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Review Article

Endoscopic ultrasound-guided tissue acquisition: Needle types, technical issues, and sample handling

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A B S T R A C T

Endoscopic ultrasound (EUS)-guided tissue acquisition is an established method for the pathologic diagnosis of solid pancreatic masses due to its high accuracy and safety. Currently, EUS-guided biopsy is applied to any lesions adjacent to the gastrointestinal tract that can be visualized with EUS. In this review, conventional and novel types of needles for EUS-guided tissue acquisition are introduced and their diagnostic performance is compared. In addition, technical issues and sampling handling methods to improve diagnostic accuracy are discussed.

Keywords: Biopsy; Diagnosis; Instrumentation; Needles; Pathology

Introduction

Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) is a pivotal method for the pathologic diagnosis of solid pancreatic masses due to its high accuracy and safety.[1] EUS-FNAB is now applied to any lesions adjacent to the gastrointestinal tract that can be visualized with EUS. In the past, only cytological evaluations were performed through EUS-guided fine needle aspiration (EUS-FNA), but some lesions require an evaluation of tissue architecture, including immunohistochemical staining, for an accurate pathologic assessment.[2] For pancreatic neuroendocrine tumors, it is important to obtain a histologic core to determine the mitosis count and Ki-67 index, which are important prognostic factors.[3] Therefore, various types of needles have been developed to obtain adequate core tissue samples to conduct a further histologic evaluation and improve the diagnostic yield.[4–8] In addition, many techniques and devices for EUS-guided tissue acquisition and handling methods of obtained tissue have been introduced, and clinical studies have been conducted to prove their efficacy.[9–11] Methods for the on-site evaluation of sample adequacy, such as rapid on-site evaluation (ROSE) by a cytopathologist and macroscopic on-site evaluation (MOSE) by an endoscopist, have been introduced to confirm whether an obtained sample is adequate for interpretation, and several supplementary methods have also been introduced in sample handling to increase sample adequacy.[12,13] In this review, various types of needles for EUS-guided tissue acquisition, sampling techniques for better diagnostic yield, and methods for processing obtained samples are discussed.

Needles for EUS-Guided Tissue Acquisition

Conventional needles

At first, all EUS-FNA needles had a similar basic design for a cytopathological diagnosis only. About a decade ago, new needles designed to obtain core tissue with preserved tissue architecture for histologic evaluations and molecular profiling were developed.[14] The names of the biopsy needles currently in use and their features are described in Table 1. By using a biopsy needle, it is theoretically possible to obtain a core tissue sample that can be identified by gross inspection, which is expected to reduce the number of needle passes and increase the diagnostic yield without ROSE. However, in real-world clinical practice, the shape and material of the aspiration needle have been developed together, so that sufficient core tissue can also be obtained with the aspiration needle. Since reverse-bevel biopsy needles showed only minimal benefits in tissue acquisition, antegrade-bevel biopsy needles were introduced to obtain a larger amount of tissue because they hold the tissue while pushing the needle forward, which is the most ef-

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fective movement during the procedure.[15] Biopsy needles showed better diagnostic adequacy and accuracy, with fewer needle passes in solid gastrointestinal lesions, but not in solid pancreatic lesions.[16] Franseen and fork-tip biopsy needles have been compared in a meta-analysis, which found that both Franseen and fork-tip needles demonstrated a similarly high diagnostic yield of over 90%, with comparable adverse events.[17]

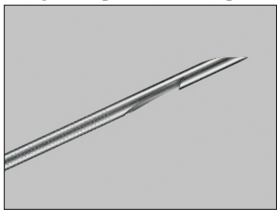
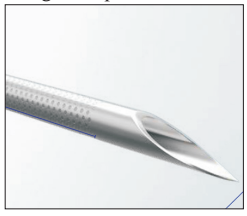
Interestingly, a recent meta-analysis compared the accuracy of needles in solid pancreatic masses according to the needle shape.[18] In 16 randomized controlled trials with 1,934 patients, Franseen, Menghini-tip, reverse-bevel, antegrade-bevel, and fork-tip needles were compared with respect to the diagnostic performance using FNA as reference values. Among them, Franseen and fork-tip needles, particularly those of a 22-gauge size, showed the best results in diagnostic accuracy and sample adequacy.

Regarding needle size, a systematic review with a network meta-analysis was performed, and no specific gauge was superior among 19-, 22-, and 25-gauge needles in terms of diagnostic accuracy, sample adequacy, and histologic core procurement; however, these results were based on low-quality evidence.[19] Nonetheless, 25-gauge needles seem to be more convenient to handle, and they are especially useful when there is acute angulation to access the lesion, as is the case for lesions in the uncinate process or medial aspect of the pancreas head.[20] A previous meta-analysis suggested that 25-gauge needles were more sensitive than 22-gauge needles for diagnosing solid pancreatic masses because 25-gauge needles retrieve less bloody aspirate and show better handling in needle passage and actuation.[20] However, in real clinical practice, when 25-gauge needles are used, it is often not easy to push out the obtained tissue by reinserting the stylet. In addition, recent developments in needles have made them more flexible, and the advantages of the 25-gauge needle have been diluted because the diagnosis is usually made based on a biopsy specimen rather than cytology. The use of 19-gauge needles is limited because of their stiffness, but these large-bore needles seem to be useful for establishing organoids with tissues obtained through EUS or performing molecular profiling for precision medicine.

