Review Article

Basic technique in endoscopic ultrasound-guided fine needle aspiration for solid lesions: What needle is the best?

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ABSTRACT

Basic technique for endoscopic ultrasound (EUS) of solid lesions has developed during 30 years of EUS, as endoscopes and accessory equipment, particularly needles, have been developed. Systematic high-quality examinations require understanding and planning. Needles used for EUS-guided fine needle aspiration (FNA) have gone through many improvements; some 18 characteristics of any needle are presented and these come under consideration whenever choosing the best needle for each procedure. The bright future of EUS and FNA for solid lesions currently still leaves much room for continued developments.

Key words: Endoscopic ultrasound, fine needle aspiration, needles, solid lesion

INTRODUCTION

Having had first-hand experience with endoscopic ultrasound (EUS) since 1986, the development of the basic technique has evolved and continues to evolve, mostly along predictable paths. Most if not all most of endoscopy has begun with new diagnostic techniques which, over time, become more and more invasive and useful and then eventually become replaced by less invasive or less dangerous procedures as these become available.

The basic techniques involved in EUS began with the radial instruments in the 1980s. Because these instruments had mechanically moving images, they were and are considered unsafe for guiding fine needle aspiration (FNA) procedures-thus their use was limited to imaging for diagnosis. Many of the pioneers of EUS first learned EUS techniques using radial instruments and some continue to use these instruments as a first-line imaging technique, having some advantages in imaging 360° around the scope.

However, over time, the linear EUS instruments have become much more widely available and have essentially replaced radial instruments. In 2013, there may be some slight use for the radial instruments in very well-stocked EUS units, which continue to maintain their radial echoendoscopes. Due to the high maintenance costs of EUS instruments, relatively very few of the EUS units world-wide utilize the entire gamut of scopes. Miniprobes have essentially replaced the advantages of radial echoendoscopes. Even so, the miniprobe is not found in most of the EUS centers, is of considerable expense and has only very infrequent unique indications, such as advancing past non-dilatable stricturing tumors in the esophagus to do

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EUS scanning through the stomach for staging of an esophageal lesion.

The most cost-efficient approach chosen by most centers world-wide is to purchase only linear EUS echoendoscopes, the versatility of which continues to evolve and improve. To learn basic EUS techniques for solid lesions, the first step is thorough familiarity with the EUS guidelines set forth by the American and European Societies for Gastrointestinal Endoscopy.[1-4]

Basic technique for EUS begins with getting sufficient information to make a well-informed decision that the EUS is indicated and that the patient is fit for the procedure. From Chicago to Israel to China, many or most of the EUS procedures are done through an open-access program in which the first face-to-face contact between the Echoendoscopist and the patient is on the day of the planned procedure. Triage of referral letters, imaging studies and calls for procedures is often performed by EUS trainees, nurses and additional support staff of the EUS unit. In our own center, about 90% of the EUS exams are arranged through open access. Open access has been found to be less than ideal in terms of the satisfaction of the referring physicians.[5] Open access is efficient in terms of saving the patient and the co-payers the time and costs of a pre-EUS visit. However, costs of sometimes partially inappropriate procedures and complications, which might have been avoided, are some partially hidden disadvantages of the open-access system. The focused medical history and physical examination preceding EUS exams are brief. The ideal consent process, both from medical and legal perspectives, is best completed before the day of the procedure, but this is often not practical and benefits are generally weighed against costs. Triage and consent culminate in a medical plan in which the Echoendoscopist understands the solid lesion/s being considered and the implications for echoendoscopy.

The aphorism "actions last, first comes thought" applies to EUS exams. Basic technique begins with considering the various possible targets for FNA, not just the primary solid lesion but the likely areas to which metastases might occur and which will be sought. For example: Esophageal, pulmonary and mediastinal solid lesions may metastasize to the adrenal glands, which would change the therapeutic plan for many patients from a surgical to non-surgical approach. For the common indication of examining patients for suspected pancreatic solid lesions, the EUS study should plan to include a careful examination of the celiac axis and other lymph node areas, including those parts of liver accessible by EUS and the mediastinal nodes — all of which may render diagnostic information critical to proper medico-surgical management of a pancreatic tumor. Interrogating the relations of tumors to local vasculature, as well as noting vascular problems such as thrombi, may have management implications. The option of taking biopsies from such distant but relevant organs or lesions may best be gently discussed in the consenting as well as the planning process before beginning a EUS exam.

