EUS tissue acquisition: From A to B

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ABSTRACT

EUS-guided tissue acquisition (EUS-TA) has made rapid development since its introduction in the early 1990s. The technique is widely accepted and invaluable for staging and diagnosis of a variety of upper gastrointestinal and mediastinal lesions. Fine-needle aspiration (FNA) has long been the gold standard, but due to its limitations such as the inability to retain stroma and associated cellular architecture, novel biopsy needles (FNB) were designed. Overall, FNA and FNB needles perform seemingly equally in terms of diagnostic accuracy, however, the second-generation FNB needles require less passes. The third-generation FNB needles (crown-cut needle types) seem to be preferable to FNA needles as well as to the second-generation FNB needles, when larger histological specimens and preserved tissue architecture are required. EUS-TA is constantly under development, and new applications of this technique include tumor risk stratification according to its genetic profile as well as minimally invasive creation of patient-derived organoids, hallmarks of personalized medicine. It remains yet to be shown, whether these applications will lead to a decisive shift from aspiration to biopsy, i.e., from A to B.

Key words: EUS, FNA, fine-needle biopsy, pancreas

INTRODUCTION

The idea of coupling an ultrasound transducer with an endoscope came in the early 1980s when the first EUS examinations were performed.1,2 Early echoendoscopes were equipped with radial scanning mechanical transducers, providing good imaging resolution of the gastrointestinal wall as well as of the neighboring organs. However, EUS did not become universally accepted until the development of echoendoscopes with a linear array transducer, providing a possibility for ultrasonic guidance of a biopsy needle during the scanning procedure. The first dedicated biopsy instrument for EUS-FNA was developed by our group in the early 1990s [Figure 1], allowing for a precise diagnosis of even diminutive lesions, which was previously impossible unless surgery was performed.3-8 During this pioneering era, indications of EUS-guided tissue sampling were defined and include today staging of upper gastrointestinal and lung cancer, as well as investigation of lymph nodes, submucosal tumors, adrenals, pancreas, and the biliary tract.9 EUS-guided tissue acquisition (EUS-TA) was also proven to have a major clinical impact in aforementioned indications.

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sparing the patients for invasive diagnostic procedures, such as thoracoscopy, mediastinoscopy, laparoscopy, or open surgery. Today, EUS-TA is widely accepted and is the cornerstone of the diagnostic process both in gastroenterology and in pulmonology when combined with endobronchial ultrasound-guided transbronchial needle aspiration biopsy.\[^{[10]}\] Since the early 1990s, design of conventional needles has been further improved with the aim of harvesting more tissue for histology, challenging standard aspiration techniques. The aim of this review is to discuss new trends and directions in EUS-TA while reviewing data from previous studies with EUS-FNA and discuss results from recent studies with these novel fine-needle biopsy (FNB) needles.

NEEDLE DESIGN

The first needles developed for EUS-TA were aspiration needles (FNA) with a simple tip bevel.\[^{[8]}\] Studies evaluating these devices demonstrated a high overall diagnostic accuracy (87%–92%).\[^{[9,11]}\] However, while EUS-FNA performed very well in case of lymph nodes and extraluminal masses, the diagnostic accuracy in case of gastrointestinal wall lesions was moderate (67%–84%).\[^{[9,11]}\] This, together with the need for specimens with preserved architecture (in case of lymphoma, autoimmune pancreatitis, neuroendocrine tumors, or other), led to the development of biopsy needles (FNB) with a beveled side-slot (Quick-Core\(^{\text{®}}\), ProCore\(^{\text{®}}\), Cook Medical, Bloomington, IN, USA), considered to be the first and second generation of FNB needles, respectively. The beveled side-slot design was modified to be forward-facing (20G ProCore\(^{\text{®}}\), Cook Medical, Bloomington, IN, USA) in an attempt to improve TA, but no data supporting this are currently available. The third-generation FNB needles incorporate various designs but may be uniformly characterized as crown-cut needles. The cutting tip of one of these needles is shaped as a fork-tip, with two opposite cutting edges, and became available in 2014 (SharkCore\(^{\text{TM}}\), Medtronic, Minneapolis, MN, USA). Another needle has three cutting edges symmetrically distributed at the tip – a Franseen needle type (Acquire\(^{\text{TM}}\), Boston Scientific, Marlborough, MA, USA). Recently, another Franseen-type needle has become available with a slight adjustment of the angle of the cutting edges of the spikes compared to the Acquire\(^{\text{TM}}\) needle (TopGain\(^{\text{®}}\), MediGlobe, Achenmühle, Germany). An overview of different needle designs is presented in Figure 2.

EUS needles are composed of different materials, including aluminum, stainless steel, chromium-cobalt, and nitinol, offering various degrees of hardness and tensile properties. Nitinol needles, for example, are more flexible and preferred in cases where the echoendoscope is fully angulated, for example, with lesions in the uncinate process. Furthermore, all needles undergo postproductional modifications (by polymer coating, laser etching, mechanical dimpling, or sandblasting) in order to enhance the visualization of the needle tip during TA. The echogenicity of the currently available needles is dissimilar, but the difference has not been shown to impact neither tissue quality nor quantity.\[^{[12]}\]

Performance of different needle designs has been tested in several randomized controlled trials (RCT) and meta-analyses as well as numerous low-quality studies. The reversed bevel design has been most thoroughly examined in several RCTs and two meta-analyses evaluating needle performance in various solid lesions and lymph nodes.\[^{[13,14]}\] Compared to the FNA-needle, no overall difference was observed in sample adequacy, histologic core procurement rate, and adverse event rate.\[^{[13,14]}\] However, in order to reach a diagnostic sample, fewer passes were needed with the reversed bevel FNB needle compared to FNA needle.\[^{[13,14]}\] Subgroup analysis of nonpancreatic lesions, for example, submucosal tumors, are lacking in the

