Endoscopic Ultrasound-Guided Fine-Needle Aspiration of Pancreatic Lesions: A Systematic Review of Technical and Procedural Variables

Bhairvi S. Jani, Fadi Rzouq, Shreyas Saligram, Diego Lim, Amit Rastogi, John Bonino, Mojtaba Olyaee

Department of Internal Medicine, Division of Gastroenterology, University of Kansas Medical Center, Kansas City, Kansas, USA

Abstract

Endoscopic ultrasound (EUS)-guided tissue acquisition has emerged over the last decade as an invaluable diagnostic tool in approaching the different pancreatic lesions. Given the safety and minimal invasiveness of this approach combined with the high diagnostic yield, it became the standard of care when dealing with different pancreatic pathologies. However, some variables regarding this procedure remain not fully understood. These can influence the diagnostic yield of the procedure and include the presence of the on-site cytopathologist, the type and size of the needle used as well as obtaining aspiration versus core biopsy, the number of passes and the sampling technique, and the role of suction and stylet use among others. We performed a comprehensive literature search using PubMed, Google Scholar, and Embase for studies that assessed these variables. Eligible studies were analyzed using several parameters such as technique and procedure, with the aim of reviewing results from an evidence-based standpoint.

Keywords: Cytopathology, endoscopic ultrasound (EUS), fine-needle aspiration (FNA), fine-needle biopsy (FNB), pancreatic lesions

Address for correspondence: Dr. Mojtaba Olyaee, Department of Internal Medicine, Division of Gastroenterology, University of Kansas Medical Center, Kansas City - 66160, Kansas, USA. E-mail: Molyaee@kumc.edu

Introduction

Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) has become an indispensable tool for acquiring pancreatic lesion tissue. Since the first studies of EUS-FNA in the early 1990s, the relative safety and accuracy of EUS-FNA have led to most academic and community centers replacing percutaneous FNA.[1,2] The high spatial resolution of EUS compares favorably with traditional cross-sectional imaging modalities such as magnetic resonance imaging and computed tomography scanning, particularly for lesions measuring <2 cm. EUS provides detailed information regarding vascular involvement, and allows for simultaneous staging.[3] The resulting cytopathological diagnoses may have a major impact on therapeutic decision and on the prognosis of patients.[4]

The use of EUS-FNA in clinical practice has been steadily increasing. Roy *et al*. analyzed a 5-year trend (2006-2010) in tissue acquisition in pancreatic diseases in the United States using the Medicare database.[5] The use of EUS-FNA increased by 69.3%, surgical biopsy declined by 41.7%, and the use of percutaneous biopsy remained stable.

A meta-analysis of 33 studies between 1997 and 2009 recently showed that EUS-FNA has a pooled sensitivity for malignant cytology of 85-91%, specificity of 94-98%, positive predictive value of 98-99%, and negative predictive value of 65-72%.[6,7] False-negative results for malignancy may occur in up to 20-40% of the cases.[8,9] In another recent meta-analysis, the accuracy of EUS-FNA in diagnosing solid pancreatic masses was analyzed. Pooled sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were 86.8% [95% confidence interval (CI), 85.5-87.9], 95.8% (95% CI, 94.6-96.7), 15.2 (95% CI, 8.5-27.3), and 0.17 (95% CI, 0.13-0.21), respectively. Repeat EUS-FNA is recommended as the second-line test in patients in whom there is a strong clinical suspicion of malignancy but a nondiagnostic finding on initial examination.[6,10-12]

In previous studies, the accuracy of EUS in obtaining pancreatic and lymph node biopsy specimens has varied from 71% to 98%.[13-17] More recently, improvements have been made in the technique and equipment that serve to optimize specimen positivity. The diagnostic yield of EUS-FNA-guided biopsies depends on the location, size, and type of target lesion, in addition to procedural and technical factors such as type of needle, biopsy technique, experience of the endosonographer, and presence of on-site cytopathologist.[18-25]

There are several variables that can influence the diagnostic yield and sensitivity of EUS-FNA-guided biopsies of pancreatic lesions. We performed a comprehensive literature search using PubMed, Google Scholar, and Embase for studies that assessed these variables. Eligible studies were analyzed using several parameters such as technique and procedure. The aim of our study is to discuss these variables from an evidence-based point of view as detailed below.

Presence of on-Site Cytopathologist

The rapid on-site evaluation (ROSE) process involves the evaluation of the direct smears obtained at the point of care in the endoscopy suite.[26] This is usually done by a cytopathologist using a light microscope with immediate feedback given to the endosonographer about the diagnostic quality of the aspirates, and additional EUS-FNA passes may be required or a modification in the method of FNA such as altering use of suction or changing the needle size. This information will simply not be available if the EUS-FNA is performed without ROSE. More than a decade ago, Klapman *et al.*[27] compared the outcomes of EUS-FNA with and without ROSE in procedures performed by the same endoscopist. Patients treated with ROSE had their cytology positive or negative for malignancy more frequently than those who underwent EUS-FNA without ROSE (*P* = 0.001)

and were less likely to have an unsatisfactory specimen (*P* = 0.04) or undergo a repeat procedure (*P* = 0.16). Since then, numerous studies have confirmed the superiority of ROSE in terms of increasing the diagnostic yield by limiting the number of passes and decreasing the number of inadequate samples.[24,27-29]

Collins *et al.*,[30] over a consecutive 3-year period analyzed 379 patients who underwent ROSE and 377 patients who did not. The percentage of repeat procedures on the non-ROSE group was 5.8%, which was slightly higher than in the ROSE group (2.9%). The use of ROSE decreased the number of repeated procedures by approximately 50% (*P* = 0.024). In patients requiring an additional procedure, the use of ROSE provided a higher number of definitive diagnoses.

The diagnostic yield of cytology obtained by EUS-FNA with ROSE in most studies exceeds 90%.[31] In a meta-analysis involving 34 studies evaluating EUS-FNA, the pooled sensitivity and specificity for EUS-FNA of pancreatic ductal adenocarcinoma were 88.6% and 99.3%, respectively[32] A meta-regression model demonstrated that ROSE remained a significant determinant of the accuracy of EUS-FNA (*P* = 0.001) after correcting the study population number and reference standard. In a second, more recent meta-analysis, ROSE was associated with a 3.5% improvement in adequacy rates for EUS-FNA of solid pancreatic lesions.[33] ROSE assessor type was found to have no impact on adequacy rates but it was found to be an effective modifier on the relationship between needle passes and per-case adequacy for EUS-FNA of solid pancreatic lesions.

ROSE is most frequently used in the United States but its popularity is growing in Europe too. The European Society of Gastrointestinal Endoscopy (ESGE) technical guidelines state that ROSE provides a highly reliable diagnosis with an excellent agreement with the final cytopathological diagnosis.[31] Although there is growing evidence to support the efficacy of ROSE, there have been some concerns about the cost-effectiveness of implementing an on-site cytopathologist, especially at centers with financial constraints or limited resources. Wani *et al.*[34] performed a study in which two strategies for EUS and ROSE were evaluated. In the first, an on-site cytotechnologist performed ROSE to assess adequacy of sampling. In the second strategy, multiple passes were performed by the endosonographers until it was decided, in their opinion that an adequate sample been obtained and ROSE was not carried out. The factors assessed included diagnostic yield with offsite cytopathology, incremental diagnostic yield with ROSE, incremental adequacy of sampling with ROSE, cytotechnician salary, and incremental diagnostic yield of a second EUS-FNA

after an initial nondiagnostic procedure. The results indicated that the overall cost per patient was less with ROSE (US$2061) compared with when ROSE was not used (US$2,228).

