Review Article

A quarter century of EUS-FNA: Progress, milestones, and future directions

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ABSTRACT

Tissue acquisition using EUS has considerably evolved since the first EUS-FNA was reported 25 years ago. Its introduction was an important breakthrough in the endoscopic field. EUS-FNA has now become a part of the diagnostic and staging algorithm for the evaluation of benign and malignant diseases of the gastrointestinal tract and of the organs in its proximity, including lung diseases. This review aims to present the history of EUS-FNA development and to provide a perspective on the recent developments in procedural techniques and needle technologies that have significantly extended the role of EUS and its clinical applications. There is a bright future ahead for EUS-FNA in the years to come as extensive research is conducted in this field and various technologies are continuously implemented into clinical practice.

Key words: EUS, EUS-FNA, tissue acquisition

THE HISTORY OF EUS AND EUS-FNA DEVELOPMENT

It has been a remarkable journey for EUS over the past three decades. EUS has evolved from a technical curiosity into a procedure with a significant impact on gastrointestinal and pulmonary diseases. Thus, EUS has radically changed the ability to diagnose and stage gastrointestinal cancers and to evaluate the pancreas. It permits high-resolution imaging of the gastrointestinal wall and abdominal organs, as well as real-time EUS-FNA. The development of EUS-FNA changed the landscape of EUS, upgrading its diagnostic power. Its greatest advantage is that it allows safe and accurate sampling of lesions that were earlier not accessible or required more invasive techniques for pathological diagnosis. This ability has also expanded its role in diagnosis and staging of pulmonary diseases.

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Flexible endoscopy was developed in 1911[1] and the ultrasound (US) arrived later in 1956.[2] By combining ultrasonography and endoscopy, EUS opened an entirely new dimension in imaging. EUS was designed in the early 1980s in an attempt to improve US imaging of the pancreaticobiliary system. The addition of US probes to endoscopes[3] allowed an improved visualization of the gastrointestinal wall and its surrounding structures. The resolution of the images was enhanced due to the closeness of the US transducer to the lesion and the use of a high-frequency US probe. The first radial echoendoscope was designed and provided by Olympus (Tokyo, Japan) in 1982. The initial commercial EUS instrument was mechanical radial type, where the US transducer located on the tip of the instrument was rotated by a motor in the endoscope handle. A 360° image was obtained, perpendicular to the insertion shaft of the endoscope, and that allowed an easier interpretation of anatomy in real time.[4] In 1984, Tio and Tytgat depicted the possibility of using the biopsy channel for cytological puncture, which would increase the diagnostic value of EUS.[5] Rösch and Classen[6] presented the advantages of EUS along with its limitations, the most important being the lack of specificity in distinguishing between benign and malignant changes.

In the early 1990s, Pentax Medical, in cooperation with Hitachi, developed the first commercially available linear-array echoendoscope.[7] Linear echoendoscopes brought a new landscape to EUS due to the ability to track a needle in real time across the image plane into a target lesion. The electronic instruments also permitted the use of Doppler technology to assess vascular flow. Vilmann *et al.* in collaboration with Medi-Globe GmbH created a special biopsy equipment, and this was a major step leading to the clinical application of the biopsy method.[8-10] In 1992, Vilmann *et al.*[8] reported the first case of EUS-FNA of a pancreatic head lesion, using a curved linear array (CLA) echoendoscope. EUS-FNA of various lesions from upper and lower gastrointestinal tract was further described by Wiersema *et al.*,[11] who published the first EUS-FNA report performed in the United States.

In 1993, a new steel needle, with Teflon sheath, for the upper gastrointestinal tract lesions was described by Vilmann *et al.*[12] Since then, many indications for EUS-FNA have been reported worldwide [Figure 1].[3,8,11-38]

In 1994, Wiersema *et al.*[18,39] and Chang *et al.*[19] described the importance of an on-site cytopathologist during the procedure to assess if the collected samples were adequate or whether further puncture attempts were necessary. Giovannini *et al.*[40] showed that EUS-FNA was safe, with no significant complications.

Vilmann and Hancke reported the development of a new biopsy handle instrument (type Hancke/Vilmann) in 1996.[20] A year later, Binmoeller *et al.*[21] first described an automated biopsy device for pancreatic lesions that could not be punctured with a conventional aspiration needle. The automatic spring-loaded biopsy needle allowed tissue sampling of indurated pancreatic lesions. However, this instrument never gained success. Subsequently, numerous extended indications for EUS-FNA have been reported.[22-28] In 2002, Wiersema *et al.*[29] described the initial experience with EUS-guided biopsies of perigastric organs. The initial core biopsy EUS needle used was the 19-G Tru-Cut needle. Although in some cases high diagnostic yields were obtained,[41] there were several complaints about the limited flexibility of the needle. The Tru-Cut was replaced by the ProCore fine-needle biopsy (FNB) needles, which are available in a range of sizes (19, 20, 22, 25-G).[42,43]

The history of EUS needles is dynamic, with new needles coming out annually [Figure 2].[44] New needles such as fork-tip needle (SharkCore; Medtronic),[30] Franseen-type needle (Acquire; Boston Scientific)[45] or 20-G FNB needle with antegrade core trap (ProCore 20-G; Cook Medical)[46] have been recently released on the market.