General statements about the techniques of EUS for solid lesions precede specific comments regarding specific lesions. Although some favor a site-specific or targeted examination, a systematic EUS exam which surveys all of the organs expected to be evaluated adds a very few minutes of time to the exam and is an expression of professionalism-going beyond the minimum required for the potential benefit. The reasonable counter-argument has been put forth that the complications of EUS might be minimized by avoiding, for example, examining through the duodenum, for patients with more proximal lesions. While the dangers including fatalities of EUS particularly in the duodenum have been documented,[6] doing a partial EUS may also be likened to doing a gastroscopy for heartburn and examining only the esophagus and gastroesophageal junction, without a complete examination of the stomach or duodenum — a completely unacceptable practice. A comprehensive and thoroughly documented EUS exam instead of a narrowly focused targeted exam has been determined to be a higher quality study by both the American Society for Gastrointestinal Endoscopy (ASGE) and European Society of Gastrointestinal Endoscopy (ESGE) and mandatory quality indicator compliance is increasingly required by healthcare systems.

Based on quality guidelines described by the ASGE ESGE and others, a systematic and thorough approach is advised, which carefully balances risks and benefits, with systematic self-assessment and improvements which have been found to be attainable.[7]

The basic technique of EUS exams, as for colonoscopy and gastroscopy, ideally involves first manipulating the echoendoscopy as far into the gastrointestinal tract as maximally planned and then to review the specific organs and finally the target solid lesion/s. After the basic

imaging is completed, planning goes to the sequence of FNAs. If suspected metastatic lesion/s to visceral organs or nodes is to be biopsied, these may be sampled first using the needle which might then be re-used for the primary target lesion. Working in the opposite sequence would cause a risk of seeding of tumor cells into benign lymph nodes, or into areas of the liver which might have only focal sparing of fatty liver infiltration, for example and thus should be avoided.

The risks of EUS-FNA have been described at length in the literature; larger needles seem to have more likelihood of causing more damage. In a recent meta-analysis of 45 studies by Varadarajulu *et al.* concluded: "The choice of needle size for EUS-FNA of pancreatic lesions should be based on the clinical indication and patient or disease-specific characteristics."[8] Specifically, the smaller 25-G needles may penetrate hard lesions with abundant desmoplasia more easily than larger needles.

Before performing FNA, the solid lesion to be biopsied should be examined from whatever different approaches are possible. For example-pancreatic lesions are often visible through the stomach and also through the duodenum. Although seeding of tumors into the stomach wall has rarely been a problem, transduodenal biopsy when feasible may avert gastric seeding. The section of duodenum through which a needle passes will likely be excised if the patient has operable disease. The different perspectives/views of the same solid lesion should be considered so as to allow ideal targeting of the lesion for FNA.[9] In 2013 the Wallace *et al.* group, concluded that, among 256 pancreatic cancer patients, EUS-FNA was deemed safe and not associated with increased risk of needle-track seeding.[10]

Regarding seeding of gastrointestinal stromal tumors (GISTs), despite the textbook comments that EUS-FNA may cause dissemination of submucosal lesions such as a GIST - this author was unable to find any evidence of this theoretical risk neither in practice nor via literature review on Medline despite the many thousands of such biopsies which have been performed. Trajectories that include going through blood vessels are best avoided, so as to minimize the likelihood of bleeding. Despite the distant medical past of intra-aortic injections and of intracardiac punctures, such events are generally left to experts and not for the faint-hearted. The different views of a solid lesion also provide opportunities to assess the stage of the lesion. Sometimes penetration of a lesion into adjacent organs or structures, such as of a pancreatic tumor invading the portal vein, or encasing an artery, will be an image which has great impact on further medical/surgical/onocological care.

While each Echoendoscopist may have pre-settings which allow for viewing which is most comfortable and versatile, the consoles of the ultrasounds afford an array of options for getting optimal views and maximal information. Altering the frequency, the depth and the distance at which there is greatest focus, may all enhance standard imaging. For larger lesions, the depth of penetration applications may be used to image as much of the lesion as possible, not just that which is closest to the probe, to obtain an effective measurement of the maximal size and distal consistency of the solid lesion.