Alternative strategies have been suggested for ROSE although most have failed to show any benefit. Gross inspection and ROSE performed by an endosonographer was shown to be worse (70%) compared to ROSE by a cytotechnician (89%) in the diagnosis of malignancy (*P* = 0.001).[35] However, Hikichi T *et al.*[36] found no difference in sample adequacy, number of needle passes, or EUS-guided FNA performance characteristics in two different 2-year periods. In one period, ROSE was performed by endosonographers and in the other by cytopathologists. Additionally, Hayashi *et al.*[37] examined whether diagnostic accuracy increased through ROSE by endosonographer training using predefined cytological criteria such as identification of anisonucleosis, nuclear membrane irregularity, overlapping, and enlargement. The rate of inconclusive diagnoses, interpreted as "suspicious," "atypical," and "inadequate for diagnosis" was reduced from 26.4% to 8.2% (*P* = 0.004). Moreover, diagnostic accuracy was increased from 69.2% to 91.8% (*P* < 0.001).

The need for an onsite cytopathologist may be obviated if reliable core tissue can be procured for histologic assessment.[38] Currently, none of the specially designed biopsy needles or a 19G needle can guarantee reliable histologic core tissue procurement or demonstrate a diagnostic accuracy of greater than 95%[28,29,39] although some studies have shown a benefit. In a recent study by Krishnan *et al.*,[40] the utility of ROSE in achieving EUS-guided core biopsy specimens was evaluated. Sixty consecutive patients referred for EUS-fine-needle biopsy (FNB) were evaluated to determine the additive value of ROSE on diagnostic accuracy. On-site specimen adequacy and final diagnostic accuracy were 58% (95% CI: 45.1-71.2%) and 83% (95% CI: 71.9-91.5%), respectively. The results obtained were found to be superior to those of standard EUS-guided FNA. In his study comparing EUS-FNB alone with EUS-FNA with ROSE followed by EUS-FNB, Keswani *et al.*[41] showed that in nonpancreatic adenocarcinoma lesions, EUS-FNB without cytology provided a high diagnostic accuracy with no additional benefit of prior EUS-FNA. The overall diagnostic accuracy was similar between the EUS-FNB and EUS-FNA/B groups (83.7% vs 84.9%; *P* = 1.0).

To summarize, from the data reported in the literature, ROSE may decrease the number of needle passes and increase the diagnostic yield of EUS-FNA specimens by 10-30% and thus, diagnostic accuracy. EUS-guided FNA combined with ROSE is able to diagnose pancreatic lesions with a high sensitivity and specificity by obtaining cytological and/or histological samples. Using core biopsy needles may increase specimen adequacy and diagnosis although more studies are needed to explore this. Alternatives to ROSE have failed to show any significant benefit in terms of diagnosis and sample adequacy.

Needle Type and Size

Three commonly used sizes of EUS-FNA needles are commercially available — 19G, 22G, and 25G. The most commonly used needle is a 22G needle, which is flexible and enables cytologic assessment without a significant risk for complications.[42] Nevertheless, it has been suggested that the 25G needle may actually pose a benefit when performing FNA of the pancreatic head or uncinate process, due to its flexibility and thus facilitate ease of use when compared to a higher gauge needle.[43] For uncinate process lesions, the use of a 22G needle has been reported to be unsuccessful in up to 33% of the cases.[18] The diagnostic accuracy for pancreatic masses when using the 22G needles is up to 95%[13] and as low as 68%[44] for subepithelial lesions. The overall rates of diagnostic adequacy for sampling pancreatic masses for cytology using the 22G needle are variable compared with histology (82-93% vs 84-87%).[45] The overall diagnostic accuracy for histology on each pass is only 60% for the 25G needle and 75% for the 22G needle.

Gimeno-Garcia *et al.* performed a randomized controlled trial comparing stylet-free EUS-guided FNA with 22G and 25G needles.[46] There were no significant differences between the 22G and the 25G FNA needles in sample adequacy, bloodiness, ease of puncture, FNA failure, visibility, number of passes, and complications; and no significant differences between the needles were found in relation to lesion site. Similar outcomes were found in a Danish study.[47] A recent randomized controlled trial by Ramesh J *et al.* comparing flexible 19G and 25G needles for EUS-FNA of solid pancreatic masses also found that there was no significant difference in the performance of flexible 19G and 25G needles in terms of technical failure (0% vs 2%, *P* = 0.99) or adverse events (2% vs 0%, *P* = 0.99) between the two cohorts.[48] While most of these studies have failed to show any superiority of one needle over another, a meta-analysis of EUS-FNA of solid pancreatic lesions noted that the 25G needle was associated with a higher sensitivity (*P* = 0.0003) but comparable specificity (*P* = 0.97) to the 22G needle.[49] Affolter *et al.* also noted in their meta-analysis that in the evaluation of pancreatic and peripancreatic lesions by EUS-FNA, 25G needles may confer an advantage in adequacy relative to 22G needles but confer no advantages with respect to accuracy, number of passes, or complications.[50]

It remains to be said that Bang J *et al.* have proposed an algorithm to choose needles for aspiration: 25G needles for transduodenal FNA, 22G or 25G needles for other FNAs, 19G flexible needles for transduodenal interventions, and standard 19G needles for interventions for other routes.[51]

While the 22G needle is the most commonly used needle size, no single needle size has shown superiority over another in recent studies. 25G needles may have a slightly greater sensitivity and adequacy than 22G needles but confer no advantages with respect to accuracy, number of passes, or complications. Needle selection is a complex process and will ultimately depend on lesion morphology, location, and presence of an on-site cytopathologist.

Endoscopic Ultrasound Core Biopsy

In recent years, many studies have focused on the use of the EUS-core biopsy needle to obtain more tissue. These needles allow a larger amount of tissue and better preservation of cell architecture than an aspirate. Possible indications for the use of a FNB include failure of FNA with a 22G or 25G needle, suspicion of metastatic tumors requiring special studies for identification, and diagnosis of neuroendocrine tumors, lymphoma, or autoimmune pancreatitis.[6] Therefore, in theory, a FNB specimen should have greater diagnostic accuracy and provides more tissue for ancillary testing than a typical FNA sample. In this setting, FNB may offset the limitations of FNA wherein the diagnostic sensitivity is reliant on the availability of an onsite cytopathologist.[26] Without a cytopathologist in endoscopic room, combining EUS-FNA cytology and histology significantly increases the sensitivity for malignancy diagnosis compared with cytology or histology alone (89.9% vs 68.1% for cytology *P* = 0.007 and 60% for histology *P* < 0.001).[19]

Conventionally, obtaining such a core histopathological specimen required the use of a large 19G FNA needle or a "tru-cut" spring-loaded biopsy needle (e.g., Quick Core, Cook Medical). However, these have had a high rate of failure due to needle stiffness and scope angulation.[52] Tru-cut biopsy has not been shown to be superior to FNA with respect to overall diagnostic accuracy except perhaps in certain situations such as autoimmune pancreatitis and lymphoma.[6,53,54] A new nitinol-based 19G flexible needle (Flex 19; Boston Scientific, Natick, MA, USA) and the latest 19G histologic needles (ProCore, Wilson-Cook) have largely replaced the 19G tru-cut needle for performing core biopsies.

