-site assistance significantly improves diagnostic accuracy [55]. Short intensive EUS-cytopathology courses for endosonographers have also been shown to provide effective training in specimen handling and evaluation for diagnostic adequacy [56]. Integrating

focused EUS-cytopathology didactics into advanced endoscopy fellowships has been suggested, knowing that many newly trained graduates join practices without cytopathology support. This is becoming increasingly common as changes in medical reimbursement, such as bundled payments, changes in capitations, and global budgets, are taking place.

Many cytotechnologists and pathologists utilize gross visible assessment as a valuable mechanism to determine if cellular material has been obtained and to reduce procedural time. The slide that macroscopically appears to be cellular is evaluated first under the microscope. This may allow a diagnosis to be reached more quickly, minimize the number of unnecessary passes, and allow for diagnostic material to be diverted to cell block. In addition to gross assessment of smears, experienced personnel who routinely handle the aspirated material become adept in evaluating core biopsy specimens and aspirated material collected in solution for ancillary testing, and may come to recognize bloody, dilute, sclerotic, and mucoid samples based upon the consistency and manner that the material extrudes from the needle. As an example, both sclerotic and necrotic material may appear tan-white in color, but sclerotic stromal tissue is firm, not easily smeared, and as a core may sink to the bottom of the collection tube. Likewise, the experienced eye often can discriminate between red hemorrhagic “core” tissue, and tan-pink material that has a higher diagnostic yield. Gross visual assessment is not equivalent to microscopic evaluation, but with experience personnel can readily predict an inadequate specimen [57]. This can be invaluable, particularly when determining if sufficient material has been collected for cell block.

Cell block is often the best material for ancillary testing. With concurrent advances in

minimally invasive procedures and molecular diagnostics, cell blocks are often required for multiple tissue sections for immunohistochemical and special staining, molecular testing, and research. Cell blocks typically maintain architectural integrity, so can be compared to other tissue specimens in a given patient. There is no standard method for cell block preparation, and multiple methods may exist even within a single institution. The quality and cellular yield of the more commonly used methods of cell block preparation, such as plasma-thrombin, histogel, or Cellient™ (Hologic, Inc.; Bedford, MA, USA) technology can be unsatisfactory [58]. Until the cell block preparation method is optimized, the responsibility of collecting enough material for a diagnostic cell block remains with the endoscopist.

THE FUTURE

Minimally invasive tissue acquisition is a powerful tool, and the role of EUS-FNA will continue to expand and evolve with technical advances in needle and scope design, and integration of cytopathology training and diagnostics. Tissue acquisition relies upon lesion access, which is particularly challenging from the duodenum when an unstable or acutely angulated scope position may be required. This can be improved by addressing the two key limiting factors: needle and scope design. Needles require flexibility to smoothly pass through the scope and into the lesion, and respond to manipulation with the scope tip and elevator to sample in different trajectories through the lesion. Further advances in scope design may include developing a thinner, more flexible scope with increased range of movement at the tip of the scope. Such

changes may also improve the safety of passing the scope through the pharynx and from the first to second part of the duodenum. Integration of a mechanism to provide variable stiffness through the shaft of the scope, analogous to colonoscopes, may also increase the scope stability and functionality. Development of a scope capable of performing both EUS and endoscopic retrograde cholangiopancreatography, to allow combined procedures without exchanging scopes, would be a game changer. Essential to EUS performance is the quality of ultrasound imaging, and further advances include expanding the angle of EUS imaging and increasing image resolution. Finally, the march toward in-room pathology diagnosis continues, and providing high-quality EUS-FNA specimens is the first step. Integration of basic cytopathology training and utilization is the next step, and in the future this may be followed by an increased shift of pathological evaluation from the laboratory into the endoscopy room to allow real-time diagnosis.

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Conflict of interest. Bronte Holt declares that she has no conflict of interest. Shantel

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Compliance with ethics guidelines. The analysis in this article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

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