New concepts of needles

New needle concepts, including EUS-guided through-the-needle (TTN) microforceps and confocal laser endomicroscopy (CLE), have been introduced.[21–25] These devices are usually used for the diagnosis of pancreatic cysts, for which conventional EUS-

Table 1 A List of Needles for EUS-Guided Tissue Acquisition

Product	Company	Shape	Needle size (gauge)
ProCore	Cook	Menghini-tip with core trap 	19, 20, 22, 25
Acquire	Boston Scientific	Franseen 	22, 25
EZ Shot 3 Plus	Olympus	Menghini-tip with side hole 	19, 22
SharkCore	Medtronic	Fork-tip 	19, 22, 25

EUS, endoscopic ultrasound.

FNA and cyst fluid analysis have low diagnostic yield.[26] The major limitations of EUS-FNA result from obtaining insufficient fluid for biochemical analysis and inadequate sample cellularity.[27,28]

TTN biopsy demonstrated a higher diagnostic yield for pancreatic cysts than EUS-FNA; however, concerns about safety issues have been raised, including bleeding, leakage, infection, and pancreatitis.[29,30] A recent multicenter retrospective analysis of 506 patients who underwent TTN biopsy with microforceps revealed that the incidence of adverse events was 11.5%, and intraductal papillary mucinous neoplasms sampled with multiple microforceps passes were classified as being at high risk for adverse events (28%).[31] Therefore, the risk-benefit balance of TTN biopsy should be carefully considered, and TTN biopsy should be performed in selected patients.

Needle-based CLE allows for a real-time *in vivo* microscopic evaluation of epithelial and vascular patterns of the cyst wall, with studies demonstrating better diagnostic accuracy than EUS with cyst fluid analysis for the differential diagnosis of pancreatic cysts.[28,32,33] CLE uses an intravenous fluorophore, fluorescein, and a fiber-optic confocal laser to achieve higher magnification and reveal cellular and subcellular structures in the epithelium.[34] The CLE probe can be advanced through a 19-gauge needle into pancreatic cysts or parenchyma. In a meta-analysis of 10 studies with 547 individuals with pancreatic cysts, the pooled sensitivity and specificity were 90% and 96%, respectively.[35] The overall rate of CLE-related adverse events was 2.7%, including pancreatitis, bleeding, pruritus, infection, and peri-pancreatic fluid collection[34,35] Besides pancreatic cysts, attempts have been made to use CLE to differentiate pancreatic adenocarcinoma, neuroendocrine tumors, chronic pancreatitis, and autoimmune pancreatitis, but the images of solid lesions are static and image interpretation is challenging.[34,36,37]

TTN biopsy and CLE were compared in the diagnosis of pancreatic cysts, and CLE showed higher diagnostic yield than biopsy (85% vs. 74%, $P < 0.0001$), while sensitivity (80% vs. 86%) and specificity (80% vs. 83%) were comparable in a recent meta-analysis.[38]

Technical Issues during EUS-Guided Tissue Acquisition

Technical factors associated with diagnostic accuracy

Endoscopists have adopted diverse techniques to improve the diagnostic accuracy of EUS-guided tissue acquisition, and numerous studies have tried to determine the ideal techniques for EUS-guided tissue acquisition. The following technical factors can be considered in EUS-FNAB: suction or non-suction, applying capillary sampling with a stylet slow-pull, the number of to-and-fro movements or actuations, the door-knocking technique, the fanning technique, the torque technique, the number of needle passes, and the use of a stylet.[39–41]

The basic maneuvers in EUS-FNAB are as follows. The echoendoscope should be positioned as straight as possible to facilitate needle insertion. Before puncture, the target lesion should be carefully inspected and located at the 5- to 7-o'clock position on the real-time EUS image. Color Doppler should be applied to avoid puncturing intervening vessels. Excessive movements of the tip of echoendoscope or the elevator should be avoided, as they increase the resistance to movements of the needle.

Although applying suction seems to lead to a better diagnostic yield rate than non-suction, the value of suction may vary depending on the target site and disease.[9,11] There seem to be little difference in diagnostic adequacy between suction and capillary sampling with the stylet slow-pull technique.[11,42] The accumulation of blood and blood contamination can reduce the specimen quality and result in blood clogging inside the needle lumen.[11,43,44] A recent prospective study investigated the optimal number of needle actuations to obtain adequate cellularity with minimizing blood contamination. Significant blood contamination was observed with 20 actuations compared with 15 actuations when suction was applied, whereas 10 actuations showed a lower diagnostic yield than 15 or 20 actuations when suction was not applied.[11] Therefore, 15 actuations were recommended for EUS-FNA of solid pancreatic masses.