There are a number of studies evaluating the use of 19G, 22G, and more recently 25G fine-biopsy needles with a reverse bevel design (EchoTip ProCore, Cook Medical).

An early multicenter study noted that the ProCore 19G needle was technically feasible in 45 (95.7%) of 47 pancreatic tumor cases, with a diagnostic accuracy rate of 89.4%.[55] Technical failures occurred in only 2 out of 35 cases from the duodenum. Following the release of the 22G core biopsy needle, Beroza *et al.* compared its use with a standard 25G FNA needle in sampling the same pancreatic lesion during EUS.[56] There are no significant differences in the diagnostic yield between EUS-guided 22G core biopsy and the standard 25G FNA for diagnosing pancreatic lesions but core biopsy required a fewer number of passes. There was a nonsignificant incremental diagnostic yield when using both needles during the same procedure.

Another study showed no significant difference in the yield or quality of the histologic core between 22G FNA and 22G biopsy needles for EUS-guided sampling of solid pancreatic masses.[39] Diagnostic sufficiency, technical performance, and safety profiles of both types of needles were comparable. Similarly, Hucl *et al.* noted that there was no statistically significant difference between a 22G core needle and a standard 22G FNA needle in terms of sensitivity, specificity, and positive and negative predictive values although the core needle required fewer passes to provide an adequate sample, offering a potentially shorter procedure time.[57] A retrospective study by Iwashita *et al.* evaluating the diagnostic yield of EUS-FNB of pancreatic lesions using a 25G core biopsy needle showed a high cytological yield (cumulative sensitivity of 83%, 91%, and 96% when cytologic analysis was performed on passes 1, 2, and 3, respectively), similar to 25G FNA needles while also providing some histologic core tissue.[58]

At present, therefore, most studies have shown no appreciable benefit in using a core biopsy needle over a FNA needle during EUS of pancreatic lesions, with the exception of less needle passes needed to obtain an adequate sample. Sensitivity, specificity, negative and positive predictive values, yield, and safety are comparable to 22G and 25G FNA needles.

Pancreatic Cystic Lesions

Pancreatic cystic neoplasms (PCNs) include a spectrum of pathology, covering intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasms (MCN), and serous cystadenomas. PCNs are often classified into mucinous versus nonmucinous cysts because the appearance of cyst fluid obtained by EUS-FNA. Mucinous cysts are usually malignant or potentially malignant.[59] Peripheral calcification (egg shell calcification) can be seen in 10-25% of MCNs and help to differentiate them from serous cysts.[60] EUS-FNA can provide information

on the viscosity, cytology, chemistry, tumor markers and molecular markers of the cystic lesions.

Endoscopic ultrasonography with FNA has been shown to be superior to CT and MRI in accurately classifying a cyst as neoplastic ($P < 0.0001$).[61] Moreover, the addition of EUS-FNA to abdominal imaging significantly increases the overall accuracy for diagnosis of neoplastic pancreatic cysts. This may be related to the fact that EUS has low invasiveness and high resolution as well as anatomical proximity to the pancreas and upper gastrointestinal tract in comparison to other modalities such as endoscopic retrograde cholangiopancreatography (ERCP).[59]

A recent large multicenter study evaluated the factors influencing the yield of EUS-FNA of pancreatic cystic lesions.[62] It found that on univariate analysis, factors associated with higher cytologic yield included vascular involvement on EUS, presence of solid cystic component, and increased number of needle passes during EUS-FNA. In addition, for pancreatic cysts with a solid component, the diagnostic yield of EUS-FNA increased significantly from 44% with one pass to 78% with more than one pass ($P = 0.016$). However, it must be noted that the decision to use EUS-FNA for PCN is dependent on a number of factors, with a significant one being the size of the PCN. A very small cyst may not provide an adequate sample for analysis. Walsh *et al*. concluded that a minimum size of 1.5 cm was needed for successful analysis of EUS-FNA of PCN.[63]

FNA of pancreatic cystic lesions involves very similar techniques to FNA of solid lesions. For suspected mucinous cysts, a 22G needle is used because of the high viscosity of the fluid. The cyst fluid from serous cystadenomas is thin and easily aspirated and serous cystadenomas and cystic neuroendocrine tumors should be aspirated with a 25G needle in order to minimize the risk of bleeding.[64] Brugge *et al*. recommend that pseudocysts be aspirated with a 22G or 19G needle in order to evacuate the entire lesion.[65] Through-the-needle imaging is an exciting new concept that allows direct visualization within the cyst via a needle probe. Two types of through-the-needle imaging are currently being explored: Cystoscopy and confocal laser endomicroscopy (CLE). Recent studies have been promising although further work is needed to explore these technologies further.[66-68]

Cyst fluid analysis is fraught with limitations but in the appropriate clinical and radiologic context can facilitate diagnosis.[69] The classic study by Brugge *et al*. evaluating various cyst fluid markers in 112 patients had identified CEA as having the highest clinical utility in discriminating mucinous from nonmucinous cystic lesions,[70] although a recent large multicenter study showed that cyst fluid CEA levels have a clinically suboptimal accuracy level in differentiating MCNs from non-MCNs.[71] Amylase below 250 U/L can rule out a pseudocyst with 98% specificity.[72] Typically, amylase levels are higher in IPMN than in MCN although they can be similar as well.[73] Other tumor markers such as CA19-9 and the lipase enzyme have also been evaluated although more studies are needed to confirm their clinical utility. Molecular analysis of aspirated cyst fluid for DNA mutations may help to distinguish MCN from non-MCNs. KRAS mutations favor a mucinous diagnosis, whereas a GNAS mutation favors a diagnosis of IPMNs over MCNs.[74] A recent study of the cyst fluid proteome demonstrated that proteomic profiling of mucin in cyst fluid (obtained by EUS-FNA) was 98% accurate for premalignant and malignant cysts.[75]

EUS-FNA for PCN is relatively safe. In a systematic review, the overall complication rate of EUS-FNA for pancreatic cysts was higher than that of pancreatic solid lesions (2.75% vs 0.82%).[76] Pancreatitis was seen in 1.1%, bleeding in 0.3%, infection in 0.2%, abdominal pain in 0.8% and fever in 0.3% patients. It is to be noted that the incidence of pancreatitis is considerably lower than that of pancreatitis after ERCP with pancreatic duct maneuver.[59] In addition, a concern when considering aspiration of a cystic lesion is the introduction of infection. Multiple aspirations could potentially increase this risk.[60] However, there have been no randomized trials conducted to determine the need for prophylactic antibiotics in the setting of EUS-FNA. The risk of bacteremia after EUS-FNA is low (0-6%) and comparable with that of diagnostic endoscopy.[77] The American Society of Gastrointestinal Endoscopy (ASGE) suggests antibiotics before EUS-FNA of mediastinal cysts and advises against the administration of prophylactic antibiotics before EUS-FNA of pancreatic and peripancreatic cystic lesions.[78] Prophylaxis, when deemed necessary, involves administration of an antibiotic such as a fluoroquinolone administered before the procedure and continued for 3-5 days after the procedure.