EUS-FNA was introduced at the beginning of the 1990s due to a echoendoscope that allowed real-time visualization of aspiration needles.[13,47] The design of CLA echoendoscopes and the technique of EUS-FNA have not substantially changed since then. In 2007, Voermans *et al.*[48] described the first use of a prototype forward-viewing (FV) echoendoscope. The FV echoendoscope was initially created for therapeutic procedures, particularly for pseudocyst drainage.[49,50] However, it has become evident that it can be very

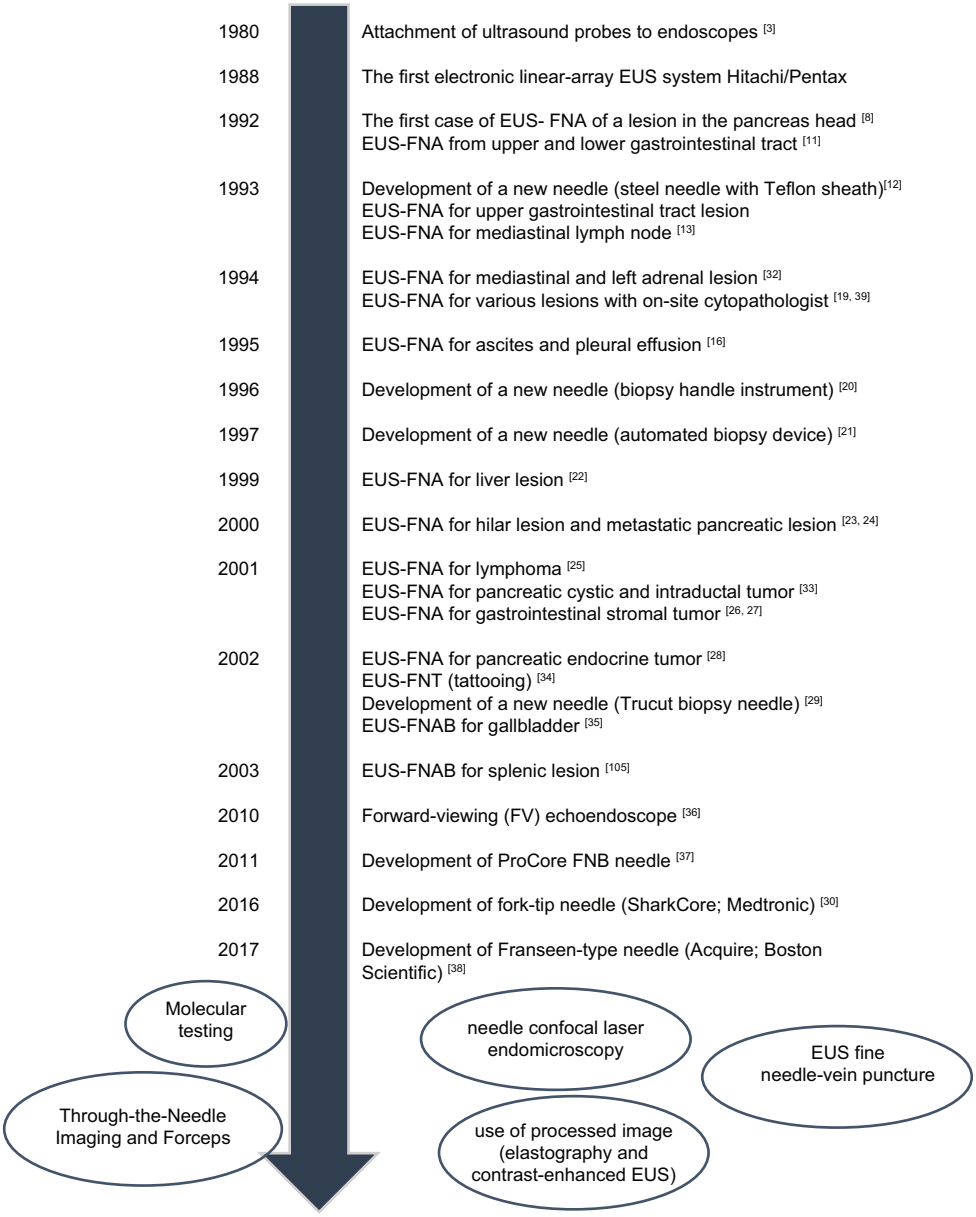

Figure 1. The history of EUS-guided fine-needle aspiration

useful for other therapeutic interventions besides EUS-FNA.[51,52]

Older mechanical radial echoendoscopes have been substituted by electronic radial echoendoscopes that produce significantly better images.[53] Radial-array echoendoscopes are used only for diagnostic EUS examinations and consequently have limited applications because tissue sampling and therapeutic interventions are not feasible.[54,55]

Technical advances have significantly extended the role of EUS and its clinical applications. The diagnostic and therapeutic capabilities of EUS continue to emerge and improve. EUS has become more than a tool to distinguish different tissue densities;[56] tissue can now be depicted in great detail using modalities such as elastography;[57,58] the tissue vascularity can now be described with increasing precision.[58] Using these various techniques, targets for biopsy can be precisely pinpointed. On reaching the target, tissue can then be examined microscopically in real time, ensuring optimal targeting and diagnosis.[59]

There is a bright future ahead for EUS in the years to come as extensive research is conducted in this field and various technologies are continuously implemented into clinical practice.

DIAGNOSTIC ROLE OF EUS-FNA

Since its initial report 25 years ago, EUS-FNA has now become part of the diagnostic and staging algorithm for the evaluation of benign and malignant diseases of the gastrointestinal tract and of bordering organs. Its introduction represents an important breakthrough in the endoscopic field. The EUS-FNA technique has remarkably enhanced the diagnostic potential of EUS, decreasing the number of surgical interventions for diagnostic sampling.

The use of EUS-FNA in clinical practice has been increasing lately. Roy *et al.*[60] investigated the tendency in tissue acquisition (TA) in pancreatic diseases in the United States over a period of 5 years (2006–2010). The use of EUS-FNA increased by 69.3%, surgical biopsy decreased by 41.7%, and the use of percutaneous biopsy remained stable.