The use of a stylet with EUS-FNA did not increase the diagnostic yield, whereas stylet use was associated with poorer sample quality in a previous prospective study.[45] Expressing aspirate from the needle by air flushing seems to be preferred over reinserting the stylet because bloodiness was lower with air flushing than with stylet reinsertion. Moreover, expressing the aspirate by air flushing is easier and safer.[9]

Needle priming with saline or heparin was introduced to reduce blood clogging.[43,46] These techniques provide better cellularity and specimen adequacy without negative effects on histologic interpretation and immunohistochemical staining.

The needle speed of actuations during EUS-FNA may be related to the diagnostic yield. Faster movement of the needle may increase tissue fracture and cause more cell detachment.[47] Mukai et al[48] introduced the "door-knocking technique," named for the sound made by the needle handle hitting the stopper during a quick and forceful forward push. Although this technique did not improve the accuracy of the histologic diagnosis, a larger amount of tissue acquisition was possible. When the actual needle speed was measured during EUS-FNA, the diagnostic accuracy and specimen quality were higher when the acceleration was greater than 9.8 m/s^2.[49] The fanning technique, which is defined as using the needle to sample multiple areas within a lesion using the up/down knob of the echoendoscope, has been introduced, and showed superiority because fewer passes were required to confirm the pathologic diagnosis.[50] The torque technique, which is defined as applying torque by twisting the shaft of the echoendoscope in the clockwise or counterclockwise direction without using the left/right control knob during EUS-FNAB, was evaluated in a prospective study of 124 patients with solid pancreatic masses, and the authors concluded that the torque technique enabled better histologic core procurement.[41]

The minimum number of needle passes to obtain adequate tissue is critical. The more needle passes are performed, the higher the diagnostic yield could be; however, performing numerous needle passes requires a long time and the probability of procedure-related adverse events might increase. In addition, if the number of passes is increased beyond a certain threshold, the diagnostic yield may not further increase, and time can be wasted unnecessarily. In the absence of ROSE, at least 5 to 7 passes were suggested for EUS-FNA of pancreatic malignancies.[51,52] However, in a recent prospective study of 239 patients with solid pancreatic masses, performing more than four passes of EUS-FNA did not increase the sensitivity of detection.[39] With the development of biopsy needles, as MOSE is widely implemented, the number of needle passes could be further reduced.

Targeting under contrast-enhanced harmonic EUS

Because 80% to 100% of false-negative cases in EUS-FNA are correctly classified by contrast-enhanced harmonic EUS (CEH-EUS), CEH-EUS plays a complementary role in the diagnosis of

solid pancreatic masses.[53] In addition, CEH-EUS has been proposed as a method to improve lesion targeting because the contrast agent may enable better recognition of the puncture site by helping to avoid the necrotic area.[53,54] EUS-FNAB under CEH-EUS may improve the diagnostic accuracy and adequacy with fewer needle passes.[55–58]

ROSE versus MOSE

The presence of a cytopathologist to perform ROSE during EUS-FNA can improve the diagnostic performance by reducing inadequate samples and the need for additional passes.[14,59–61] However, ROSE requires medical resources, including an on-site cytopathologist, as well as additional time and costs. A recent study reported the efficacy of MOSE for ensuring core tissue acquisition with a minimal number of needle passes and a high diagnostic yield.[62] A macroscopic visible core larger than 4 mm is an indicator of an adequate sample that improves diagnostic accuracy.[63] A recent international, multicenter, prospective, randomized clinical trial revealed that EUS-FNAB with MOSE required fewer needle passes to achieve an adequate diagnostic yield similar to that of the conventional method.[64] MOSE should be implemented in real-world practice in terms of obviating the need for an on-site cytopathologist and saving additional costs and time for slide staining and interpretation.[7]

Adverse events and contraindications of EUS-guided tissue acquisition

EUS-guided tissue acquisition is minimally invasive and safe, with an adverse event rate ranging from 0% to 3%.[65] Adverse events include abdominal pain, acute pancreatitis, perforation, infection, bleeding, and tumor seeding. Most unpredictable adverse events are mild in severity and self-limiting.[65] Although there is a concern that biopsy needles may cause more bleeding, it has been reported that there was no difference in the risk of adverse events, including bleeding, because biopsy needles could reduce the number of needle passes. A recent meta-analysis showed no difference in the incidence of adverse events between aspiration needles and the biopsy needles (1.8% vs. 2.3%, respectively; pooled risk ratio, 1.13; 95% confidence interval, 0.40–3.22; $P = 0.64$).[66]

There is no absolute contraindication to EUS-guided tissue acquisition, but caution is required in cases of cardiopulmonary instability, bleeding tendency with coagulopathy or thrombocytopenia, and recent use of anticoagulants or antiplatelets.[67]

Optimal Handling of the EUS-Guided Obtained Sample

It is important to obtain an adequate tissue sample, but it is also very important to process the obtained tissue appropriately to make a pathological interpretation. In addition to the pathological analysis, the detection of molecular alterations may be helpful for improving diagnostic accuracy.