EUS-FNA appears to be a safe and effective procedure for the evaluation of PCNs, especially when combined with ROSE. It should be considered in cysts that are at least 1.5 cm in diameter. 22G and 25G needles appear to be the most effective for PCN, with the addition of a 19G needle for pseudocysts. Updated guidelines from the ASGE suggest that antibiotic prophylaxis of PCN before EUS-FNA is not necessary although they may be given in high-risk patients.

Role of Stylet Use

Utilization of a stylet has been a point of debate in the literature. A removable stylet is usually included in

EUS-FNA needle systems. There was an assumption that the use of a stylet during EUS-FNA prevents clogging of the needle lumen by gastrointestinal (GI) wall tissue as the needle traverses this to reach the target lesion, which could limit the ability to aspirate cells from the target lesion.[79] Based on this theoretical belief of improving the quality of specimens obtained, the use of a stylet is routine practice for some endosonographers during EUS-FNA.[80]

Several randomized controlled trials have noted that stylet use increases the bloodiness of the specimen and does not increase the diagnostic yield in FNA.[79,81-83] Air flushing in a controlled fashion has been demonstrated to be superior to reinsertion of the stylet to express EUS-FNA aspirates. The traditional technique of reinserting the stylet to express EUS-FNA aspirates may be required only in cases where the aspirates cannot be expelled due to clotting or drying.[80] In a recent cross-sectional survey of American endosonographers, DiMaio *et al*. found that 91% (*n* = 192) of all practitioners utilized a stylet during initial FNA.[84]

The use of a stylet has, therefore, not been shown to have any clinical benefit during EUS-FNA.

Role of Suction

Similarly, the role of suction varies and there is no consensus on its use. Suction is supposed to improve the diagnostic yield during EUS-FNA by holding the tissue against the cutting edge of the needle as it is moved through the target lesion and drawing up cells. EUS-FNA without suction uses the fine-needle capillary sampling technique to achieve the same result.[80] Lee *et al*.[21] noted in their randomized control trial that the diagnostic yield during EUS-FNA of pancreatic masses with suction was higher than without suction (72.8% vs 58.6%, *P* = 0.001). The EUS-FNA was performed with a 22G or 25G needle with suction being applied with a 10 mL syringe. In another study, EUS-FNA with suction was associated with increased number of pathology slides, higher sensitivity, and negative predictive value (85.7% vs 66.7%, *P* = 0.05) and no difference in the bloodiness of each sample.[85] In a pilot study by Larghi *et al*.,[68,86] continuous high negative pressure mechanical suction (35 mL of a 60 mL syringe) with a 22G needle yielded a tissue core adequate for histologic evaluation in 96% of solid masses.

However, Wallace *et al*. found that the use of suction during EUS-FNA of lymph nodes was associated with excessive bloodiness [odds ratio (OR): 4.7, 95% CI: 1.9-11.2].[87] The European Society of Gastrointestinal Endoscopy (ESGE) technical guidelines recommend the application of continuous suction for EUS-FNA of solid masses but no suction for lymph nodes.[31]

A different suction technique, which uses capillary aspiration by slow withdrawal of the fine-needle stylet, may have higher sensitivity and a better negative predictive power than conventional suction. Chen *et al*. compared the diagnostic yield of EUS-FNA of pancreatic lesions with stylet capillary sampling compared with conventional suction.[88] They found that the quality of the cytology specimen graded by blinded pathologists was abundantly cellular in 55% of capillary sampling specimens compared to only 33% of the conventional syringe suction samples.

Other studies have evaluated the efficacy of the slow-pull technique, mostly in the setting of core-biopsy needles. This involves repetitive to-and-fro movements with simultaneous minimum negative pressure provided by pulling the needle stylet slowly and continuously. One such study showed a higher diagnostic yield using a 25G core biopsy needle and the slow-pull technique.[89] Nakai *et al*. compared slow pull versus suction in EUS-FNA of pancreatic masses and found that the slow-pull technique was associated with less blood contamination and increase in the diagnostic yield, especially when used with a 25G needle.[90] In contrast, Kin *et al*. found no difference between suction and slow pull in EUS-FNA of solid pancreatic lesions using a standard 22G needle.[91]

In summary, suction has not been shown to consistently improve diagnostic yield although it could increase the bloodiness of the tissue sample. The capillary aspiration and slow-pull technique have shown benefit in some studies but not in others.

Sampling Technique

There are a number of techniques used when sampling lesions for biopsy, and no single method is unquestionably superior. However, several of these techniques have recently gained popularity. For large lesions that may be centrally necrotic, targeting the periphery is recommended although this runs the risk of sampling reactive desmoplasia and inflammatory debris.[89]

As opposed to sampling just one region of the lesion, the "multipass" technique involves sampling widely through the lesion many times, before removing the needle from the scope. The needle is moved through the entire diameter of the lesion for 5-10 strokes, and the needle is withdrawn from the lesion and moved to a different region of the lesion. Approximately, five regions per lesion are sampled before processing the sample.[92] In contrast, the fanning technique involves sampling multiple areas within a lesion with each pass. A sufficient number of passes must be performed to provide enough material for analysis, and in the case of failure the procedure needs to be repeated.[11] In a randomized

trial comparing fanning to the conventional "to-and-fro" technique, there was no significant difference in diagnostic accuracy between both the techniques (76% vs 96%) although the fanning technique facilitated a first-pass diagnosis in 85% of the patients compared with less than 60% with the standard technique.[93]

More prospective trials are needed to conclusively evaluate and compare sampling techniques before recommendations can be made.

Experience of Endoscopist

There does exist a steep learning curve in performing satisfactory EUS-FNA of pancreatic lesions. Current ASGE guidelines recommend 25 supervised EUS-FNA for the diagnosis of pancreatic adenocarcinoma. As endoscopists perform more EUS-FNA, sensitivity rises.[20] A single operator study tracked the performance of one endoscopist over the course of the first 300 EUS-FNAs, showing improved performance when comparing the last 100 procedures performed to the first 100.[94] Most experts recommend a 6-24 month "hands-on" training in EUS before achieving competency.[31] Early hand-on exposure to EUS-FNA for trainees with attending physician supervision has been shown to be safe and have comparable performance characteristics to experienced endoscopists including number of passes, diagnostic yield, and complications.[95]

Number of Needle Passes

The number of needle passes needed to obtain diagnostic material varies by site, size, and type of lesion, and potentially may be optimized by immediate cytological assessment of the adequacy of specimens,[4] as described above. If ROSE is not available, a number of studies have analyzed the number of needle passes needed to achieve a good diagnostic yield. An early study by Erickson *et al*.[24] showed that five to six passes were required for pancreatic masses, with the caveat that a general policy of five passes meant that too few or too many passes would be made in about 50% of the patients. In addition, well-differentiated pancreatic adenocarcinoma required a higher number of passes as compared to moderately and poorly differentiated tumors. In tumors that are very vascular, increasing the number of passes may decrease the diagnostic yield due to increasing blood contamination.[89]

LeBlanc *et al*. demonstrated an increase in sensitivity from 17% to 87% when more than seven passes were made with a 22G needle into pancreatic masses during EUS-FNA.[96] Interestingly, a German study involving three contributing centers found that gross examination of the specimens by the EUS endoscopist for cytological and histological sensitivities required only one to two passes in 92% of the cases with solid pancreatic masses.[19] The macroscopic assessment was unsuccessful in 7% (cytology) and 13.5% (histology) of the cases. Similarly, Turner *et al*. showed in their cohort of patients that only two to three passes were necessary to achieve a diagnostic accuracy of 80% although ROSE was available in about 44% of the cases.[97] In a prospective study involving a 25G needle, four passes were found to be sufficient for EUS-FNA of solid pancreatic lesions.[98] The diagnostic accuracy was similar irrespective of an onsite cytopathologist was present or absent.

