

Endoscopic ultrasonography in pancreatic diseases: advances in tissue acquisition



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ABSTRACT

Background and study aims Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) improved the diagnostic performance and upgraded the role of endoscopic ultrasonography (EUS) into an interventional modality, able to guide patient management and treatment. This review aimed to highlight the advances, emerging practices, procedural techniques and technological innovations in EUS tissue acquisition in pancreatic diseases.

Methods A thorough review of the literature was performed using PubMed to identify articles that describe techniques, advances, and practices in EUS tissue acquisition in gastrointestinal diseases.

Conclusion Since the first EUS-FNA procedure, EUS guided-tissue acquisition has been evolving continuously. Development of needles with innovative tip design enabled procurement of larger samples with preserved histological architecture. Moreover, sampling techniques and complementary methods, such as contrast harmonic imaging and EUS-elastography, have been introduced in an effort to improve diagnostic performance and sample adequacy.

Introduction

The innovative combination of ultrasonography and flexible endoscopy resulted in development of endoscopic ultrasound (EUS) [1], which revolutionized our ability to visualize lesions of the gastrointestinal tract, adjacent organs and structures whose assessment was difficult by means of conventional diagnostic modalities and especially transabdominal ultrasound.

EUS was a breakthrough in endoscopic evaluation of benign and malignant diseases, but development of EUS-guided fine-needle Aspiration (EUS-FNA) [2], improved EUS diagnostic performance [3] and upgraded its role into an interventional modality, able to guide patient management and treatment.

Today tissue acquisition is routinely performed during EUS procedures and has been evolving continuously beyond EUS-FNA as use of newly developed needles with innovative tip design enables collection of larger tissue samples with preserved

histological architecture. Nevertheless, acquisition of adequate tissue samples can be influenced by various factors, such as needle type, geometry, and size; sampling technique; use of needle stylet, use and type of suction, availability of rapid on-site cytopathologist evaluation; sample handling; and endoscopographer experience.

This review aimed to highlight the advances, emerging practices, procedural techniques and technological innovations in EUS tissue acquisition in pancreatic diseases.

Literature search

A thorough review of the literature was performed using PubMed to identify articles that describe techniques, advances, and practices in EUS tissue acquisition in gastrointestinal diseases.

The search was performed using the search string: ("tissue acquisition" OR "EUS-FNA" OR "EUS-FNB" OR "EUS guided" OR "core tissue" OR "core biopsy" OR "biopsy" OR "biopsies" OR "histology" OR "cytology" OR "microforceps" OR "ProCore" OR "Sharkcore" OR "Acquire") AND ("EUS" OR "endosonography" OR "endoscopic ultrasound"). Only articles in English with relevant titles were reviewed, as well as guidelines and publications from gastroenterological and endoscopic societies regarding EUS procedures and EUS-tissue acquisition.

Needles

A significant body of literature is available regarding the role of different needle types and needle sizes in diagnostic performance, sample adequacy, and technical difficulties.

A summary of the available needles can be found in ► **Table 1**.

FNA needles

Needles primarily designed to collect cells from suspected lesions are known as EUS-FNA needles and information regarding their performance is mainly gained from studies evaluating pancreatic lesions [4]. EUS-FNA needles are characterized by similar safety profiles [5] and are available in 19-, 22- and 25-gauge (G) size.

The smaller 22- and 25-G needles predominantly allow procurement of cytological material, whereas the larger 19G allows aspiration of both cellular and histological material [4]. However, the inherent rigidity of 19-G EUS-FNA needles results in scope torque, loss of maneuverability, increased rate of technical failure, and limited tissue sample procurement in areas where the echoendoscope is flexed (e.g. pancreatic head, or uncinate process lesions) [6–8]. For these reasons, their use in transduodenal approach is not recommended [4].