Conventional smear versus liquid-based cytology

A conventional smear with Papanicolaou or Diff-Quick stain is the usual method for the cytologic preparation of EUS-FNA specimens. However, conventional smears have problems such as bloody smears, dry artifacts, crushing artifacts, and thick tissue fragments, which obscure cytologic features and lead to a suboptimal diagnosis.[12,55] Therefore, liquid-based cytology was introduced to solve the problems of conventional smears.[68] In liquid-based cytology with EUS-FNA specimens, the ThinPrep method and SurePath system have mainly been studied.[12] Prior studies comparing the ThinPrep method with conventional smears found that conventional smears were superior to ThinPrep in diagnosing pancreatic malignancies.[69–71] However, the SurePath system showed similar diagnostic performance to conventional smears and reduced the blood background, thereby facilitating cell observation and justifying the use of liquid-based cytology for EUS-FNA over conventional smears when available.[12]

Identification of molecular alterations

Molecular diagnostic techniques can enhance the diagnostic yield through various types of immunohistochemical staining and molecular analyses.[72–74] EUS-guided tissue acquisition can provide cancer DNA for sequencing analysis. Although target sequencing is feasible even with cytology specimens, it can be difficult to proceed with whole-genome or whole-exome sequencing if the obtained samples are too small.[75] EUS-guided tissue acquisition using a large-bore needle is more likely to achieve successful next-generation sequencing.[76] A recent randomized crossover clinical trial reported that the specimen adequacy for genetic profiling was significantly better with biopsy needles than with aspiration needles.[77]

Additional genetic profiling using EUS-guided obtained samples, including *KRAS*, *TP53*, *SMAD4*, and *CDKN2A/P16* mutation analysis, can improve the diagnostic accuracy of pancreatic cancer.[14] Ancillary testing, including *KRAS*, *GNAS*, *HVL*, and *CTNB1* may be helpful in the differential diagnosis of pancreatic cysts.[78] Besides diagnosis, molecular profiling of an obtained tissue sample can predict the prognosis and help determine the management

Table 2 A Summary of Optimal EUS-Guided Tissue Acquisition and Processing Methods

Needle shape	Franseen, fork-tip, and Menghini-tip needles are all recommended. Through-the-needle microforceps are promising for pancreatic cysts.
Needle gauge	A 22-gauge needle is recommended considering its convenience and clinical evidence. The 19-gauge needle seems to be in the spotlight again because more tissue is better for genetic profiling.
Tissue acquisition technique	Rapid insertion and slow withdrawal of needle under negative suction, 15 times. The fanning technique or torque technique.
Confirmation of adequacy of the obtained sample	Macroscopic on-site evaluation to confirm histologic core.
Cytology preparation	Liquid-based cytology (SurePath).
Ancillary test	Addition of molecular profiling.

EUS, endoscopic ultrasound.

plan, such as using PARP inhibitors, immune checkpoint inhibitors, TRK inhibitors, or RAS GTPase family inhibitors targeting *KRAS*^{G12C} [79–82].