The ESGE recommends performing three needle passes when sampling lymph nodes and liver lesions, five passes when sampling solid pancreatic masses, and a single pass when sampling pancreatic cysts due to the potential for introducing an infection.[99]

The optimal number of needle passes to obtain a high diagnostic yield seems to be three to seven. Ultimately, the number of passes will highly depend on the availability of a cytopathologist during the biopsy procedure and level of cytolopathologist experience available.

Safety

The total complication rate of EUS-FNA in published series ranges from 0% to 13%.[100-102] Complications most commonly include perforation, pancreatitis, infection, tumor seeding, or clinically significant bleeding.[103] A multicenter study in the United States demonstrated a complication rate of 0.28%[104] while a more recent prospective study noted the complication rate as 0.85%.[102] No definite association was found between the occurrence of a complication and the type and size of the pancreatic lesion, number of passes, or history of chronic pancreatitis.

EUS-guided FNA of solid pancreatic masses is a generally safe and effective modality for tissue acquisition of solid pancreatic lesions with acute pancreatitis being the most common complication but infrequently encountered in clinical practice.

Conclusion

EUS-FNA is an excellent modality for evaluating and sampling solid and cystic pancreatic lesions. It has high accuracy, sensitivity, specificity, and positive predictive value. A majority of studies have shown benefit in using an on-site cytopathologist although implementation of this resource may be an issue in smaller hospitals.

If ROSE cannot be provided, three to seven passes for solid pancreatic masses, three to seven passes for peripancreatic lymph nodes, and a single pass for pancreatic cystic lesions will provide the optimum yield. No single needle size including 19G, 22G, and 25G sizes has shown superiority over another in terms of yield, accuracy, or complication rate. However, the 25G needle remains a popular choice due to its flexibility in accessing the head of the pancreas and uncinate lesions. A core biopsy needle does not appear to have an advantage over 22G or 25G FNA needles except for a reduced number of passes needed to obtain an adequate sample. Currently, the use of stylet, suction, different sampling technique, and passes >7 to obtain FNA have not consistently shown to increase the yield of FNA. EUS-FNA is a useful technique to evaluate pancreatic cysts larger than 1.5 cm, using 22G or 25G needles. Newer technologies such as needle-based confocal endomicroscopy used during EUS can be used to improve diagnostic yield via FNA by providing *in vivo* histology of pancreatic cysts; however, further validation is needed before recommendations can be made.

EUS-guided FNA of solid pancreatic lesions is a generally safe procedure, with acute pancreatitis being one of the more frequently cited complications; however, the overall incidence remains very low. EUS-FNA of cystic lesions in the pancreas has been shown to have a slightly higher risk of pancreatitis and other complications than solid lesions but the risk still remains low. Preprocedure prophylaxis for cystic lesions is currently not recommended. Currently, a minimum of 25 supervised EUS-guided FNA procedures of pancreatic lesions are recommended to achieve confidence in diagnosing pancreatic adenocarcinoma.

There has been tremendous progress over the past decade to overcome the limitations of EUS-FNA due to sampling technique, procedure and equipment. Efforts to increase diagnostic yield and ease of interpretation of biopsy samples may herald a shift from cytological to histological analysis, allowing an increased availability and use of EUS. Refined techniques such as molecular analysis of EUS-FNA aspirate are also being more commonly investigated. Awareness of the variables affecting sampling in EUS-FNA and incorporating evidence-based best practice will undoubtedly help improve clinical outcomes.

Financial support and sponsorship

Conflicts of interest

There are no conflicts of interest.

References

1. Wiersema MJ, Hawes RH, Tao LC, Wiersema LM, Kopecky KK, Rex DK, *et al*. Endoscopic ultrasonography as an adjunct to fine needle aspiration cytology of the upper and lower gastrointestinal tract. Gastrointest Endosc 1992;38:35-9.

2. Vilmann P, Jacobsen GK, Henriksen FW, Hancke S. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. Gastrointest Endosc 1992;38:172-3.

3. Forcione DG. On-site cytopathology for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses: Is it time to make it standard of care? Cancer Cytopathol 2013;121:471-2.

4. Jenssen C, Dietrich CF. Endoscopic ultrasound-guided fine-needle aspiration biopsy and trucut biopsy in gastroenterology — An overview. Best Pract Res Clin Gastroenterol 2009;23:743-59.

5. Roy AK, Kim M, Hawes R, Varadarajulu S. 196 changing trends in tissue acquisition in pancreatic diseases. Gastrointest Endosc 2013;77:AB134.

6. Weston BR, Bhutani MS. Optimizing diagnostic yield for EUS-guided sampling of solid pancreatic lesions: A technical review. Gastroenterol Hepatol (N Y) 2013;9:352-63.

7. Hewitt MJ, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: A meta-analysis. Gastrointest Endosc 2012;75:319-31.

8. DeWitt J, McGreevy K, Sherman S, LeBlanc J. Utility of a repeated EUS at a tertiary-referral center. Gastrointest Endosc 2008;67:610-9.

9. Iglesias-Garcia J, Dominguez-Munoz E, Lozano-Leon A, Abdulkader I, Larino-Noia J, Antunez J, *et al*. Impact of endoscopic ultrasound-guided fine needle biopsy for diagnosis of pancreatic masses. World J Gastroenterol 2007;13:289-93.

10. Eloubeidi MA, Varadarajulu S, Desai S, Wilcox CM. Value of repeat endoscopic ultrasound-guided fine needle aspiration for suspected pancreatic cancer. J Gastroenterol Hepatol 2008;23:567-70.

11. Tadic M, Kujundzic M, Stoos-Veic T, Kaic G, Vukelic-Markovic M. Role of repeated endoscopic ultrasound-guided fine needle aspiration in small solid pancreatic masses with previous indeterminate and negative cytological findings. Dig Dis 2008;26:377-82.

12. Bhutani MS, Gress FG, Giovannini M, Erickson RA, Catalano MF, Chak A, *et al*.; No Endosonographic Detection of Tumor (NEST) Study. The No Endosonographic Detection of Tumor (NEST) Study: A case series of pancreatic cancers missed on endoscopic ultrasonography. Endoscopy 2004;36:385-9.

13. Eloubeidi MA, Jhala D, Chhieng DC, Chen VK, Eltoum I, Vickers S, *et al*. Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. Cancer 2003;99:285-92.

14. Savides TJ, Donohue M, Hunt G, Al-Haddad M, Aslanian H, Ben-Menachem T, *et al*. EUS-guided FNA diagnostic yield of malignancy in solid pancreatic masses: A benchmark for quality performance measurement. Gastrointest Endosc 2007;66:277-82.

15. Gress FG, Hawes RH, Savides TJ, Ikenberry SO, Lehman GA. Endoscopic ultrasound-guided fine-needle aspiration biopsy using linear array and radial scanning endosonography. Gastrointest Endosc 1997;45:243-50.

16. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. Am J Gastroenterol 2002;97:1386-91.