Introduction of flexible 19G EUS-FNA needles made of nitinol, a metal alloy of nickel and titanium, exhibiting the properties of shape memory effect and super elasticity, were demonstrated to overcome the aforementioned disadvantages of the standard 19G EUS-FNA needles while allowing acquisition of adequate cytological as well as histological material in the majority of patients (100% and 94.7%, respectively) [9].

Nevertheless, several meta-analyses investigating the performance of different EUS-FNA needles [6,10–12] reached similar conclusions; in pancreatic and peripancreatic lesions, where cytology is sufficient to reach a diagnosis, 19G EUS-FNA needles were not demonstrated to confer a diagnostic advantage over the thinner and more commonly used 22 and 25G needles [13] with respect to diagnostic accuracy, number of passes, and complications. However, in two of the aforementioned meta-analyses, the thinner 25G EUS-FNA needles demonstrated a marginal advantage regarding sample adequacy [6] and diagnostic sensitivity [11] compared to the 22G needles.

The diagnostic performance of EUS-FNA is maximized with rapid onsite cytopathology evaluation. When that is not available, cell-block histological sections for off-site cytopathology result in moderate diagnostic accuracy (75–80%) [14,15]. Moreover, the negative predictive value of EUS-FNA with 22 and 25G needles was demonstrated to be weak (29.7% [95% CI 18.9–42.4%] vs. 6.9% [95% CI 1.9–16.7%], $P=0.001$) [16],

► **Table 1** Available EUS needles.

Needle type	Available sizes
EUS-FNA	19, 22, 25-gauge
EchoTip ProCore Needle, Cook Medical, Indiana, United States	19, 22, 25-gauge (Reverse bevel)
	20-gauge (Antegrade Core Trap)
Acquire EUS-FNB Needle, Boston Scientific Co., Natick, Massachusetts, United States	19, 22, 25-gauge
SharkCore EUS-FNB Needle, Medtronic, Minneapolis, Minnesota, United States	19, 22, 25-gauge
EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; EUS-FNA, endoscopic ultrasound-guided fine-needle biopsy	

thus, a normal result might not correctly indicate that the patient is disease-free, as the risk of a false negative diagnosis is high.

FNB needles

While EUS-FNA cytology is suitable for evaluation of pancreatic masses, lymph nodes, and subepithelial tumors, its role is limited in investigation of disorders for which histological samples with preserved tissue architecture are necessary to reach a diagnosis, such as autoimmune pancreatitis [17], lymphomas [18,19], well-differentiated adenocarcinomas, and mesenchymal tumors [20]. Moreover, the yield of true histological samples and quality of the sample material for performance of ancillary testing, for example, identification of specific neoplasm-related molecular biomarkers, DNA sequencing and characterization of neoplastic features such as desmoplastic fibrosis, was shown to be suboptimal, especially when the smaller 25G EUS-FNA needles were used [21] or when rapid onsite cytopathology evaluation was unavailable [22,23].

The same paradigm was not shown to apply for difficult-to-use 19G EUS-FNA needles, as they were demonstrated to provide superior histological yield (>98%), with excellent sensitivity, specificity, and diagnostic accuracy (91.8%, 100%, and 93.2%, respectively) [24]. The first EUS core tissue biopsy needle, the 19G Tru-Cut biopsy needle [25], employed a spring-loaded mechanism built into the needle handle, which allowed the automatic procurement of true histological samples. However, its use was technically demanding, especially when a transduodenal approach was required [26] and it was linked to increased procedure costs and complication rate [27]. For the aforementioned reasons, use of the 19G Tru-Cut biopsy needle was quickly abandoned.

Needles with hollowed reverse bevel architecture, namely the 19, 22 and 25G EchoTip ProCore (Cook Medical, Indiana, United States) needles, were developed to enable true histological sample procurement.

A significant number of studies investigated performance of the 22 and 25G ProCore needles in evaluation of solid masses, pancreatic masses, lymph nodes, and subepithelial lesions,

without being able to firmly establish their superiority with respect to diagnostic accuracy over EUS-FNA needles [28–41].