Conclusions

It will be crucial to obtain a larger amount of high-quality tissue in line with current medical trends, which are progressing toward precision medicine and targeted therapy. Table 2 summarizes the EUS-guided tissue acquisition and processing methods that can produce the highest diagnostic performance based on the results of previous clinical studies and meta-analyses.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Chang KJ, Nguyen P, Erickson RA, Durbin TE, Katz KD. The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. *Gastrointest Endosc.* 1997;45:387-93.
2. Paik WH, Park Y, Park DH, Hong SM, Lee BU, Choi JH, et al. Prospective evaluation of new 22 gauge endoscopic ultrasound core needle using capillary sampling with stylet slow-pull technique for intra-abdominal solid masses. *J Clin Gastroenterol.* 2015;49:199-205.
3. Paik WH, Lee HS, Lee KJ, Jang SI, Lee WJ, Hwang JH, et al. Malignant potential of small pancreatic neuroendocrine neoplasm and its risk factors: a multicenter nationwide study. *Pancreatology.* 2021;21:208-14.
4. 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.
5. Iwashita T, Nakai Y, Samarasena JB, Park DH, 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.
6. Larghi A, Iglesias-Garcia J, Poley JW, Monges G, Petrone MC, Rindi G, et al. Feasibility and yield of a novel 22-gauge histology EUS needle in patients with pancreatic masses: a multicenter prospective cohort study. *Surg Endosc.* 2013;27:3733-8.
7. Pausawasdi N, Cheirsilpa K, Chalermwai W, Asokan I, Sriprayoon T, Charatcharoenwitthaya P. Endoscopic ultrasound-guided fine-needle biopsy using 22G franseen needles without rapid on-site evaluation for diagnosis of intraabdominal masses. *J Clin Med.* 2022;11:1051.
8. Chen YI, Chatterjee A, Berger R, Kanber Y, Wyse J, Lam E, et al. Endoscopic ultrasound (EUS)-guided fine needle biopsy alone vs. EUS-guided fine needle aspiration with rapid onsite evaluation in pancreatic lesions: a multicenter randomized trial. *Endoscopy.* 2022;54:4-12.
9. 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.
10. Lee KY, Cho HD, Hwangbo Y, Yang JK, Han SJ, Choi HJ, et al. Efficacy of 3 fine-needle biopsy techniques for suspected pancreatic malignancies in the absence of an on-site cytopathologist. *Gastrointest Endosc.* 2019;89:825-31.e1.
11. Paik WH, Choi JH, Park Y, Lee JB, Park DH. Optimal techniques for EUS-guided fine-needle aspiration of pancreatic solid masses at facilities without on-site cytopathology: results from two prospective randomised trials. *J Clin Med.* 2021;10:4662.
12. Chun JW, Lee K, Lee SH, Kim H, You MS, Hwang YJ, et al. Comparison of liquid-based cytology with conventional smear cytology for EUS-guided FNA of solid pancreatic masses: a prospective randomized noninferiority study. *Gastrointest Endosc.* 2020;91:837-46.e1.
13. Lewin D. Optimal EUS-guided FNA cytology preparation when rapid on-site evaluation is not available. *Gastrointest Endosc.* 2020;91:847-8.
14. Cazacu IM, Luzuriaga Chavez AA, Saftoiu A, Vilmann P, Bhutani MS. A quarter century of EUS-FNA: progress, milestones, and future directions. *Endosc Ultrasound.* 2018;7:141-60.
15. Armellini E, Manfrin E, Trisolini E, Andorno S, Ballarè M, Bernardoni L, et al. Histologic retrieval rate of a newly designed side-bevelled 20G needle for EUS-guided tissue acquisition of solid pancreatic lesions. *United European Gastroenterol J.* 2019;7:96-104.
16. Li Z, Liu W, Xu X, Li P. A meta-analysis comparing endoscopic ultrasound-guided fine-needle aspiration with endoscopic ultrasound-guided fine-needle biopsy. *J Clin Gastroenterol.* 2022. doi: 10.1097/MCG.0000000000001702. [Epub ahead of print]
17. Mohan BP, Shakhatreh M, Garg R, Asokkumar R, Jayaraj M, Ponnada S, et al. Comparison of Franseen and fork-tip needles for EUS-guided fine-needle biopsy of solid mass lesions: a systematic review and meta-analysis. *Endosc Ultrasound.* 2019;8:382-91.
18. Gkolfakis P, Crinò SF, Tziatzios G, Ramai D, Papaefthymiou A, Papanikolaou IS, et al. Comparative diagnostic performance of end-cutting fine-needle biopsy needles for EUS tissue sampling of solid pancreatic masses: a network meta-analysis. *Gastrointest Endosc.* 2022;95:1067-77.e15.
19. Facciorusso A, Wani S, Triantafyllou K, Tziatzios G, Cannizzaro R, Muscatiello N, et al. Comparative accuracy of needle sizes and designs for EUS tissue sampling of solid pancreatic masses: a network meta-analysis. *Gastrointest Endosc.* 2019;90:893-903.e7.