Doppler imaging generally is used as part of basic technique before FNA, not only to assess for the presence or absence of vessels along the planned trajectory of the needle, but also to assess for the vascularity of the targeted solid lesion. This is important as sampling of vascular areas may lead to more bleeding and less useful samples being obtained. Many sampled lesions may have areas within them which are less solid; including necrotic areas or cystic areas may yield less useful cellularity.

The stance of the Echoendoscopist can be mentioned as being one involving personal preference. Facing the patient and external rotation of the left arm holding the scope is one preferred stance, which allows facing the patient directly. The alternative stance, facing away from the patient, allows for a straighter scope, less curving within the patient, more direct transmission of the torque of the scope to the tip position. However, this stance involves not seeing the patient during most of the proceedings. The degree to which the Echoendoscopist may wish to see the patient may depend on whether an anesthesiologist is present or whether, as is the case in many centers, the Endoscopist is also the person responsible for safe delivery of the sedation and monitoring of the vital signs of the patient, tasks which may be more continuously achieved if the Endoscopist faces the patient. For those who have an anesthesiologist, nurse, or MD present and delivering the sedation, the facing-away stance is facilitated.

The balloon and or water instillation may be used to improve acoustic coupling of the echoendoscope to

the solid lesion. With experience, the balloon often is superfluous and experienced endosonographers use the balloon infrequently, which is not to be understood that use of the balloon would be a sign of inexperience. One may use deaerated water to fill parts of the esophagus or stomach or rectum to improve acoustic coupling and to afford a clear image, reducing the need for pressing the scope against the lesion. The water immersion technique has as a drawback that air may enter the water causing it to be bubbly during the instillation process and also the water tends to move out of the area of interest quickly, spreading throughout the gastrointestinal tract. Thus, while the technique was used more frequently in the past it is infrequently seen in use today. Endoscopist-controlled button-initiated water filling of the balloon has made this faster and yet it is only infrequently needed. Some of the echoendoscopes have been manufactured without balloons.

SPECIFIC COMMENTS REGARDING SEVERAL OF THE SOLID LESIONS COMMONLY REFERRED FOR EUS

For rectal lesions our practice is to prepare a patient by fasting overnight and patient undergoing two separate sodium phosphate ("Fleet") enemas given at least 30 min apart. Special attention is given to allergies to antibiotics and an antibiotic such as one gram of intravenous cefazolin is given at the time of FNA and patients are given metronidazole and ciprofloxacin for 3 days after FNA of rectal lesions, as is the local practice for biopsies of the prostate. Strong evidence-based guidelines for antibiotic prophylaxis for patient undergoing transrectal FNA are lacking. Overall, the recommendations for antibiotic prophlaxis for EUS-FNA and for endoscopy appear in various well-established guidelines and are part and parcel of basic techniques for EUS of solid and cystic lesions.[3]

Esophageal solid lesions are best examined in their entirety, which sometimes necessitates pre-EUS dilatation of the strictured esophagus. An alternative approach is to use miniprobes, which pass the stricture and image the tumor and can assess depth of tumor invasion, but which do not afford the possibility of FNA distal to the tumor. Esophageal solid lesions commonly arise in the muscle layer and represent leiomyomas or GISTs. EUS often provides staging information for such lesions, including depth of penetration.

Mediastinal solid lesions may be easily accessible to EUS and FNA. Few centers have added endobronchial ultrasound (EBUS) to the EUS services, or in collaboration with the EUS service. Both EUS and EBUS are necessary to thoroughly assess all of the mediastinal nodal zones. Notably, because the endoscope is in a straight or neutral position, esophageal and mediastinal lesions are among the easier solid lesions to biopsy with FNA and these lesions are often the first on which trainees learn to do FNA. While most of the endosonographers using basic technique can identify and aspirate cells from the mediastinum, it is important to acquire at least rudimentary understanding of the anatomy of this region so as to perform thorough and useful examinations.

Gastric wall solid lesions are a common indication for EUS and FNA. Staging of gastric tumors is increasingly important as neoadjuvant therapy becomes established as effective. Position of the intra-gastric lesions may be important, as patient position may be changed to facilitate placement of the probe on the lesion.