Similarly, a recent meta-analysis [42] concluded that neither the 19G ProCore needles were able to produce an advantage over EUS-FNA needles, with respect to sample adequacy (75.2% vs. 89.0%, odds ratio [OR] 0.39, $P=0.23$), diagnostic accuracy (85.8% vs. 86.2%, OR 0.88, $P=0.53$) and core specimen acquisition (77.7% vs. 76.5%, OR 0.94, $P=0.85$). However, ProCore needles were shown to reduce the required number of needle passes compared to EUS-FNA needles, to reach a diagnosis (standardized mean difference -1.2 , $P<0.001$) [42].

Data evaluating the diagnostic performance of the 20G ProCore needle, which employs an antegrade core trap instead of reverse bevel architecture, and studies comparing its diagnostic performance against other needles are scarce [43, 44].

New EUS-FNB needles with innovative tip geometry, namely the fork-tip (Sharckore; Medtronic, Minneapolis, Minnesota, United States) and Franseen-tip (Acquire, Boston Scientific Co., Natick, Massachusetts, United States) recently have been introduced. The ability of the Acquire needle to procure histological and cytological samples was reported to be superior compared to EUS-FNA needles of the same size (22G) [45–47]. Moreover, their high rate of tissue adequacy was not associated with an increase in adverse events [48], despite concerns regarding their use in borderline coagulopathic patients and in pancreatic cyst lesions [49].

Apart from the improved histological yield, both the Franseen-tip and the Fork-tip needle demonstrated excellent diagnostic accuracy ($>90\%$), with or without presence of rapid on-site evaluation (ROSE) [46]. In addition, they were shown to alleviate the need for ROSE, for both solid pancreatic and non-pancreatic lesions, as well as to reduce the required number of needle passes, with a single pass resulting in an onsite diagnostic accuracy in more than 95% of patients [46, 47, 50–56].

Nevertheless, results of a recent meta-analysis could not establish a clear benefit of EUS-FNB over EUS-FNA in the investigation of pancreatic lesions, with regards to diagnostic yield and accuracy [57].

Rapid on-site cytological evaluation

The rationale behind rapid on-site cytological evaluation of EUS-guided acquired samples is enhancement of sample adequacy and diagnostic yield, with fewer needle passes [58–63]. However, ROSE entails several limitations: it is unavailable in a significant number of institutions due to shortage of experienced pathologists and it results in prolonged procedure duration and increases costs [64]. Meta-analyses based on observational studies resulted in varying results regarding its value in EUS-guided tissue acquisition [65–68], however, the outcome of recent randomized controlled trials indicated that apart from reduction in required needle passes, no benefit could be established regarding diagnostic yield, the proportion of adequate specimens and accuracy in pancreatic masses [69, 70]. In addition, rapid on-site cytological evaluation during EUS-FNA procedures was not shown to offer a diagnostic advantage over EUS-FNB procedures alone [71–74]. For the aforementioned reasons, use of ROSE could not be rendered mandatory [64].

► **Table 2** Required needle passes for differentiation of pancreatic lesions.

Needle type	Required needle passes
Solid pancreatic masses	
▪ EUS-FNA	3–4
▪ ProCore	2–3
▪ Franseen-tip & fork-tip EUS-FNB needles	1–2
Pancreatic cystic lesions	
▪ EUS-FNA	1

EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; EUS-FNS, endoscopic ultrasound-guided fine-needle biopsy

Number of needle passes

As mentioned above, rapid on-site cytological evaluation is not available in a number of institutions [75]. Thus, a minimum number of needle passes is required to increase the possibility of acquiring adequate samples and reaching accurate diagnoses. Various studies have evaluated the minimum number of required passes [76–79], which is shown to differ between EUS-FNA and EUS-FNB procedures.