20. 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.
21. Robles-Medranda C, Olmos JI, Puga-Tejada M, Oleas R, Baquerizo-Burgos J, Arevalo-Mora M, et al. Endoscopic ultrasound-guided through-the-needle microforceps biopsy and needle-based confocal laser-endomicroscopy increase detection of potentially malignant pancreatic cystic lesions: a single-center study. *World J Gastrointest Endosc.* 2022;14:129-41.
22. Mittal C, Obuch JC, Hammad H, Edmundowicz SA, Wani S, Shah RJ, et al. Technical feasibility, diagnostic yield, and safety of microforceps biopsies during EUS evaluation of pancreatic cystic lesions (with video). *Gastrointest Endosc.* 2018;87:1263-9.
23. Yang D, Samarasena JB, Jamil LH, Chang KJ, Lee D, Ona MA, et al. Endoscopic ultrasound-guided through-the-needle microforceps biopsy in the evaluation of pancreatic cystic lesions: a multicenter study. *Endosc Int Open.* 2018;6:E1423-30.
24. Cho SH, Song TJ, Seo DW, Oh D, Park DH, Lee SS, et al. Efficacy and safety of EUS-guided through-the-needle microforceps biopsy sampling in categorizing the type of pancreatic cystic lesions. *Gastrointest Endosc.* 2022;95:299-309.
25. Kohoutova D, Zar S, Repak R, Vlavianos P, Bures J. Pancreatic cysts: diagnostic role of EUS-guided microforceps biopsy and confocal laser endomicroscopy. *Gastroenterol Res Pract.* 2019;2019:3431048.
26. Crinò SF, Bernardoni L, Gabbrielli A, Capelli P, Salvia R, Rusev BC, et al. Beyond pancreatic cyst epithelium: evidence of ovarian-like stroma in EUS-guided through-the-needle micro-forceps biopsy specimens. *Am J Gastroenterol.* 2018;113:1059-60.
27. de Jong K, Poley JW, van Hooft JE, Visser M, Bruno MJ, Fockens P. Endoscopic ultrasound-guided fine-needle aspiration of pancreatic cystic lesions provides inadequate material for cytology and laboratory analysis: initial results from a prospective study. *Endoscopy.* 2011;43:585-90.
28. Cheesman AR, Zhu H, Liao X, Szporn AH, Kumta NA, Nagula S, et al. Impact of EUS-guided microforceps biopsy sampling and needle-based confocal laser endomicroscopy on the diagnostic yield and clinical management of pancreatic cystic lesions. *Gastrointest Endosc.* 2020;91:1095-104.
29. Crinò SF, Bernardoni L, Brozzi L, Barresi L, Malleo G, Salvia R, et al. Association between macroscopically visible tissue samples and diagnostic accuracy of EUS-guided through-the-needle microforceps biopsy sampling of pancreatic cystic lesions. *Gastrointest Endosc.* 2019;90:933-43.
30. Kovacevic B, Klausen P, Rift CV, Toxværd A, Grossjohann H, Karstensen JG, et al. Clinical impact of endoscopic ultrasound-guided through-the-needle microbiopsy in patients with pancreatic cysts. *Endoscopy.* 2021;53:44-52.
31. Facciorusso A, Kovacevic B, Yang D, Vilas-Boas F, Martínez B, Stigliano S, et al. Predictors of adverse events after endoscopic ultrasound through-the-needle biopsy of pancreatic cysts: a recursive partitioning analysis. *Endoscopy.* 2022. doi: 10.1055/a-1831-5385. [Epub ahead of print]
32. Napoléon B, Lemaistre AI, Pujol B, Caillol F, Lucidarme D, Bourdariat R, et al. A novel approach to the diagnosis of pancreatic serous cystadenoma: needle-based confocal laser endomicroscopy. *Endoscopy.* 2015;47:26-32.
33. Napoleon B, Palazzo M, Lemaistre AI, Caillol F, Palazzo L, Aubert A, et al. Needle-based confocal laser endomicroscopy of pancreatic cystic lesions: a prospective multicenter validation study in patients with definite diagnosis. *Endoscopy.* 2019;51:825-35.
34. Kim DH, Krishna SG, Coronel E, Kröner PT, Wolfsen HC, Wallace MB, et al. Confocal laser endomicroscopy in the diagnosis of biliary and pancreatic disorders: a systematic analysis. *Clin Endosc.* 2022;55:197-207.
35. Wang X, Hu J, Yang F, Yang F, Sun S. Needle-based confocal laser endomicroscopy for diagnosis of pancreatic cystic lesions: a meta-analysis. *Minim Invasive Ther Allied Technol.* 2022;31:653-63.
36. Giovannini M, Caillol F, Monges G, Poizat F, Lemaistre AI, Pujol B, et al. Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy in solid pancreatic masses. *Endoscopy.* 2016;48:892-8.
37. Hao S, Ding W, Jin Y, Di Y, Yang F, He H, et al. Appraisal of EUS-guided needle-based confocal laser endomicroscopy in the diagnosis of pancreatic lesions: a