Figure 2. (a) The BNX system with 19-gauge (G), 22G, and 25G needles allows multiple needle exchanges through the outer sheath (Image courtesy of Beacon Endoscopic and used with permission). (b) The Echo Tip ProCore needle has a reverse bevel design for acquiring a tissue specimen. The 22G and 25G needles are shown. (c) A close-up view of the tip of the ProCore 25G needle (Image courtesy of Cook Medical and used with permission). (d) The nitinol-based Expect Flex 19G fine aspiration needle is more flexible than its stainless steel predecessors and appears more promising for use in the duodenum. (e) An extreme close-up view of the expect 19G needle (Image courtesy of Boston Scientific and used with permission). (f) The clear view EUS-guided fine aspiration needle. The distal 2 cm of the needle are laser-etched to enhance visibility (Image courtesy of ConMed Endoscopic Technologies and used with permission)

EUS-FNA proved to be an effective technique, superior, and safer than computed tomography (CT)-guided or ultrasonography-guided percutaneous TA in the evaluation of small lesions.[61-64]

The indications for EUS-TA include diagnosis and staging of lesions, solid or cystic, within and proximal to the gastrointestinal tract, including esophageal, gastric, rectal, and pancreaticobiliary malignancies [Table 1].[65-84] It is also used in case of gastrointestinal subepithelial lesions (SELs), intra-abdominal and mediastinal lymphadenopathy, lung lesions, or adrenal masses.[85,86]

EUS-FNA represents the standard method for the pathological diagnosis of solid pancreatic masses [Figure 3].[44] It has been proven to be very accurate (sensitivity 85%–89% and specificity 96%–99%) according to three meta-analyses.[87-89] Furthermore, EUS is also an advanced staging method as it allows

Tabel 1. Common indications for EUS-FNA

1. Pancreatic lesions (solid and cystic)[65-67]
2. Biliary strictures[68,69]
3. Liver lesions (metastasis, hepatocellular carcinoma)[70]
4. Esophageal or gastric wall thickening[71-73]
5. Lymph node staging in case of esophageal, gastric, rectal, or lung cancer[74-76]
6. Subepithelial gastrointestinal tumors[77,78]
7. Assessment of mediastinal and abdominal lymph nodes[79]
8. Left adrenal gland lesions[80,81]
9. Splenic mass[82]
10. Peritoneal carcinomatosis[83]
11. Perivascular tumor extension and tumor thrombus[84]

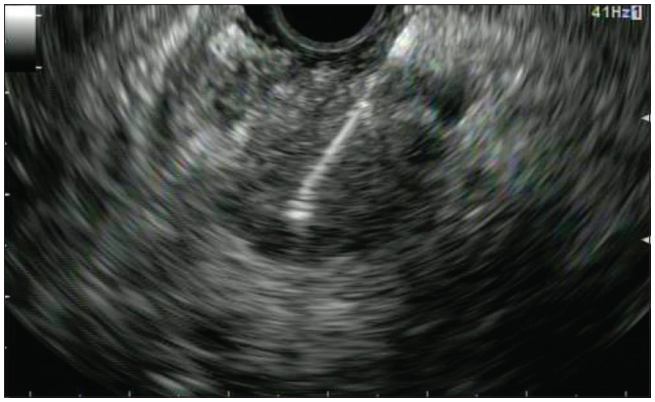

Figure 3. EUS-FNA of a pancreatic mass (re-used with permission)

the sampling of locoregional and distant lymph nodes, liver lesions, and ascites usually undetected by other imagining techniques.[90]

A meta-analysis comprising 18 studies and 1438 patients showed that cytopathological examination of pancreatic cystic lesions has a pooled sensitivity and specificity of 54% and 93%, respectively.[91] Another meta-analysis[92] revealed good specificity but poor sensitivity for EUS-FNA-based cytology in differentiating benign from malignant intraductal papillary mucinous neoplasms. Major pitfalls in diagnosing pancreatic cystic lesions by EUS-FNA are the frequency of insufficient aspirates and difficulty in differentiating pathological mucin from gastrointestinal contaminant, secondary to a transgastric or transduodenal approach of EUS-FNA. EUS morphology and FNA-based cytology combined with cyst fluid analysis for potential molecular markers may help improve the overall accuracy of EUS-FNA in the diagnosis of pancreatic cystic lesions.[93]

EUS-FNA is an unsterile procedure. This is important in the puncture of cystic lesions because the esophagus and stomach are not sterile. This can be neglected if the target is solid and perfused, taking into consideration the multiple defense strategies of the human body.[94] The circumstances are different in cystic lesions. Without a blood supply, the cystic fluid is vulnerable to infection, and consequently, peri-interventional antibiotic treatment is recommended.[95]

Many studies have reported the importance of performing EUS-FNA for mediastinal and abdominal lymph nodes.[96] One indication is the diagnosis of an enlarged lymph node at a distance from a known tumor [Figure 4]. Biopsying a lymph node through an area of a tumor should be carefully avoided because of the risk of false-positive results and needle track seeding.[95] According to a meta-analysis,[97] in the case of mediastinal lymph nodes, EUS-FNA had a higher sensitivity (88% *vs.* 84.7%) and specificity (96.4% *vs.* 88%) compared to EUS imaging alone. For abdominal lymph nodes, fewer studies have been conducted and the results showed that EUS-FNA is viable and safe. For instance, a prospective study with 142 patients with inconclusive or unfeasible percutaneous image-guided sampling showed that EUS-FNA was possible in 92% of the patients and it led to a diagnosis in 91% of cases.[98] Furthermore, when tuberculosis was not diagnosed using routine

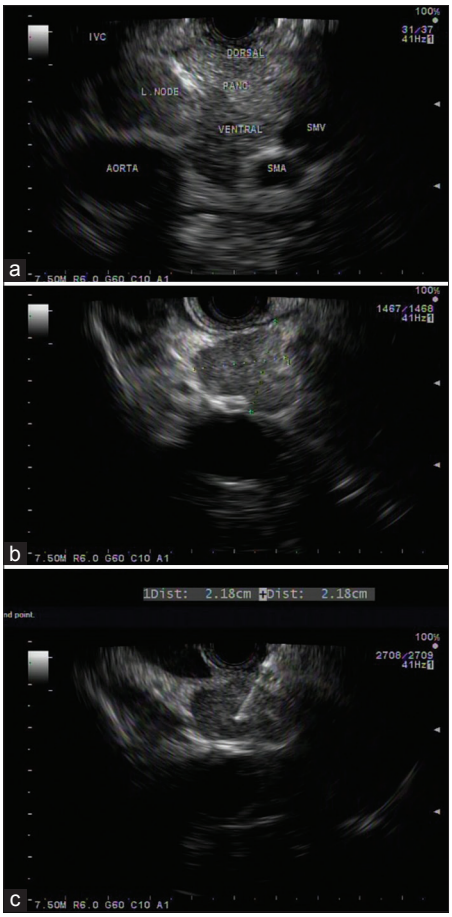

Figure 4. Patient with a history of colon cancer with aorto-caval lymph node on computed tomography positron emission tomography, underwent EUS-FNA for pathological diagnosis. (a) Hypoechoic, irregular, oval-shaped lymph node between the aorta and inferior vena cava; (b) close-up view of the lymph node; (c) EUS-FNA of the lymph node using a 25-gauge needle. Cytology: metastatic carcinoma. IVC: Inferior vena cava, SMA: Superior mesenteric artery, SMV: Superior mesenteric vein, L. node: Lymph node, PANC: Pancreas

methods, EUS-FNA of mediastinal or abdominal lymph nodes was extremely accurate.[98,99] EUS-FNA has also proved its benefit in diagnosing sarcoidosis[100,101] and lymphomas.[102-104]