17. Mitsuhashi T, Ghafari S, Chang CY, Gu M. Endoscopic ultrasound-guided fine needle aspiration of the pancreas: Cytomorphological evaluation with emphasis on adequacy assessment, diagnostic criteria and contamination from the gastrointestinal tract. Cytopathology 2006;17:34-41.

18. Sakamoto H, Kitano M, Komaki T, Noda K, Chikugo T, Dote K, *et al.* Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. J Gastroenterol Hepatol 2009;24:384-90.

19. Moller K, Papanikolaou IS, Toermer T, Delicha EM, Sarbia M, Schenck U, *et al.* EUS-guided FNA of solid pancreatic masses: High yield of 2 passes with combined histologic-cytologic analysis. Gastrointest Endosc 2009;70:60-9.

20. Mertz H, Gautam S. The learning curve for EUS-guided FNA of pancreatic cancer. Gastrointest Endosc 2004;59:33-7.

21. Lee JK, Choi JH, Lee KH, Kim KM, Shin JU, Lee JK, *et al.* A prospective, comparative trial to optimize sampling techniques in EUS-guided FNA of solid pancreatic masses. Gastrointest Endosc 2013;77:745-51.

22. Lee YN, Moon JH, Kim HK, Choi HJ, Lee SH, Choi MH, *et al.* A triple approach for diagnostic assessment of endoscopic ultrasound-guided fine needle aspiration in pancreatic solid masses and lymph nodes. Dig Dis Sci 2014;59:2286-93.

23. Wiersema MJ, Vilmann P, Giovannini M, Chang KJ, Wiersema LM. Endosonography-guided fine-needle aspiration biopsy: Diagnostic accuracy and complication assessment. Gastroenterology 1997;112:1087-95.

24. Erickson RA, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. Gastrointest Endosc 2000;51:184-90.

25. Mesa H, Stelow EB, Stanley MW, Mallery S, Lai R, Bardales RH. Diagnosis of nonprimary pancreatic neoplasms by endoscopic ultrasound-guided fine-needle aspiration. Diagn Cytopathol 2004;31:313-8.

26. Petrone MC, Arcidiacono PG. Basic technique in endoscopic ultrasound-guided fine needle aspiration for solid lesions: How many passes? Endosc Ultrasound 2014;3:22-7.

27. Klapman JB, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. Am J Gastroenterol 2003;98:1289-94.

28. Iglesias-Garcia J, Dominguez-Munoz JE, Abdulkader I, Larino-Noia J, Eugenyeva E, Lozano-Leon A, *et al.* Influence of on-site cytopathology evaluation on the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) of solid pancreatic masses. Am J Gastroenterol 2011;106:1705-10.

29. Alsohaibani F, Girgis S, Sandha GS. Does onsite cytotechnology evaluation improve the accuracy of endoscopic ultrasound-guided fine-needle aspiration biopsy? Can J Gastroenterol 2009;23:26-30.

30. Collins BT, Murad FM, Wang JF, Bernadt CT. Rapid on-site evaluation for endoscopic ultrasound-guided fine-needle biopsy of the pancreas decreases the incidence of repeat biopsy procedures. Cancer Cytopathol 2013;121:518-24.

31. Polkowski M, Larghi A, Weynand B, Boustière C, Giovannini M, Pujol B, *et al.*; European Society of Gastrointestinal Endoscopy (ESGE). Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline. Endoscopy 2012;44:190-206.

32. Hebert-Magee S, Bae S, Varadarajulu S, Ramesh J, Frost AR, Eloubeidi MA, *et al.* The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: A meta-analysis. Cytopathology 2013;24:159-71.

33. Matynia AP, Schmidt RL, Barraza G, Layfield LJ, Siddiqui AA, Adler DG. Impact of rapid on-site evaluation on the adequacy of endoscopic-ultrasound guided fine-needle aspiration of solid pancreatic lesions: A systematic review and meta-analysis. J Gastroenterol Hepatol 2014;29:697-705.

34. Wani S, Rastogi A, Early DS, Mullady D, Collins BT, Wang JF, *et al.* Sa1540 Cost minimization analysis of onsite cytopathologist (CyP) evaluation during EUS FNA of solid pancreatic lesions (SPL). Gastrointest Endosc 2013;77(Suppl):AB243-4.

35. Savoy AD, Raimondo M, Woodward TA, Noh K, Pungpapong S, Jones AD, *et al.* Can endosonographers evaluate on-site cytologic adequacy? A comparison with cytotechnologists. Gastrointest Endosc 2007;65:953-7.

36. Hikichi T, Irisawa A, Bhutani MS, Takagi T, Shibukawa G, Yamamoto G, *et al.* Endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses with rapid on-site cytological evaluation by endosonographers without attendance of cytopathologists. J Gastroenterol 2009;44:322-8.

37. Hayashi T, Ishiwatari H, Yoshida M, Ono M, Sato T, Miyanishi K, *et al.* Rapid on-site evaluation by endosonographer during endoscopic ultrasound-guided fine needle aspiration for pancreatic solid masses. J Gastroenterol Hepatol 2013; 28:656-63.

38. Varadarajulu S, Hawes RH. The changing paradigm in EUS-guided tissue acquisition. Gastrointest Endosc Clin N Am 2014;24:1-7.

39. Bang JY, Hebert-Magee S, Trevino J, Ramesh J, Varadarajulu S. Randomized trial comparing the 22-gauge aspiration and 22-gauge biopsy needles for EUS-guided sampling of solid pancreatic mass lesions. Gastrointest Endosc 2012;76:321-7.

40. Krishnan K, Dalal S, Nayar R, Keswani RN, Keefer L, Komanduri S. Rapid on-site evaluation of endoscopic ultrasound core biopsy specimens has excellent specificity and positive predictive value for gastrointestinal lesions. Dig Dis Sci 2013;58:2007-12.

41. Keswani RN, Krishnan K, Wani S, Keefer L, Komanduri S. Addition of endoscopic ultrasound (EUS)-guided fine needle aspiration and on-site cytology to eus-guided fine needle biopsy increases procedure time but not diagnostic accuracy. Clin Endosc 2014; 47:242-7.

42. Vilmann P, Seicean A, Săftoiu A. Tips to overcome technical challenges in EUS-guided tissue acquisition. Gastrointest Endosc Clin N Am 2014;24:109-24.

43. Varadarajulu S, Bang JY, Holt BA, Hasan MK, Logue A, Hawes RH, *et al.* The 25-gauge EUS-FNA needle: Good for on-site but poor for off-site evaluation? Results of a randomized trial. Gastrointest Endosc 2014;80:1056-63.

44. Watson RR, Binmoeller KF, Hamerski CM, Shergill AK, Shaw RE, Jaffee IM, *et al.* Yield and performance characteristics of endoscopic ultrasound-guided fine needle aspiration for diagnosing upper GI tract stromal tumors. Dig Dis Sci 2011;56:1757-62.

45. Itoi T, Itokawa F, Kurihara T, Sofuni A, Tsuchiya T, Ishii K, *et al.* Experimental endoscopy: Objective evaluation of EUS needles. Gastrointest Endosc 2009;69:509-16.

46. Gimeno-García AZ, Elwassief A, Paquin SC, Gariépy G, Sahai AV. Randomized controlled trial comparing stylet-free endoscopic ultrasound-guided fine-needle aspiration with 22-G and 25-G needles. Dig Endosc 2014;26:467-73.