Pancreatic solid lesions, a dominant indication in many EUS centers, require various basic techniques to be applied. The uncinate process is best imaged through the second part of the duodenum; the pancreatic head may also be best imaged through the duodenal view. However, the head body and tail regions are also all accessible in most cases through the gastric wall, at about 50 cm from the teeth. The strait scope in the stomach allows for relatively easy imaging of the pancreas, by rotating the scope tip controls.

The size of a lesion which is minimal to justify FNA varies based on several parameters, the key issue being importance to the patient. Biopsy of pancreatic lesions with FNA is often hindered when in the intra-gastric position, as the splenic vessels tend to be between the pancreas and the stomach. Many small (<2 cm) lesions are identified using EUS, which have not been identified on previous computed tomography scans.

EUS imaging and sampling of solid nodes and other solid lesions alongside the digestive tract are common goals for EUS exams. The basic technique for nodes has been the subject of lively discussion and proponents have alternately suggested reducing or increase of suction when sampling nodes to reduce bloodiness of samples and to increase cellularity. In

studying EUS attempts to focus on the center versus the periphery of nodes, neither site has been found to be more accurate.[11]

FNA

The next issue involves techniques of FNA. Somewhat tongue-in-cheek, some physicians have quipped that the main purpose of all endoscopy is to collect material for cyto-histo-pathological evaluation. Endosonographers increasingly agree that "tissue is the issue," although some of the old-school endosonographers of the radial scope era continue to base decisions on imaging even when FNA was feasible. The belief that negative FNA results might lead to delay in proper treatment, rendering FNAs as undesirable, competes with the potent desire to avoid unnecessary surgery for cases of lesions which are not dangerous to our patients. The relative acceptability of a major operation such as the Whipple operation resulting a diagnosis of pancreatitis (chronic, autoimmune or other) is a cultural and medicolegal issue. Patients along with their families and respective surgeons want to be confident that the preoperative diagnoses rendered by Echoendoscopists of potentially operable cancer do not turn out to be an over-reading of imaging findings. One recent study of 985 patients with pancreatic masses found that pre-operative EUS-FNA leads to "significantly fewer benign lesions resected" compared with in the surgery-only without EUS group ($P = 0.024$).[12] Overall, there is less than perfect agreement amongst the relevant specialists treating patients with solid lesions considering EUS-FNA, i.e., among surgeons, oncologists and gastroenterologists, as to the optimal uses of EUS-FNA.[13]

Rather than continuing the debate as whether one prefers to miss optimal timing of surgery versus to undergo unnecessary surgeries, it may be best in a review for echoendoscopists to focus on the need for more accurate FNA. Vitally important, is that more futile operations may be obviated by increasing the diagnostic accuracy of EUS-FNA.

The issue of targeting of different areas within solid lesions in order to get maximal accuracy is an issue of great interest for Echoendoscopists. The imaging of a lesion is clearly not sufficient to establish, which part of the lesion is most likely to reveal the worst or most advanced lesion. The imaging of a solid lesion or node is often insufficient to determine if a lesion is neoplastic. Elastography is one technique which attempts to determine the relative "hardness" of a lesion, using ultrasound technology to relate to the firmness usually found in tumors with desmoplasia, a sign of neoplasia. Early, basic, qualitative elastography has not made the grade for Food and Drug Administration (FDA) approval, but is widely available world-wide. Our own experience has not found the elastography to be very helpful in determining which site within a tumor is likely to be cancerous.

Sonovue is another non-FDA approved technique for increasing diagnostic certainty, using microbubbles to add contrast enhancement to differentiate between types of solid lesions. Thus, imaging provides some limited information about the nature and internal consistency of solid lesions and future developments are sought to locate ideal sites for FNA. However, the ability to control a needle and to pinpoint the areas biopsied from within a lesion is also limited. Variations of needles have been used to attempt to address this limitation.