EUS-FNA has a high accuracy for patients with lung cancer and metastatic mediastinal lymph nodes.[105,106] When EUS is available, it is safer and less invasive than mediastinoscopy,[107] and according to the European guidelines, the combination of EUS-FNA and endobronchial US-guided transbronchial needle aspiration is at present considered the first choice for staging of nonsmall cell lung cancer.[108] In nondiagnosed pulmonary lesions, EUS-FNA can be useful in diagnosing tumors with lymph node involvement if the tumors cannot be reached directly by bronchoscopy. Non-small cell carcinoma and small

cell carcinoma can be distinguished by FNA of the involved lymph nodes.[109] The resulting material can be used for further testing, such as epidermal growth factor receptor analysis, for more targeted therapy.[110]

Two meta-analyses have shown that EUS-FNA had 66% and 80% overall sensitivities and 100% and 80% specificities in diagnosing malignant biliary strictures.[111,112] Recent studies that were not included in the meta-analyses reported similar results.[113] However, a major concern regarding EUS-FNA of biliary strictures is the potential for needle track tumor seeding. For patients with unresectable cholangiocarcinoma limited to the liver and bile ducts, liver transplantation is one of the most successful therapies.[114] Immunosuppression following transplantation may increase the risk of tumor recurrence in patients with peritoneal tumor seeding. Accordingly, performing EUS-FNA of a primary unresectable bile duct tumor is considered contraindicated in case of potential liver transplantation.[115] On the other hand, another study showed that performing EUS-FNA in patients with cholangiocarcinoma has no impact on overall survival or progression-free survival. Even though tumor cell dissemination can occur along the needle track during EUS-FNA, its clinical impact was not considered significant.[116] This is a controversial issue, and many centers continue to avoid EUS-FNA of biliary strictures that may be treated with curative surgical intent or liver transplantation.

In addition, EUS-FNA is a valuable tool for diagnosing SELs of the upper gastrointestinal tract.[117] The sensitivity, specificity, and accuracy of EUS-FNA for diagnosing subepithelial mesenchymal tumors of the upper gastrointestinal tract were 82.9, 73.3, and 80%, respectively.[118] In a retrospective study including 121 patients, using a FV linear echoendoscope and a 19-G needle, the diagnostic accuracy for SELs of the stomach, duodenum, and rectum was 93%.[52] Furthermore, a new core needle (SharkCore, Medtronics) showed superior diagnostic yield, compared with a standard aspiration needle, for obtaining material suitable for the immunohistochemical differentiation of benign subepithelial gastrointestinal tumors from potentially malignant gastrointestinal stromal tumors.[119]

A meta-analysis has also shown that EUS-FNA improves the sensitivity (from 84.7% to 96.7%) and specificity (from 84.6% to 95.5%) compared to EUS imaging alone in assessing nodal stage of esophageal cancer.[120] EUS-FNA targets lymph nodes that are not in the proximity of the tumor (the needle should not puncture the tumor because of the risk of false-positive results and needle track seeding). Regional and distant lymph nodes as well as metastases can be the targets as well.[121,122]

There is an increasing interest in using EUS-FNA for liver lesions. According to a multicenter survey, EUS-FNA of the liver diagnosed malignancy in 89% of cases after inconclusive FNA under transabdominal US guidance.[123] Liver biopsy represents an important feature in the diagnosis of liver diseases. Recently, EUS was used to obtain liver biopsy.[124] Studies have shown that EUS-guided biopsy using a 19-G needle was safe, yielded adequate tissue for diagnostic purposes of liver disease.[41]

EUS-FNA of splenic lesions represents an indication for EUS when CT- or US-guided FNA is nondiagnostic or not feasible. EUS-FNA can diagnose splenic tuberculosis, sarcoidosis, Hodgkin's disease, colon cancer metastasis, abscess, and infarction.[125]

The potential utility of EUS-FNA in case of posterior mediastinitis was described[31] to provide material to identify specific agents, such as bacteria or fungi, followed by culture and selection of appropriate therapy.

A new application of EUS-FNA has recently been described for patients with severe gastroparesis. EUS-FNA of the antral muscularis propria with a 19-G needle provided adequate samples for the evaluation of the loss of interstitial cells of Cajal in 11 of 13 patients; a positive correlation between results obtained with surgical and endoscopic specimen was noticed.[126]

Several studies[127-129] have shown the utility of bedside EUS in critically ill patients. The bedside EUS-FNA was feasible and could offer an alternative in life-threatening situations. Consequently, endosonographers should consider using EUS in Intensive Care Unit patients when clinically indicated.

HOW TO ACHIEVE EXCELLENCE?