single Chinese center experience. *Endosc Ultrasound.* 2020;9:180-6.
38. Kovacevic B, Antonelli G, Klausen P, Hassan C, Larghi A, Vilmann P, et al. EUS-guided biopsy versus confocal laser endomicroscopy in patients with pancreatic cystic lesions: a systematic review and meta-analysis. *Endosc Ultrasound.* 2021; 10:270-9.
39. Mohamadnejad M, Mullady D, Early DS, Collins B, Marshall C, Sams S, et al. Increasing number of passes beyond 4 does not increase sensitivity of detection of pancreatic malignancy by endoscopic ultrasound-guided fine-needle aspiration. *Clin Gastroenterol Hepatol*. 2017;15:1071-8.e2.
40. Kedia P, Gaidhane M, Kahaleh M. Technical advances in endoscopic ultrasound (EUS)-guided tissue acquisition for pancreatic cancers: how can we get the best results with EUS-guided fine needle aspiration? *Clin Endosc*. 2013;46:552-62.
41. Park SW, Lee SS, Song TJ, Koh DH, Hyun B, Chung D, et al. The diagnostic performance of novel torque technique for endoscopic ultrasound-guided tissue acquisition in solid pancreatic lesions: a prospective randomized controlled trial. *J Gastroenterol Hepatol.* 2020;35:508-15.
42. Weston BR, Ross WA, Bhutani MS, Lee JH, Pande M, Sholl AB, et al. Prospective randomized comparison of a 22G core needle using standard versus capillary suction for EUS-guided sampling of solid pancreatic masses. *Endosc Int Open.* 2017; 5:E505-12.
43. Diehl DL, Mok SRS, Khara HS, Johal AS, Kirchner HL, Lin F. Heparin priming of EUS-FNA needles does not adversely affect tissue cytology or immunohistochemical staining. *Endosc Int Open.* 2018;6:E356-62.
44. Varadarajulu S, Hasan MK, Bang JY, Hebert-Magee S, Hawes RH. Endoscopic ultrasound-guided tissue acquisition. *Dig Endosc.* 2014;26(Suppl 1):62-9.
45. 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. Erratum in: *Endoscopy*. 2011;43:168.
46. Attam R, Arain MA, Bloechl SJ, Trikudanathan G, Munigala S, Bakman Y, et al. "Wet suction technique (WEST)": a novel way to enhance the quality of EUS-FNA aspirate. Results of a prospective, single-blind, randomized, controlled trial using a 22-gauge needle for EUS-FNA of solid lesions. *Gastrointest Endosc.* 2015;81:1401-7.
47. Mahvash M, Dupont PE. Mechanics of dynamic needle insertion into a biological material. *IEEE Trans Biomed Eng.* 2010;57:934-43.
48. Mukai S, Itoi T, Ashida R, Tsuchiya T, Ikeuchi N, Kamada K, et al. Multicenter, prospective, crossover trial comparing the door-knocking method with the conventional method for EUS-FNA of solid pancreatic masses (with videos). *Gastrointest Endosc.* 2016;83:1210-7.
49. Ciocîrlan M, Gheorghiu A, Bilous D, Cruceru M, Mănăilă G, Tianu E, et al. Monitored needle acceleration in endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses improves sample quality and diagnostic accuracy: a randomized trial. *Endoscopy*. 2022;54:389-93.
50. 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.
51. 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.
52. 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.
53. Kitano M, Kamata K, Imai H, Miyata T, Yasukawa S, Yanagisawa A, et al. Contrast-enhanced harmonic endoscopic ultrasonography for pancreatobiliary diseases. *Dig Endosc.* 2015;27(Suppl 1):60-7.
54. Kitano M, Sakamoto H, Kudo M. Contrast-enhanced endoscopic ultrasound. *Dig Endosc.* 2014;26(Suppl 1):79-85.
55. Yamabe A, Irisawa A, Bhutani MS, Shibukawa G, Fujisawa M, Sato A, et al. Efforts to improve the diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for pancreatic tumors. *Endosc Ultrasound.* 2016;5:225-32.
56. Sugimoto M, Takagi T, Hikichi T, Suzuki R, Watanabe K, Nakamura J, et al. Conventional versus contrast-enhanced harmonic endoscopic ultrasonography-guided fine-needle aspiration for diagnosis of solid pancreatic lesions: a prospective randomized trial. *Pancreatology*. 2015;15:538-41.
57. Sugimoto M, Takagi T, Suzuki R, Konno N, Asama H, Watanabe K, et al. Contrast-enhanced harmonic endoscopic ultrasonography in gallbladder cancer and pancreatic cancer. *Fukushima J Med Sci.* 2017;63:39-45.
58. Seicean A, Samarghitan A, Bolboacă SD, Pojoga C, Rusu I, Rusu D, et al. Contrast-enhanced harmonic versus standard endoscopic ultrasound-guided fine-needle aspiration in solid pancreatic lesions: a single-center prospective randomized trial. *Endoscopy*. 2020;52:1084-90.
59. Hébert-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.
60. Ramesh J, Varadarajulu S. How can we get the best results with endoscopic ultrasound-guided fine needle aspiration? *Clin Endosc*. 2012;45:132-7.
61. Haba S, Yamao K, Bhatia V, Mizuno N, Hara K, Hijioka S, et al. Diagnostic ability and factors affecting accuracy of endoscopic ultrasound-guided fine needle aspiration for pancreatic solid lesions: Japanese large single center experience. *J Gastroenterol.* 2013;48:973-81.