A variety of needles have been used during the past 25 years, with innovations continuing to appear, some but not all of which become accepted as being improvements. The ergonomy of the needles has been improving, as have the flexibility of outer sheaths [see Figure 1a-e]. One notable technique which did not go far was the tru-cut technique for large-bore 19-G needles. These needles took a relatively large sample, which were not eventually proven to be more accurate than the smaller bore needles with similar accuracy. Among the traits determining which needle is best, safety looms large, convenience cost versatility all play roles and accuracy of samples is a benchmark outcome which probably all centers should monitor.[14] Table 1 below lists 16 key features for an ideal needle. The

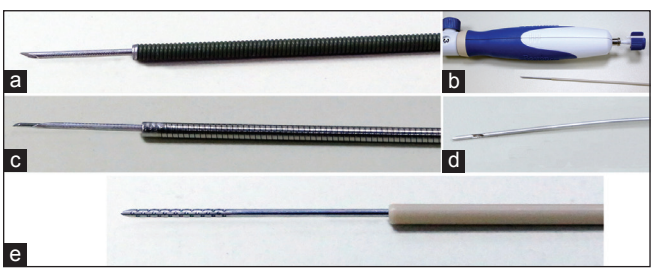

Figure 1. Recent needles from four prominent companies making the needles. (a) Wilson-Cook EUSN3 with flexible outer sheath; (b) Boston expect needle emphasizing large ergonomic handle; (c) Mediglobe 22-G needle; (d) Wilson-Cook procore with side-hole for core sampling; (e) Olympus 22-G needle

Table 1. Eighteen attributes for choosing the best needle

Attributes	Explanation
Safe	Have a proven record of comparably high safety compared to other needles
Accuracy	Have excellent results in terms mostly of minimal false-negatives
Visibility	The echo-enhancing dimpling on the needle had best be to its tip
Targettable	Needle should be manageable when deployed
Flexible	Should pass through a bent endoscope without tearing the biopsy channel
Connectors	The plastic or metal connections should not break when used
Durable	The needle should be sufficiently elastic to be usable for several passes
Available	Well-stocked distributor so that endoscopic ultrasound center not need to warehouse
Ergonomic	User-friendly handles help
Bevelled stylets	With a stylet, the tip should be sharp enough and do least damage
Suctioning	The suction apparatus is ideally manageable by the Endoscopist alone, it ideally affords flexible levels of suction according to the specific situation
Adjustable length	Convenient length adjustments to fit the various scopes
Cost	The current costs of needles are high, a consideration in much of the world
Disposability	Needles should be easily safely disposable
Shelf-life	Long shelf-life approved by regulators is an advantage
Re-sterilizable	If a package is opened but not used, resterilizability is advantageous
Nitinol	An alloy with metallic memory has recently been introduced so that needles will return to their original shape after being bent inside of a scope and inside of a tumor. Nitinol may increasingly replace stainless steel, despite its higher cost, if the needles prove more flexible, durable and re-usable
Special-purpose needles	Tru-cut needles, celiac plexus neurolysis and other injection-intentioned needles with side-holes and/or closed tips, and side-holed needles for collecting cores each present advantages and disadvantages. For example- the surgical adage preferring cutting not tearing to minimize bleeding is a consideration when using side-holed needles which may tear or shear off samples

problem that EUS needles tend to return to a tract and thus the same part of a solid lesion gets to be sampled again and again, has been addressed in several ways. The fanning maneuver of using the elevator to try to deploy the needle along different paths inside of a lesion is often but not always successful. Not uncommonly, solid tumors may have necrotic parts which yield acellular samples unsuitable for making diagnoses. Thus achieving multiple targeting within solid lesions remains a challenge which future needles need to address. Even acellular specimens can be of value when staining for mucin or when there is sufficient fluid to test for carcinoembryonic antigen and other markers. The history of EUS needles is dynamic, with new and usually better needles coming out nearly annually over the past 20 years.

Needle withdrawal technique has various attributes

The techniques involved in suction have various options. Proponents of no-suction, especially for lymph nodes, have claimed that a less-bloody sample is obtained and that this leads to a higher diagnostic yield. Minimal-suction proponents have suggested that the stylet may be partly withdrawn and thus used for creating minimal negative pressure and a less bloody specimen than when using standard suction. The various needles come with single- or multiple-levels of fixed suction, from 5cc to 20cc of suction on a syringe are applied. Multiple passes may be done with each pass utilizing a different technique, as the ideal technique of suctioning is not well-established. The trade-off perhaps involves obtaining dry, empty and/or inadequate specimens while not using suction, versus getting bloody specimens which prevent accurate readings, when excessive suction is applied.