Tissue acquisition using EUS has considerably advanced since the first EUS-FNA was reported 25 years ago. Numerous studies have tried to determine the ideal EUS-FNA equipment and techniques. Within this

basic technique, more complex issues to enhance the diagnostic yield of EUS-FNA have been studied.[130] Multiple factors may contribute to the results of EUS-TA. These factors include experience level of the endosonographer, sampling site, location of the lesion, sampling technique, needle size and type, presence of rapid on-site pathologist, and various methods of processing the tissue sample obtained.[131]

A first step in performing high-quality EUS-FNA is adequate training. EUS-FNA is technically challenging, with a prolonged learning curve, and requires appropriate training.[132-135] The American Society for Gastrointestinal Endoscopy (ASGE) recommends 150 supervised EUS procedures before competency should be assessed, 75 of which must evaluate the pancreatobiliary system and 50 must incorporate EUS-FNA.[133] A recent systematic review highlights that the number of EUS procedures required to achieve competency remains unclear but has clearly risen above the current ASGE recommendations.[136] Each trainee requires individualized assessment to ensure competency is achieved before entering independent clinical practice. Nonetheless, it is reasonable to assume that endoscopists performing a high volume of FNAs are likely to have more success because the procedure is highly operator dependent.[44]

Adequate sedation for EUS-FNA is important because these procedures are typically longer than standard endoscopy.[137] Sedation is usually provided with conscious sedation or with monitored anesthesia care. Propofol is preferred because often the length of the procedure may be unpredictable, especially if a lesion is difficult to find.[138] Moreover, a poorly sedated patient is predisposed to an increased risk of complications.[44] A retrospective study[139] showed that the use of general anesthesia (GA) in case of EUS-FNA of pancreatic lesions is associated with an increased diagnostic accuracy (83% with GA compared with 73% without GA). Despite these results, in most institutions, the procedure is performed using moderate sedation or monitored anesthesia care.[140] Other intravenous anesthetic agents including benzodiazepine-opioid drugs are used, but their respiratory–inhibitory effects limit their application.[141] Moreover, many EUS procedures require water injection into the digestive tract, increasing the risk of aspiration during anesthesia. In such a scenario, studies showed that nitrous oxide sedation represents a safe and effective choice in patients undergoing EUS-FNA.[142]

Another key point to improve TA is a puncture technique adapted to the target. After identifying the target lesion, it is important to determine the scope position and to decide which part of the lesion is the most suitable for sampling to obtain the best diagnostic accuracy.[140] Even though the risk of tumor seeding via the EUS needle tract is very low, cases of seeding the stomach wall have been reported.[143] A site with minimum number of blood vessels should be chosen using Doppler imaging to avoid bleeding complications.

Studies have shown that the passage of the needle with or without the stylet has no impact on diagnostic accuracy.[144-148] Suction may increase sensitivity, but it contributes to bleeding, and thus its use is also variable.[149,150] High diagnostic yields have been obtained without suction in case of lymph node sampling[151] and with the stylet pull technique (pulling the stylet while moving the needle within the lesion) for solid lesions.[152] Further studies regarding the value of the slow pull technique are needed, some have adopted this technique in their practice, while others continue to use the conventional 10–20 mL negative suction pressure for EUS-FNA.[153] A novel "wet-suction" technique (where the EUS needle is flushed with saline solution to replace the column of air within the lumen)[154] was shown to increase tissue specimen adequacy (85.5% *vs.* 75.2%) when using 22-G FNA needles.[155] Villa *et al.*[156] showed that the samples were of higher quality, and furthermore, the diagnostic yield for the wet suction technique was significantly better than the conventional method.

The methods used during FNA are also debated. Taking into consideration that inner portions of a pancreatic tumor may be necrotic, targeting the peripheral areas of the mass can improve diagnostic yield. However, obtaining adequate tissue for diagnosis is still a challenge because of the dense desmoplastic reaction at the periphery. Therefore, endosonographers can use the "fanning" technique, which involves adjusting the trajectory of the needle, using the elevator or dials on the head of the echoendoscope. Thus, instead of advancing the needle back and forth through the same portion of the mass, it samples different areas.[157] A study comparing fanning with standard TA during EUS-FNA showed superiority of the fanning technique after a single pass as compared to standard technique (86% *vs.* 58% diagnostic yield).[158] Recently, the door knocking method, where the needle is rapidly advanced within the target lesion,

has been developed. Mukai *et al.*[159] showed that the door knocking method enabled the acquisition of larger specimens compared to standard method. Nakai *et al.*[160] passed a biopsy forceps through a 19-G FNA needle to perform direct biopsy and found that the TA rate for a single puncture was 67% with the biopsy forceps alone but increased to 88%, when this technique was combined with regular EUS-FNA, indicating that this is a useful technique for acquiring tissue with a small number of needle passes.

NEEDLES

A diversity of needles has been used during the past 25 years, with innovations continuing to appear. The history of EUS needles is dynamic, with new needles coming out nearly annually.

In recent years, new needles designed to obtain samples suitable for histologic evaluation, with preserved tissue architecture, have been developed. These FNB needles feature either a special geometry of the cutting tip or a side-slot in the distal portion of the needle. Conventional needles without these refinements are referred to as FNA needles.[46]

All EUS-FNA needles have the same basic design and are currently single use. The various commercially available FNA needles have different echogenicity under EUS guidance. The visibility of the needle tip is critical when performing FNA,[157] and to improve it, needle tips are tailored using different techniques such as laser etching, mechanical dimpling, or sandblasting.[161,162] A multicenter study evaluated and graded 10 different EUS needles based on their echogenicity. A prototype needle with polymeric coating had significantly higher overall ranking, indicating that this coating to the needle tip and shaft may enhance visualization.[163]

Needles with a side hole at the tip have been developed as core biopsy needles, and numerous reports have investigated their utility. The EchoTip ProCore™ has allowed diagnosis with fewer needle passes than conventional needles without side holes, but with no significant difference in diagnostic adequacy and accuracy.[164,165]

NEEDLE SIZE: SMALL *VS.* LARGE

Today, four different needle sizes are available: 19-G (aspiration and core biopsy), 20-G (core biopsy), 22-G (standard size, aspiration, and core biopsy), and ultrathin 25-G needles. The most widely used needle for EUS-FNA is the 22-G needle,[166] which is flexible and enables cytologic assessment without significant risk for complications, although a 2% risk of acute pancreatitis was reported in a retrospective study.[167]

For FNA of solid lesions, 25-G or 22-G needles are the most widely used, while 22-G needles are usually used for cystic lesions.[168] Eight randomized clinical trials compared 22-G and 25-G needles in patients with solid masses and lymph nodes[103,169-171] or only with solid pancreatic masses.[172-175] One study has shown a higher accuracy for the 25-G needle;[169] the others have shown no significant difference in diagnostic accuracy. Studies comparing FNA with 25-G and 22-G needles were also subjected to four meta-analyses[176-179] that provided conflicting results. The recent meta-analysis by Facciorusso *et al.*,[176] comprising only randomized clinical trials, did not show significant differences between the needles in terms of sensitivity and specificity for pancreatic malignancy. On the other hand, Xu *et al.*[177] demonstrated higher sensitivity for 25-G needle with no significant difference in specificity for malignancy in patients with solid pancreatic masses. The other two meta-analyses were published in 2013 but did not contain recent information and had important limitations such as significant heterogeneity and inclusion of retrospective data.