According to a recently published technical guideline [64], three to four passes with EUS-FNA needles and two to three passes with reverse bevel needles are suggested in diagnostic evaluation of pancreatic masses whereas one EUS-FNA needle pass is adequate for pancreatic cystic lesions. Lymph node investigation with EUS-FNA requires fewer needle passes compared to pancreatic masses, with three needle passes reaching a sensitivity of 100%.

Studies on the recently introduced Franseen-tip and Fork-tip FNB needles indicate that diagnostic adequacy for on-site and off-site evaluation is possible from the first needle pass, for solid mass lesions [50]. A summary of the required needle passes can be found in ► **Table 2**.

Sampling techniques

Needle stylet use

The technique of lesion puncturing without removing the stylet from the needle lumen has been routinely practiced by endosonographers, as it is considered to prevent needle lumen contamination and clogging with unwanted overlying gastrointestinal tissue [64]. However, various studies were not able to establish a benefit of the aforementioned technique with respect to diagnostic accuracy, adequacy, quality, cellularity or blood contamination on EUS-FNA [80–85] or EUS-FNB procedures [86]. In addition, it was considered labor-intensive and associated with prolonged procedure duration [85–86]. Nevertheless, stylet use may be justified during EUS-FNA investigation of pancreatic cystic lesions, as cystic fluid contamination with gastrointestinal mucin could generate differentiation difficulties [87]. The value of stylet use in EUS procedures with the

new Franseen-tip and Fork-tip needles remains to be determined.

Dry suction technique

Sampling under negative pressure, applied with a 10- or 20-cc suction syringe attached to the proximal end port of the needle, is recommended during EUS-FNA of solid masses and lymph nodes [64]. Dry suction increases sample cellularity, diagnostic sensitivity, and diagnostic accuracy, however, it is associated with an increased risk of blood contamination and increased number of needle passes, especially when 22G needles are used [16].

In addition, dry suction may result in technical failure when smaller needles are used [16]. Use of larger-volume syringes was demonstrated to procure larger samples with an increased risk of blood contamination, however, it was not able to confer an advantage in diagnostic accuracy [88].

Wet suction and modified wet suction technique

The wet suction technique (WEST) involves replacement of the air column residing inside the EUS-FNA needles, with 5 cc of saline. The rationale behind this technique relies on fluid dynamics. Withdrawal of saline results in negative pressure enhancement at the needle's distal tip, as it is incompressible, as opposed to air [89].

The wet suction technique involves needle flushing with a saline solution before aspiration is performed. Consequently, attachment of a prefilled 10-cc syringe with 3 cc of saline to the needle's proximal end port allows manual intermittent aspiration after the lesion is punctured. This technique was developed [90] to overcome risk of clogging due to blood contamination, associated with the dry suction technique [91, 92].

Although preliminary data on WEST demonstrated superior performance compared to the dry suction technique with respect to sample cellularity and diagnostic yield [89, 90], its superiority in diagnostic accuracy is questioned [93, 94]. Recently, a modified (hybrid) version of the wet suction technique (MWST), was introduced [89, 95, 96]. In MWST, needle preparation is identical to the wet suction technique; however, negative pressure is applied continuously through a pre-vacuum syringe. Despite the promising results, further studies are necessary to evaluate its diagnostic advantage on EUS-FNA procedure.

Stylet slow-pull technique

The stylet slow-pull technique involves needle stylet withdrawal during EUS-FNA, which results in minimum negative pressure generation. It is well known that the dry suction technique may influence the quality of the tissue specimen, mainly by blood contamination. However, the stylet slow-pull technique was associated with improved histological sample quality, reduced blood contamination and similar, or higher, diagnostic performance compared to the dry suction technique [97 – 100].

► **Table 3** Sampling techniques.

Needle stylet use
Dry suction technique
Wet suction and modified wet suction technique
Stylet slow-pull technique
Fanning technique
Through-the-needle biopsy

Fanning technique

The fanning technique involves sampling multiple areas of the suspected lesion during every needle pass, by changing the needle track with the help of the echoendoscope dials or elevator. The fanning technique was demonstrated to reduce the number of required needle passes to reach a diagnosis without, however, conferring a statistically significant increase in diagnostic accuracy [101].