Nakai *et al.* concluded that slow pull may provide less bloody specimens without reducing cellularity in EUS-FNA for pancreatic malignant lesions.[15]

A four-center study on 74 patients concluded that applying 20 mL aspiration volume is much better in terms of diagnostic accuracy than the conventional 10 mL applying 20 mL aspiration volume was much better in terms of diagnostic accuracy than the conventional 10 mL.[16]

Another measure which might improve the yield of EUS-FNA is the reduction of residual negative pressure in the needle. A study by Aadam *et al.* found significantly less contamination when the syringe was removed rather than merely the suction process stopped by the stopcock.[17]

Regarding use of the stylet, the need for a stylet has been reviewed, in which Sahai is a leading proponent

of doing EUS without a stylet.[18] In one survey at a Chicago EUS for experts seminar, 75% of echoendoscopists reported using the stylet when putting in the needle and 25% have taken to removing it.[19] No study which clearly examined the head-to-head benefits to risks of rounded/ball-tip stylets to beveled stylets for EUS-FNA has appeared.

Histology versus cytology

The processing of FNA samples depends to a great degree on the preference of the cytology and/or pathology staff. A wide variety of regimens are practiced, with ample evidence that rapid on-site evaluation (ROSE) of cytology allows for less passes to be performed with an increased level of confidence of having a definitive diagnosis, but with considerable evidence also that equal levels of accuracy can and are achieved in various centers without using ROSE.[20] Fewer passes hopefully leads to causing fewer adverse side-effects, potentially fewer instances of bleeding, infections, pancreatitis, etc.

Some cytologists prefer that the entire sample from the needle be expressed into fixative solutions, the cells then being spun into cell blocks, fixed as for histology specimens,[21] as is done in our own center as well. An advantage to this method is that no cells are lost, such as at the far periphery of a slide prepared during smear preparation of a specimen. Because the cell block method is used, the terminology of cytology versus histology is often unclear and the benefits of a "core" sample are indefinite. A disadvantage of using the cell block method is that centrifugation, fixation and slicing and then mounting from a block is a more time and labor intensive process than compared to viewing slides that are smeared and viewed in a few minutes. When comparing slides from FNA to histopathology slides, the difference is often impossible to make, whether a specimen has been taken by FNA aspiration or from use of a biopsy forceps. The terminology of FNA versus fine needle biopsy (FNB) for FNB is thus a matter left often for discussion. Singh *et al.* in their study have reported that their results in 40 patients showed no significant difference in the rates of diagnostic yield between FNA and FNB needles. They concluded that diagnostic sufficiency of FNA and FNB needles are comparable.[22] While this study and others reported no increased rate of complications from the core needles, the samples thus far have been small and the technique of shearing core-size samples raises the potential concern from increased rates of complications from the needle, as well as increased cost.

Some of the latest literature from the 2013 Digestive Disease Week abstract attempted to compare samples from needles purporting to obtain more core samples versus needles from within the same company that obtain aspiration samples.[23-26] No rigorous head-to-head studies have appeared comparing needles from different companies. One interesting recent report comparing pathologists' point of view reviewed 80 samples. The study concluded that overall histological quality of samples obtained with the 22-G ET (echotip) is better compared with the 22-G P (procore).[27]

Among the more important aspects of having a larger and cell-clock sample is the implication that adequate material is needed for advanced analyses including molecular and immunohistochemical testing. This will no doubt be increasingly applied to augment the accuracy of FNA.[28-34] The future surely points increasingly to personalized medicine and advanced research techniques to maximize the benefits which can come from EUS-FNA.

WHICH NEEDLE IS BEST?

In summary, a core sample may be obtained with any needle, from the smallest which are currently 25-G, to the largest (19-G); and in any case, the samples may be viewed on slides, as cytology and/or from a cell block and the decision as to the optimal fixation and viewing method depends on local logistics, availability and preferences of local pathologists. At our center, different brands of needles were purposely made available so that each of the experienced echoendoscopists could try each of the different brands. As different eyes report varying levels of visibility, different hands prefer different flexibility and different ergonomic handles.

The best needle is the one which works best at meeting that mix of advantages preferred by the Echoendoscopist who sets the goals for the procedure. Future needles will hopefully improve on all of the many quality parameters set forth in the table of needle attributes seen herein, allowing for ever safer, more accurate, less expensive and higher quality EUS-FNA for our patients.

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