The 19-G needles are more rigid, and consequently, transduodenal biopsies are more difficult.[166] These devices were developed to obtain larger amounts of material from the targeted lesions; however, compared to the 22-G needle, the 19-G needle has a higher rate of technical failure. One study showed that the 19-G needle had a higher diagnostic accuracy than the 22-G needle, but technical failures were not taken into consideration.[180] Twenty-five-G needles had the highest diagnostic accuracy for uncinate masses. In case of pancreatic body and tail lesions, no significant difference between the three types of needle was found.[181]

The use of nitinol for 19-G FNA needles has increased their flexibility. A multicenter study revealed no significant difference regarding diagnostic accuracy between the 22-G and the novel 19-G flexible needle made of nitinol, but histological core tissue was obtained in a larger number of patients by using the 19-G flexible needle.[182] Nineteen-gauge FNA has been able to obtain adequate samples in case of liver

biopsies.[43,183-185] Other studies have shown higher diagnostic yields with 19-G needles when performing SEL biopsies, which typically have lower diagnostic accuracy with 25-G and 22-G FNA needles.[186]

EUS-guided TA can be obtained by EUS-FNA or EUS-FNB. The needle tip design is the distinguishing feature between FNA and FNB because the procedural techniques are comparable.

Although tissue histology has been proved to be important for the diagnosis of autoimmune pancreatitis,[187] Hodgkin's lymphoma,[188] and well-differentiated adenocarcinomas,[189] the utility of histology for pancreas sampling has been evolving.

The initial EUS-FNB needle was the 19-G Tru-Cut. It had limited flexibility and consequently was replaced by the ProCore FNB needle by the same manufacturer, which is currently available in a range (19, 20, 22, 25-G) of sizes. A multicenter randomized clinical trial showed that the ProCore 19-G needle was superior to the Tru-Cut needle, with higher diagnostic accuracy (88% *vs.* 62%; $P = 5.02$).[43] A new variant of the ProCore needle (20-G) was introduced with a forward-facing direction of the side bevel. Two newly developed needles (SharkCore, Medtronic, Dublin, Ireland; and Acquire, Boston Scientific, Natick, MA, USA) are designed with two or three opposing sharp points and a multifaceted bevel in the needle tip, aimed at capturing a core of tissue. According to the first results, the TA was significantly higher than using standard aspiration needles, and diagnosis was possible with fewer needle passes.[30,45,190,191]

There is space for improvement: intermediate-size needles (20-G or 21-G) might be more useful for combined cytology and histology sampling or use of auxiliary devices inside the sheath of the 19-G needle. Perhaps in a more innovative vision, bulky scopes could be abandoned and more flexible luminal robotic-driven devices can be used to access and puncture the targets.

PRESENCE OF ON-SITE CYTOPATHOLOGIST

The presence of a cytopathologist during EUS-FNA with rapid on-site evaluation (ROSE) of the samples has been shown to enhance the diagnostic yield by reducing inadequate samples and decreasing the need for additional passes.[87,192-197] The diagnostic accuracy of EUS-FNA was over 90% in most studies when ROSE was used.[198] One or two passes are typically made, and then, the pathologist evaluates the adequacy of the samples and decides if further passes are needed to achieve diagnostic success. If bloody aspirates are noticed by the on-site cytopathologist, a smaller gauge needle is used, without suction.[157] Limitations of ROSE include increased cost due to cytopathologist time commitment, as well as low reimbursements for ROSE.[199] The number of institutions capable of having a pathologist when EUS-FNA is performed is limited,[200,201] and this is a factor that at least hinders the widespread use of ROSE.

Another emerging concept is telecytopathology.[202] The slides are initially prepared and prescreened by a cytotechnologist or pathology resident and then analyzed by an off-site cytopathologist using real-time remotely operated system.[203] A retrospective study demonstrated the potential use of telecytopathology as a valid substitute for on-site evaluation of pancreatic tumors by EUS-FNA.[204]

Recent studies have shown that macroscopic on-site quality evaluation (MOSE) also improves diagnostic accuracy.[153] Iwashita *et al.*[205] analyzed the size of the macroscopically visible core (MVC) and the diagnostic yield of EUS-FNA. Results showed that MVC of ≥4 mm is an indicator of specimen adequacy and can enhance diagnostic yield. Moreover, MOSE represents a procedure that can be carried out by an endoscopist and can be regarded as obligatory for endoscopists who intend to improve their diagnostic accuracy.

NUMBER OF FINE-NEEDLE ASPIRATION PASSES

In the absence of ROSE, earlier studies recommended 5–7 passes in case of a pancreatic mass.[206,207] Other studies, however, reported that fewer passes are sufficient,[208,209] and Itoi *et al.*[200] found that even without ROSE, mean 2.88 needle passes were adequate for diagnosis, with 93.3% accuracy, 91.8% sensitivity, and 100% specificity.

When ROSE is available, the maximal diagnostic yield can be obtained after 2–5 passes for pancreatic masses.[210,211] Erickson and Garza[212] also showed that with ROSE, the average number of needle passes was 3.4 ± 2.2 (range 1–10), but it was influenced by

the tumor grading. Wani *et al.*[146] carried out a similar study and found that well-differentiated tumors were a predictive factor for a larger number of needle passes.