Through-the-needle biopsy

EUS-FNA plays a central role in investigation of pancreatic cystic lesions. In particular, use of 19G or 22G EUS-FNA needles is the recommended approach [64] for diagnostic evaluation of pancreatic cysts, with or without presence of a solid component. Apart from evaluation of cystic fluid, EUS-FNA allows for cytological and histological evaluation of the pancreatic cystic wall, which was demonstrated to improve diagnostic yield compared with cyst fluid analysis alone [102 – 104].

Development of micro-forceps with an outer diameter of 1 mm allowed their use through 19G EUS-FNA needles [105], introducing an alternative method for pancreatic cystic wall tissue acquisition. Although available data regarding this innovative technique are limited, results of small studies indicate that it is a promising, highly diagnostic technique, able to determine the nature of pancreatic cysts and guide management, with acceptable rates of technical and clinical success [106 – 111]. However, the recently published European evidence-based guidelines on pancreatic cystic neoplasms do not recommend use of forceps biopsy in clinical practice due to limited data [112].

A summary of the available sampling techniques can be found in ► **Table 3**.

Lesion targeting

Contrast harmonic EUS (CH-EUS) and EUS elastography (EUS-E) were shown to facilitate lesion targeting and puncture site selection during EUS procedures.

Contrast harmonic EUS

Development of a prototype echo-endoscope [113] equipped with a broadband transducer allowed application of contrast harmonic imaging during EUS procedures. The new transducer offered adequate frequency bandwidth and acoustic power output, enabling detection of harmonic signals from infused ultrasound contrast agents; perfused tissue could be identified as

an enhanced area, whereas necrotic and fibrotic hypo-perfused regions as hypo-enhanced areas.

The rationale behind its application was that ultrasound contrast agents could facilitate lesion targeting through recognition of parenchymal perfusion and microvasculature disorders, as well as through identification of neoplastic or thrombus-related vasculature obliteration [114, 115]. A recent meta-analysis [116] supported the aforementioned hypothesis by demonstrating the superior diagnostic performance of CH-EUS in differential diagnosis of pancreatic masses (sensitivity 94% [95% CI, 0.91–0.95], specificity 89% [95% CI, 0.85–0.92], and area under the curve 0.9732). Moreover, CH-EUS was demonstrated to improve EUS-FNA diagnostic sensitivity in lesions with vascular versus avascular areas (94.3% vs 72.9%, respectively, $P < 0.001$) [117], as well as to reduce the number of required needle passes to reach a diagnosis [118] and increase tissue adequacy [119]. In addition, CH-EUS was shown to be helpful in investigation of pancreatic cysts for differentiating a mural nodule from mucin and in assessing vascularity within the cyst and septations [112, 120].

Nevertheless, histopathology remains the gold-standard for pancreatic cancer diagnosis; CH-EUS should be considered as a complementary tool in evaluation of pancreatic masses, enabling direct lesion targeting [121] until randomized controlled trials provide further evidence of the role of CH-EUS in diagnostic performance and differential diagnosis of pancreatic and non-pancreatic lesions.

EUS elastography

Elastography provides a qualitative or quantitative evaluation of tissue elasticity in real time. It has been used to investigate benign and malignant lesions, as tissue structure alterations due to neoplasia or inflammation are associated with changes in tissue elasticity. In a recently published prospective study [122], Endoscopic ultrasound elastography (EUS-E) demonstrated high diagnostic accuracy (98.4%) in evaluation of solid pancreatic masses (95% confidence interval [CI]: 91.4–99.7) compared to CEH-EUS and EUS-guided tissue acquisition 85.5% (95% CI: 74.7–92.2) and 91.5% (95% CI: 83.6–99.5), respectively. However, EUS-E is far from being a substitute for EUS-FNA or EUS-FNB procedures in differentiation of pancreatic masses, as several meta-analyses have concluded that despite its high sensitivity (95–99%), it has moderate specificity (63–76%) for diagnosis of pancreatic neoplasia [123–129].