47. Vilmann P, Săftoiu A, Hollerbach S, Skov BG, Linnemann D, Popescu CF, *et al*. Multicenter randomized controlled trial comparing the performance of 22 gauge versus 25 gauge EUS-FNA needles in solid masses. Scand J Gastroenterol 2013;48:877-83.

48. Ramesh J, Bang JY, Hebert-Magee S, Trevino J, Eltoum I, Frost A, *et al*. Randomized trial comparing the flexible 19G and 25G needles for endoscopic ultrasound-guided fine needle aspiration of solid pancreatic mass lesions. Pancreas 2015;44:128-33.

49. Madhoun MF, Wani SB, Rastogi A, Early D, Gaddam S, Tierney WM, *et al*. The diagnostic accuracy of 22-gauge and 25-gauge needles in endoscopic ultrasound-guided fine needle aspiration of solid pancreatic lesions: A meta-analysis. Endoscopy 2013;45:86-92.

50. Affolter KE, Schmidt RL, Matynia AP, Adler DG, Factor RE. Needle size has only a limited effect on outcomes in EUS-guided fine needle aspiration: A systematic review and meta-analysis. Dig Dis Sci 2013;58:1026-34.

51. Bang JY, Ramesh J, Trevino J, Eloubeidi MA, Varadarajulu S. Objective assessment of an algorithmic approach to EUS-guided FNA and interventions. Gastrointest Endosc 2013;77:739-44.

52. Levy MJ, Wiersema MJ. EUS-guided trucut biopsy. Gastrointest Endosc 2005;62:417-26.

53. Thomas T, Kaye PV, Ragunath K, Aithal G. Efficacy, safety, and predictive factors for a positive yield of EUS-guided Trucut biopsy: A large tertiary referral center experience. Am J Gastroenterol 2009;104:584-91.

54. Larghi A, Verna EC, Stavropoulos SN, Rotterdam H, Lightdale CJ, Stevens PD. EUS-guided trucut needle biopsies in patients with solid pancreatic masses: A prospective study. Gastrointest Endosc 2004;59:185-90.

55. Iglesias-Garcia J, Poley JW, Larghi A, Giovannini M, Petrone MC, Abdulkader I, *et al*. Feasibility and yield of a new EUS histology needle: Results from a multicenter, pooled, cohort study. Gastrointest Endosc 2011;73:1189-96.

56. Berzosa M, Villa N, El-Serag HB, Sejpal DV, Patel KK. Comparison of endoscopic ultrasound guided 22-gauge core needle with standard 25-gauge fine-needle aspiration for diagnosing solid pancreatic lesions. Endosc Ultrasound 2015;4:28-33.

57. Hucl T, Wee E, Anuradha S, Gupta R, Ramchandani M, Rakesh K, *et al*. Feasibility and efficiency of a new 22G core needle: A prospective comparison study. Endoscopy 2013;45:792-8.

58. Iwashita T, Nakai Y, Samarasena JB, Park do H, Zhang Z, Gu M, *et al*. High single-pass diagnostic yield of a new 25-gauge core biopsy needle for EUS-guided FNA biopsy in solid pancreatic lesions. Gastrointest Endosc 2013;77:909-15.

59. Nakai Y, Isayama H, Itoi T, Yamamoto N, Kogure H, Sasaki T, *et al*. Role of endoscopic ultrasonography in pancreatic cystic neoplasms: Where do we stand and where will we go? Dig Endosc 2014;26:135-43.

60. Nelsen EM, Buehler D, Soni AV, Gopal DV. Endoscopic ultrasound in the evaluation of pancreatic neoplasms-solid and cystic: A review. World J Gastrointest Endosc 2015;7:318-27.

61. Khashab MA, Kim K, Lennon AM, Shin EJ, Tignor AS, Amateau SK, *et al*. Should we do EUS/FNA on patients with pancreatic cysts? The incremental diagnostic yield of EUS over CT/MRI for prediction of cystic neoplasms. Pancreas 2013;42:717-21.

62. Lim LG, Lakhtakia S, Ang TL, Vu CK, Dy F, Chong VH, *et al*. Factors determining diagnostic yield of endoscopic ultrasound guided fine-needle aspiration for pancreatic cystic lesions: A multicentre Asian study. Dig Dis Sci 2013;58:1751-7.

63. Walsh RM, Zuccaro G, Dumot JA, Vargo J, Biscotti CV, Hammel J, *et al*. Predicting success of endoscopic aspiration for suspected pancreatic cystic neoplasms. JOP 2008;9:612-7.

64. Rogart JN, Loren DE, Singu BS, Kowalski TE. Cyst wall puncture and aspiration during EUS-guided fine needle aspiration may increase the diagnostic yield of mucinous cysts of the pancreas. J Clin Gastroenterol 2011;45:164-9.

65. Brugge WR, De Witt J, Klapman JB, Ashfaq R, Shidham V, Chhieng D, *et al*. Techniques for cytologic sampling of pancreatic and bile duct lesions: The Papanicolaou Society of Cytopathology Guidelines. Cytojournal 2014;11(Suppl 1):2.

66. Nakai Y, Isayama H, Shinoura S, Iwashita T, Samarasena JB, Chang KJ, *et al*. Confocal laser endomicroscopy in gastrointestinal and pancreatobiliary diseases. Dig Endosc 2014;26(Suppl 1):86-94.

67. Nakai Y, Iwashita T, Park do H, Samarasena JB, Lee JG, Chang KJ. Diagnosis of pancreatic cysts: EUS-guided, through-the-needle confocal laser-induced endomicroscopy and cystoscopy trial: DETECT study. Gastrointest Endosc 2015;81:1204-14.

68. Konda VJ, Meining A, Jamil LH, Giovannini M, Hwang JH, Wallace MB, *et al*. A pilot study of *in vivo* identification of pancreatic cystic neoplasms with needle-based confocal laser endomicroscopy under endosonographic guidance. Endoscopy 2013;45:1006-13.

69. Kim TS, Fernandez-del Castillo C. Diagnosis and management of pancreatic cystic neoplasms. Hematol Oncol Clin North Am 2015;29:655-74.

70. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, *et al*. Diagnosis of pancreatic cystic neoplasms: A report of the cooperative pancreatic cyst study. Gastroenterology 2004;126:1330-6.

71. Gaddam S, Ge PS, Keach JW, Mullady D, Fukami N, Edmundowicz SA, *et al*. Suboptimal accuracy of carcinoembryonic antigen in differentiation of mucinous and nonmucinous pancreatic cysts: Results of a large multicenter study. Gastrointest Endosc 2015. [Epub ahead of print].

72. van der Waaij LA, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: A pooled analysis. Gastrointest Endosc 2005;62:383-9.

73. Kadiyala V, Lee LS. Endosonography in the diagnosis and management of pancreatic cysts. World J Gastrointest Endosc 2015;7:213-23.

74. Maker AV, Carrara S, Jamieson NB, Pelaez-Luna M, Lennon AM, Dal Molin M, *et al*. Cyst fluid biomarkers for intraductal papillary mucinous neoplasms of the pancreas: A critical review from the international expert meeting on pancreatic branch-duct-intraductal papillary mucinous neoplasms. J Am Coll Surg 2015;220:243-53.