CAN WE DO IT BETTER? FUTURE DIRECTIONS

Through-the-needle imaging and forceps

Miniaturized devices such as a biopsy forceps or confocal microscopy fiberoptic probes have been passed through 19-G EUS-FNA needles to evaluate cystic and solid lesions.[213,214]

Small forceps passed through 19-G needles have been developed for pathologic diagnosis of pancreatic cystic lesions.[160,215-217] The use of mini-forceps through an FNA needle has been proven feasible and safe for pancreatic TA.[160] While only a pilot study has been completed, this initial report suggested high diagnostic sensitivity with no device failures or complications. This may offer an attractive alternative for the future. To date, comparisons of forceps samples to other EUS-TA techniques have not been published.[168]

THE USE OF NEEDLE CONFOCAL ENDOMICROSCOPY

Confocal laser endomicroscopy (CLE) is a novel endoscopic method that allows microscopy of the gastrointestinal mucosa during ongoing endoscopy, enabling real-time optical biopsy.[218] Technical advances allowed a confocal miniprobe to be passed through the biopsy channel of the endoscope.[219]

As probe-based confocal endomicroscopy has been miniaturized, needle-based confocal laser endomicroscopy (nCLE) has become available for clinical use. The nCLE miniprobe has 0.85 mm diameter and can be passed through a 19-G EUS-FNA needle.[220] Needle-based CLE was designed to allow *in vivo* histological images using fluorescent contrast. Therefore, nCLE could show which areas are most suspicious for malignancy and require biopsy.[221] EUS-guided nCLE seems to be a promising minimally invasive technique that might be used to improve the diagnostic accuracy of EUS-FNA. Optical needle biopsy could also be useful in reducing sampling errors because it provides real-time microscopic details, especially in cystic masses.

The diagnostic of pancreatic cysts is sometimes difficult. Results of studies using n-CLE have been very promising, and in the future, nCLE may be used routinely for diagnosing pancreatic cysts as an adjunct to conventional EUS-FNA.[213,222,223] Novel vascular patterns have been described, and a classification of nCLE patterns of pancreatic cystic lesions was established, facilitating their diagnosis.[224] nCLE was found to be safe and feasible with high technical success.[225] These promising findings require validation in larger multicenter studies to justify routine use of EUS-nCLE for the evaluation of pancreatic cystic lesions.

Studies have also evaluated the feasibility and safety of nCLE for the assessment of solid pancreatic masses and lymph nodes.[226] nCLE identified 77% of the cases in which malignancy was confirmed on histology. However, more studies with various other contrast agents and targeted markers need to be performed to improve diagnostic accuracy. Given the low negative predictive value of EUS-FNA, nCLE could help rule out malignancy after a previous inconclusive EUS-FNA.[227] The benefit of nCLE in the evaluation of solid pancreatic masses and lymph nodes is still unclear, and further studies are needed. This technique may also have significant implications in the field of molecular imaging by allowing the *in vivo* visualization of pathophysiologic events in their natural environment.[228-230]

Although it is unlikely that nCLE will replace EUS-FNA cytology for pancreatic masses and lymph nodes, it can be complementary to FNA for diagnosis during EUS.[231]

TARGETED EUS-FNA DURING CONTRAST-ENHANCED HARMONIC IMAGING AND ELASTOGRAPHY

Contrast-enhanced harmonic EUS (CH-EUS) and elastography have been proposed as methods for better targeting of the lesions.[232,233]

The use of US-contrast agents may enable the recognition of better puncture sites based on the differences in blood flow patterns. Kitano *et al.*[234] have described the utility of EUS-FNA using CH-EUS. They showed that CH-EUS could identify pancreatic adenocarcinomas as hypoenhanced lesions, with a sensitivity and specificity of 88% and 94%, respectively.

Moreover, 80%–100% of false-negative EUS-FNA cases were correctly classified by CH-EUS. However, it is not possible to completely rely only on the vascular aspect of a mass to determine its nature, so FNA still represents the standard for the diagnosis of masses. Nevertheless, CH-EUS and EUS-FNA are complementary, not competitive, and are better performed together during the same investigation.[235]

According to another study,[236] EUS-FNA under CH-EUS imaging improved the diagnostic accuracy for pancreatic masses to 86.5% from 78.4% obtained with regular EUS-FNA. Furthermore, when the two methods were combined, the diagnostic accuracy increased to 94%. Sugimoto *et al.*[237] showed that EUS-FNA under CH-EUS imaging needed fewer needle passes compared with conventional EUS-FNA, whereas Hou *et al.*[238] stated that the percentage of adequate biopsy specimens in the CH-EUS group (96.6%) was greater than that in the EUS group (86.7%). These studies suggest that the use of CH-EUS in combination with EUS-FNA may enable the acquisition of adequate specimens and improve the true-positive rate.[153] CH-EUS-FNA is easy to perform and safe, with minimum added extra costs.[238] The results are promising, but the future will know more about its clinical impact and whether there is a change in the assessment of lesions.

Elastography is a technique that allows imaging and quantification of the hardness of lesions with special software.[239] By calculating the elasticity of tissue, it is possible to distinguish benign (soft) tissue from malignant (hard) tissue.[240] The most important advantage of EUS elastography is that it can be performed in real time during a diagnostic examination with immediate information provided to the endosonographer. Furthermore, it can help in selecting the site where FNA can be performed with improved diagnostic yield. This is extremely helpful in a subset of challenging patients with either necrotic tumors or possible malignancy in a background of diffuse inflammatory change.[241,242] It may therefore improve FNA targeting, at a low cost and without the need for extensive training in the use of the software, which is available on most processors.[243,244]

GOING BEYOND CYTOLOGICAL DIAGNOSIS

An emerging concept is the use of the cells aspirated during EUS to obtain information beyond a simple cytological diagnosis.