Notwithstanding that, EUS-E is a promising modality in facilitating target selection during EUS-FNA procedures in selected patients with suspected malignant lesions [130]. The combination of CH-EUS with EUS-E was shown to provide complementary information in differential diagnosis of solid pancreatic tumors, however, it results in a non-significant increase in diagnostic accuracy compared to either modality alone [122, 131].

Heparin priming

Blood contamination of the aspirated sample during EUS-FNA procedures may affect sample quality, as formation of blood clots complicates tissue expression, smearing, and microscopic

examination [132, 133]. Moreover, blood clots may complicate stylet reinsertion in the needle lumen [133].

The technique of heparin priming prior to needle puncturing was noted to offer protection against clotting and its effectiveness was evaluated in a small number of studies. It consists of flushing the needle with the heparin solution and reinserting the stylet (“dry heparin” technique), or allowing the heparin solution to remain inside the needle lumen by not reinserting the stylet and applying suction with a pre-vacuum syringe, in a fashion similar to the wet suction technique (“wet heparin” technique) [133].

The wet heparin technique was demonstrated to improve tissue adequacy compared to the dry needle techniques (dry heparin or dry suction technique), in EUS-guided [134] or percutaneous liver biopsy [135]. Nevertheless, in EUS-FNA for pancreatic and other solid lesions, there was no improvement observed with respect to diagnostic performance, tissue adequacy, and median number of passes. Moreover, data regarding its value in blood clotting are conflicting [132, 133].

Tissue expression

Stylet reinsertion is the standard method for EUS-FNA aspirate expression, however, air flushing was demonstrated to be an efficient and safe alternative, able to reduce procedure time, without entailing risk of needle stick injury.

Although both methods resulted in a comparable number of diagnostic samples, diagnostic accuracy, sample cellularity, and air-drying artifact, rates of blood contamination were higher when the stylet reinsertion technique was used [92].

Conclusion

EUS-FNA improved EUS diagnostic performance; however, the role of FNA cytology in diagnosis of well-differentiated neoplasms as well as its use in ancillary testing is limited, whereas its diagnostic performance is dependent upon, among other things, availability of rapid on-site evaluation. Nineteen-gauge EUS-FNA needles were demonstrated to facilitate procurement of adequate cytological as well as histological material. Nonetheless, their use is associated with an increased rate of technical failure.

Use of reverse-bevel needles was not shown to increase diagnostic accuracy compared to EUS-FNA needles, although a diagnostic sample was feasible with reduced needle passes. Introduction of EUS needles with innovative tip geometry, particularly the Fork-tip and Franseen-tip needles, was expected to revolutionize EUS-guided tissue acquisition. Their design was demonstrated to enable the procurement of true histological samples, resulting in high diagnostic accuracy with a minimum number of needle passes and without the need for rapid onsite evaluation. Another advantage of the Fork-tip and Franseen-tip needles is their superior tissue acquisition yield, which can facilitate ancillary testing such as molecular profiling and immunostaining in cancer patients. However, a clear benefit of EUS-FNB over EUS-FNA in investigation of pancreatic lesions, with regards to diagnostic yield and accuracy, is not yet established.

Dry suction, stylet use, and fanning technique were not shown to firmly establish an advantage during EUS-FNA procedures. On the contrary, application of ultrasound contrast agents or elastography during EUS procedures are promising, innovative, and complementary modalities that were shown to improve lesion targeting and puncture site selection.

Despite the promising future, current procedure practices appear to be dictated by needle availability and cost, lesion location and type, need to preserve tissue architecture to reach a diagnosis, and endosonographer's experience and preferences.

Competing interests

None

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