75. Jabbar KS, Verbeke C, Hyltander AG, Sjövall H, Hansson GC, Sadik R. Proteomic mucin profiling for the identification of cystic precursors of pancreatic cancer. J Natl Cancer Inst 2014;106:djt439.

76. Wang KX, Ben QW, Jin ZD, Du YQ, Zou DW, Liao Z, *et al*. Assessment of morbidity and mortality associated with

EUS-guided FNA: A systematic review. Gastrointest Endosc 2011;73:283-90.

77. Levy MJ, Norton ID, Wiersema MJ, Schwartz DA, Clain JE, Vazquez-Sequeiros E, *et al*. Prospective risk assessment of bacteremia and other infectious complications in patients undergoing EUS-guided FNA. Gastrointest Endosc 2003;57:672-8.

78. Khashab MA, Chithadi KV, Acosta RD, Bruining DH, Chandrasekhara V, Eloubeidi MA, *et al*. ASGE Standards of Practice Committee. Antibiotic prophylaxis for GI endoscopy. Gastrointest Endosc 2015;81:81-9.

79. Wani S, Gupta N, Gaddam S, Singh V, Ulusarac O, Romanas M, *et al*. A comparative study of endoscopic ultrasound guided fine needle aspiration with and without a stylet. Dig Dis Sci 2011;56:2409-14.

80. Wani S. Basic techniques in endoscopic ultrasound-guided fine-needle aspiration: Role of a stylet and suction. Endosc Ultrasound 2014;3:17-21.

81. Sahai AV, Paquin SC, Gariépy G. A prospective comparison of endoscopic ultrasound-guided fine needle aspiration results obtained in the same lesion, with and without the needle stylet. Endoscopy 2010;42:900-3.

82. Rastogi A, Wani S, Gupta N, Singh V, Gaddam S, Reddymasu S, *et al*. A prospective, single-blind, randomized, controlled trial of EUS-guided FNA with and without a stylet. Gastrointest Endosc 2011;74:58-64.

83. Wani S, Early D, Kunkel J, Leathersich A, Hovis CE, Hollander TG, *et al*. Diagnostic yield of malignancy during EUS-guided FNA of solid lesions with and without a stylet: A prospective, single blind, randomized, controlled trial. Gastrointest Endosc 2012;76:328-35.

84. DiMaio CJ, Buscaglia JM, Gross SA, Aslanian HR, Goodman AJ, Ho S, *et al*. Practice patterns in FNA technique: A survey analysis. World J Gastrointest Endosc 2014;6:499-505.

85. Puri R, Vilmann P, Săftoiu A, Skov BG, Linnemann D, Hassan H, *et al*. Randomized controlled trial of endoscopic ultrasound-guided fine-needle sampling with or without suction for better cytological diagnosis. Scand J Gastroenterol 2009;44:499-504.

86. Larghi A, Noffsinger A, Dye CE, Hart J, Waxman I. EUS-guided fine needle tissue acquisition by using high negative pressure suction for the evaluation of solid masses: A pilot study. Gastrointest Endosc 2005;62:768-74.

87. Wallace MB, Kennedy T, Durkalski V, Eloubeidi MA, Etamad R, Matsuda K, *et al*. Randomized controlled trial of EUS-guided fine needle aspiration techniques for the detection of malignant lymphadenopathy. Gastrointest Endosc 2001;54:441-7.

88. Chen AM, Park WG, Friedland S, Banerjee S. Mo1396 endoscopic ultrasound-guided fine needle aspiration versus fine-needle capillary sampling biopsy of pancreatic solid lesions: Does technique matter? Gastrointest Endosc 2011;73(Suppl):AB331.

89. Kulesza P, Eltoum IA. Endoscopic ultrasound-guided fine-needle aspiration: Sampling, pitfalls, and quality management. Clin Gastroenterol Hepatol 2007;5:1248-54.

90. Nakai Y, Isayama H, Chang KJ, Yamamoto N, Hamada T, Uchino R, *et al*. Slow pull versus suction in endoscopic ultrasound-guided fine-needle aspiration of pancreatic solid masses. Dig Dis Sci 2014;59:1578-85.

91. Kin T, Katanuma A, Yane K, Takahashi K, Osanai M, Takaki R, *et al*. Diagnostic ability of EUS-FNA for pancreatic solid lesions with conventional 22-gauge needle using the slow pull technique: A prospective study. Scand J Gastroenterol 2015;50:900-7.

92. Paquin SC, Sahai AV. Techniques for EUS-guided FNA cytology. Gastrointest Endosc Clin N Am 2014;24:71-81.

93. Bang JY, Magee SH, Ramesh J, Trevino JM, Varadarajulu S. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic mass lesions. Endoscopy 2013;45:445-50.

94. Eloubeidi MA, Tamhane A. EUS-guided FNA of solid pancreatic masses: A learning curve with 300 consecutive procedures. Gastrointest Endosc 2005;61:700-8.

95. Coté GA, Hovis CE, Kohlmeier C, Ammar T, Al-Lehibi A, Azar RR, *et al*. Training in EUS-guided fine needle aspiration: Safety and diagnostic yield of attending supervised, trainee-directed FNA from the onset of training. Diagn Ther Endosc 2011;2011:378540.

96. LeBlanc JK, Ciaccia D, Al-Assi MT, McGrath K, Imperiale T, Tao LC, *et al*. Optimal number of EUS-guided fine needle passes needed to obtain a correct diagnosis. Gastrointest Endosc 2004;59:475-81.

97. Turner BG, Cizginer S, Agarwal D, Yang J, Pitman MB, Brugge WR. Diagnosis of pancreatic neoplasia with EUS and FNA: A report of accuracy. Gastrointest Endosc 2010;71:91-8.

98. Suzuki R, Irisawa A, Bhutani MS, Hikichi T, Takagi T, Sato A, *et al*. Prospective evaluation of the optimal number of 25-gauge needle passes for endoscopic ultrasound-guided fine-needle aspiration biopsy of solid pancreatic lesions in the absence of an onsite cytopathologist. Dig Endosc 2012;24:452-6.

99. Savides TJ. Tricks for improving EUS-FNA accuracy and maximizing cellular yield. Gastrointest Endosc 2009;69(Suppl):S130-3.

100. Ho S, Bonasera RJ, Pollack BJ, Grendell J, Feuerman M, Gress F. A single-center experience of endoscopic ultrasonography for enlarged pancreas on computed tomography. Clin Gastroenterol Hepatol 2006;4:98-103.

101. Klapman JB, Chang KJ, Lee JG, Nguyen P. Negative predictive value of endoscopic ultrasound in a large series of patients with a clinical suspicion of pancreatic cancer. Am J Gastroenterol 2005;100:2658-61.

102. Eloubeidi MA, Tamhane A, Varadarajulu S, Wilcox CM. Frequency of major complications after EUS-guided FNA of solid pancreatic masses: A prospective evaluation. Gastrointest Endosc 2006;63:622-9.

103. Fujii LL, Levy MJ. Basic techniques in endoscopic ultrasound-guided fine needle aspiration for solid lesions: Adverse events and avoiding them. Endosc Ultrasound 2014;3:35-45.

104. Eloubeidi MA, Gress FG, Savides TJ, Wiersema MJ, Kochman ML, Ahmad NA, *et al*. Acute pancreatitis after EUS-guided FNA of solid pancreatic masses: A pooled analysis from EUS centers in the United States. Gastrointest Endosc 2004;60:385-9.