62. So H, Seo DW, Hwang JS, Ko SW, Oh D, Song TJ, et al. Macroscopic on-site evaluation after EUS-guided fine needle biopsy may replace rapid on-site evaluation. *Endosc Ultrasound*. 2021;10:111-5.
63. Iwashita T, Yasuda I, Mukai T, Doi S, Nakashima M, Uemura S, et al. Macroscopic on-site quality evaluation of biopsy specimens to improve the diagnostic accuracy during EUS-guided FNA using a 19-gauge needle for solid lesions: a single-center prospective pilot study (MOSE study). *Gastrointest Endosc.* 2015;81:177-85.
64. Chong CCN, Lakhtakia S, Nguyen N, Hara K, Chan WK, Puri R, et al. Endoscopic ultrasound-guided tissue acquisition with or without macroscopic on-site evaluation: randomized controlled trial. *Endoscopy*. 2020;52:856-63.
65. Chung MJ, Park SW, Kim SH, Cho CM, Choi JH, Choi EK, et al. Clinical and technical guideline for endoscopic ultrasound-guided tissue acquisition of pancreatic solid tumor: Korean Society of Gastrointestinal Endoscopy. *Korean J Gastroenterol.* 2021;78:73-93.
66. Renelus BD, Jamorabo DS, Boston I, Briggs WM, Poneros JM. Endoscopic ultrasound-guided fine needle biopsy needles provide higher diagnostic yield compared to endoscopic ultrasound-guided fine needle aspiration needles when sampling solid pancreatic lesions: a meta-analysis. *Clin Endosc.* 2021;54:261-8.
67. Nabi Z, Lakhtakia S, Chavan R, Asif S, Basha J, Gupta R, et al. Diagnostic utility of EUS-guided tissue acquisition in children: a tertiary care center experience. *Endosc Ultrasound.* 2021;10:288-93.
68. Linder J. Recent advances in thin-layer cytology. *Diagn Cytopathol.* 1998;18:24-32.
69. Yeon MH, Jeong HS, Lee HS, Jang JS, Lee S, Yoon SM, et al. Comparison of liquid-based cytology (CellPrepPlus) and conventional smears in pancreaticobiliary disease. *Korean J Intern Med.* 2018;33:883-92.
70. LeBlanc JK, Emerson RE, Dewitt J, Symms M, Cramer HM, McHenry L, et al. A prospective study comparing rapid assessment of smears and ThinPrep for endoscopic ultrasound-guided fine-needle aspirates. *Endoscopy.* 2010;42:389-94.
71. Lee JK, Choi ER, Jang TH, Chung YH, Jang KT, Park SM, et al. A prospective comparison of liquid-based cytology and traditional smear cytology in pancreatic endoscopic ultrasound-guided fine needle aspiration. *Acta Cytol.* 2011;55:401-7.
72. Park JK, Paik WH, Song BJ, Ryu JK, Kim MA, Park JM, et al. Additional K-ras mutation analysis and Plectin-1 staining improve the diagnostic accuracy of pancreatic solid mass in EUS-guided fine needle aspiration. *Oncotarget.* 2017;8:64440-8.
73. Laurell H, Bouisson M, Berthelemy P, Rochaix P, Dejean S, Besse P, et al. Identification of biomarkers of human pancreatic adenocarcinomas by expression profiling and validation with gene expression analysis in endoscopic ultrasound-guided fine needle aspiration samples. *World J Gastroenterol.* 2006;12:3344-51.
74. Mishra G, Zhao Y, Sweeney J, Pineau BC, Case D, Ho C, et al. Determination of qualitative telomerase activity as an adjunct to the diagnosis of pancreatic adenocarcinoma by EUS-guided fine-needle aspiration. *Gastrointest Endosc.* 2006;63: 648-54.
75. Ashida R, Kitano M. Endoscopic ultrasound-guided tissue acquisition for pancreatic ductal adenocarcinoma in the era of precision medicine. *Dig Endosc.* 2022. doi: 10.1111/den.14344. [Epub ahead of print]
76. Park JK, Lee JH, Noh DH, Park JK, Lee KT, Lee JK, et al. Factors of endoscopic ultrasound-guided tissue acquisition for successful next-generation sequencing in pancreatic ductal adenocarcinoma. *Gut Liver.* 2020;14:387-94.
77. Kandel P, Nassar A, Gomez V, Raimondo M, Woodward TA, Crook JE, et al. Comparison of endoscopic ultrasound-guided fine-needle biopsy versus fine-needle aspiration for genomic profiling and DNA yield in pancreatic cancer: a randomized crossover trial. *Endoscopy.* 2021;53:376-82.
78. Jhala N, Srimunta P, Jhala D. Role of ancillary testing on endoscopic us-guided fine needle aspiration samples from cystic pancreatic neoplasms. *Acta Cytol.* 2020; 64:124-35.
79. Ryozawa S, Iwano H, Taba K, Sen-yo M, Uekitani T. Genetic diagnosis of pancreatic cancer using specimens obtained by EUS-FNA. *Dig Endosc.* 2011;23(Suppl 1): 43-5.
80. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N Engl J Med.* 2019;381:317-27.
81. Doebele RC, Drilon A, Paz-Ares L, Siena S, Shaw AT, Farago AF, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol.* 2020;21:271-82. Erratum in: *Lancet Oncol*. 2020;21:e70. Erratum in: *Lancet Oncol*. 2020;21:e341. Erratum in: *Lancet Oncol*. 2020;21:e372. Erratum in: *Lancet Oncol*. 2021;22:e428.
82. Hong DS, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, et al. $KRAS^{G12C}$ inhibition with sotorasib in advanced solid tumors. *N Engl J Med.* 2020;383:1207-17.