Along with the technical advances that increased the safety and accuracy of the procedure, there has been significant progress in understanding the biology of many tumors that are biopsied. Pancreatic cancer can be a good example to illustrate this idea. It is characterized by various molecular alterations, and therefore, identification and quantification of potential molecular markers for pancreatic cancer on the samples obtained by EUS-FNA could be a promising concept for the diagnosis of malignancy.[245,246]

Advances in molecular diagnostic techniques have made it possible to carry out various types of immunostaining and gene analyses using a small amount of specimen obtained by EUS-FNA.[65,247,248] Potential genes and proteins with roles in pancreatic carcinogenesis have been identified and it would be important to determine their clinical usefulness.[247,249]

Whole-genome sequencing and whole-exome sequencing analyses have confirmed that mutations in four genes are most commonly seen in pancreatic adenocarcinoma: K-ras, SMAD4, TP53, and CDKN2A/p16.[250,251]

K-ras

Several studies have shown that combining cytopathological and K-ras mutation analysis improves the diagnosis of pancreatic cancer in EUS-FNA samples.[252-258] Based on this combination, a medical or surgical conservative treatment can be applied, thus avoiding unnecessary pancreatic resection.[252] K-ras analysis seems important, especially in case of inconclusive EUS-FNA. In these cases, discovery of a K-ras gene mutation can spare an unnecessary repeat EUS-FNA procedure.[259] Moreover, in cases of doubtful cytopathological results, a K-ras mutation assay, when positive, can reduce false-negative results and thus avoids any delay in decision-making.[260] In direct EUS-FNA, sequencing techniques such as next-generation sequencing or pyrosequencing should be used. 5–10 ng of DNA is necessary to detect K-ras mutations using next-generation sequencing.[261,262] The detection of K-ras mutation could be useful not only in the diagnosis but also in staging, prognosis, evaluation of response to therapy, and follow-up of pancreatic cancer patients.[263] Even though further studies are certainly required, the presence of a K-ras mutation seems to negatively influence the prognosis.[264] One study reported that para-aortic lymph nodes diagnosed for K-ras mutation were independent pancreatic cancer prognostic markers in multivariate analysis.[265]

P53

TP53 status evaluation can also improve the sensitivity of EUS-FNA to diagnose pancreatic cancer.[266-268] According to a study, by combining p53 protein evaluation and cytopathological examination, the sensitivity increased to 90% (compared with 76%) and specificity remained equal to 91%.[266] In another study by Oshima *et al.*, loss of p53 protein was correlated with poor prognosis mainly if also combined with the loss of expression of p16 and SMAD4 protein.[269] Immunohistochemistry is most commonly used to detect p53 protein accumulation when the TP53 gene is mutated. Sequencing can also detect TP53 mutations.[270]

CDKN2A/P16

As for p53, the loss of p16 was correlated with poor prognosis in pancreatic cancer patients.[269] In a study performed on 101 pancreatic EUS-FNA samples, the allelic losses of CDKN2A/P16 gene, detected by analyzing the loss of heterozygosity (LOH) at 9p, revealed a sensitivity of 85% and a specificity of 64% in diagnosing patients with resectable pancreatic cancer.[271]

SMAD4

The allelic loss of the SMAD4 gene analyzed in EUS-FNA specimens by LOH at 18q showed a sensitivity and specificity of 78% and 57%, respectively.[271] The loss of SMAD4 expression is an independent prognostic factor and seems to be associated with tumor progression. Preoperative stratification based on SMAD4 could lead to appropriate treatment strategy.[272]

In the last 20 years, microRNAs (miRNAs) have become one of the most promising classes of diagnostic and prognostic biomarkers for human cancers. Numerous studies have shown the diagnostic reliability of miRNAs expression profiling in pancreatic cytology specimens.[273-276] In addition, cell-free cyst-fluid miRNAs have been demonstrated to be promising biomarkers for pancreatic cancer early diagnosis and for assessing high-risk pancreatic cysts.[277,278]

Numerous studies have also reported the usefulness of immunostaining of S100P,[246,279-285] mesothelin,[285-290] mucins,[287,291-295] or KOC/IMP3[279,282,296-298] in EUS-FNA samples. Those reports might help improve the efficacy of EUS-FNA for diagnosis of solid pancreatic masses.

Moreover, the tumor material obtained by EUS-FNA can be used to perform molecular investigations to better understand the physiopathology, carcinogenesis, and response to treatment of pancreatic cancer. Gemcitabine is the standard of care for the treatment of advanced pancreatic adenocarcinoma. However, gemcitabine resistance was related to limited intracellular uptake of gemcitabine through a decrease in human equilibrative nucleoside transporter (hENT1) protein expression.[299] Several studies have shown that high levels of hENT1 are associated with significantly longer survival after adjuvant gemcitabine,[300] and gemcitabine should not be used for patients with low tumor hENT1 expression.[301] The expression of hENT1 is epithelial, and consequently, it can be detectable in biopsies containing malignant epithelial cells. No study has yet shown the reliability of this assessment in either FNA or core biopsy samples.

One of the most exciting developments of cancer therapy in the last few years has been the remarkable progress of immunotherapy. Despite the minimal response rates in pancreatic cancer, an immune response is still present and emerging strategies to turn this response on or identify tumors with an immune-sensitive phenotype are promising. It would be therefore interesting to be able to characterize the infiltrating immune cells in EUS-FNA samples.

Another application of molecular testing is to perform polymerase chain reaction analysis and gene promoter hypermethylation on aspirated material collected during EUS-guided puncture of lymph nodes to detect micrometastases.[302-305]

EUS-GUIDED FINE-NEEDLE VEIN PUNCTURE

Circulating tumor cells (CTCs) can enter the bloodstream and can be extracted from blood samples. The identification of CTCs in blood is called "liquid biopsy."[306-308] EUS-fine needle vein (FNV) puncture can be used to obtain a liquid biopsy. Molecular analysis can further be performed for early detection and monitoring of cancer therapy.[309] EUS-FNV of the portal venous blood using a 19-G needle introduced transhepatically has been shown to be feasible and safe. Pancreatic adenocarcinoma cells were identified in higher levels in portal venous samples as compared to peripheral blood samples.[310]

CONCLUSIONS

There has been tremendous progress over the past decade to overcome the limitations of EUS-FNA due to sampling technique, procedure, and equipment. Refined techniques such as molecular analysis of EUS-FNA samples are being continuously investigated. EUS-FNA will play a major role in personalized cancer therapy in the years to come.

Research in EUS appears to be intensive as specialists continue to study the clinical impact of the procedure, to improve and refine the current indications for it, and to pursue new applications for the method.

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Conflicts of interest

There are no conflicts of interest.

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