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**Figure 1.** Prototype biopsy instrument (GIP Medizin Technique/MediGlobe), type Hancke/Vilmann
aforementioned studies, and FNB needles may be useful in these cases. A meta-analysis by Facciorusso et al. evaluating diagnostic accuracy in subepithelial lesions, further strengthened the superiority of the FNB sampling in these lesions. In an interesting cost-analysis model originating from an RCT, FNB sampling was shown to have an overall lower cost compared to FNA, mainly caused by a higher diagnostic yield of these needles and lack of rapid on-site evaluation (ROSE) in the FNB group. As ROSE is not systematically utilized outside US, results from this study may not apply to other countries.

As for the third-generation FNB needles, no difference between the fork-tip and Franseen needles was observed in terms of diagnostic accuracy and histologic core procurement rate. Furthermore, comparison of the two needles with the standard FNA needle was only reported for sample diagnostic adequacy, an outcome with no clearly established clinical significance, where both needles outperformed the FNA needle. A retrospective comparative study found the second-generation ProCore® needle to be inferior to the third-generation SharkCore™ needle regarding sample adequacy for histological analysis. Facciorusso et al. evaluated currently available needle designs and sizes in a recent large network meta-analysis including results from 2711 patients. This study, restricted to solid pancreatic lesions, failed to show any difference between FNA and FNB needles in terms of diagnostic accuracy, sample adequacy, or histologic procurement rate. Evidence regarding the newly developed 20G ProCore® needle with forward bevel and Franseen-type TopGain® needle is scarce, with no comparative studies currently available. Even though many studies have shown that different needle designs seem to have similar rates of diagnostic accuracy and histologic procurement, only a few studies have evaluated the actual size of the tissue obtained. Bang et al. were the first to measure the size of the specimen obtained by the Acquire™ needle (median size 2.9 mm) as well as median tumor percentage in the tissue. This quality parameter was followed by Karsenti et al. who, in a prospective setting, compared the new 20G Procore® with the 22G Acquire™ needle. The length of tissue core biopsies per needle pass was significantly higher with the Acquire™ needle compared to the ProCore® needle (mean 8.2 vs. 4.2 mm, respectively).

### NEEDLE SIZE

EUS needles are available in different sizes, ranging from 25G (0.46 mm) to 19G (0.91 mm). The standard needle is considered to be a 22G (0.64 mm) FNA needle. The effect of the needle size on diagnostic performance has been evaluated in several studies. Whereas a single older meta-analysis found a trend toward higher sample adequacy for the 25G needle, other more recent meta-analyses failed to replicate this finding. Most studies compared 25G and 22G needles, and comparisons between 19G and thinner needles are sparse. In terms of diagnostic accuracy, no difference between 19G and 22G needles was observed, and the only RCT comparing the 19G with 25G needle was retracted from publication. It should be underlined that even though diagnostic accuracy as well as sample adequacy may be similar between the different needle sizes, no comparison of the size of the procured micro-cores has been reported.
TECHNICAL ASPECTS OF TISSUE SAMPLING

Rapid on-site evaluation (ROSE) involves immediate assessment of the procured tissue by a cytopathologist or a cytotechnician present during the procedure. Three available RCTs failed to show an advantage of ROSE in terms of diagnostic accuracy, sample adequacy, or quality. However, fewer FNA passes are required when ROSE is utilized. Results from meta-analyses are conflicting; whereas older studies showed increased diagnostic accuracy when using ROSE, newer studies reported no difference. Availability of ROSE service is variable, and ROSE is more utilized in US compared to Europe and Asia. Application of negative pressure during TA seems to improve diagnostic accuracy. Standard suction technique implies application of negative pressure with a 10 or 20 mL syringe, and seems to increase diagnostic accuracy and sensitivity when compared to no suction. Increasing the negative pressure (50 mL) does not seem to improve accuracy for malignancy. In a single RCT, preflushing of the needle with saline (wet-suction) improved sample adequacy and quality compared to standard suction. However, diagnostic accuracy was not reported. Effect of slow removal of the stylet (slow-pull technique) during sampling has been evaluated in several RCTs, and found comparable to standard suction. At the end of the sampling procedure, negative pressure persists in the needle and may lead to increased tissue contamination from the puncture site during needle removal. Neutralizing residual negative pressure is, therefore, recommended prior to the removal of the needle. Fanning technique involves continuous back-and-forth movements of the needle in different areas of the lesion of interest. In one RCT, the fanning technique was shown to decrease the number of passes required to establish a diagnosis, but overall difference in diagnostic accuracy was not statistically significant due to a small sample size. Another prospective, nonrandomized trial showed superiority of the fanning technique combined with “slow-pull” in terms of diagnostic accuracy and blood contamination. Similarly, the effect of the needle stylet has been evaluated in several RCTs and two meta-analyses. However, the use of the stylet does not increase diagnostic accuracy, adequacy nor yield, and current ESGE guidelines do not recommend for or against its use.

SAMPLE PREPARATION AND PROCESSING

Samples obtained during EUS-TA contain free cells, blood contaminants, and/or tissue micro-cores, and several different methods of sample processing exist which vary between different centers. Direct smears are performed by a majority of respondents in an international survey, and glass slides can be left to air-dry, immersed in alcohol, or fixated by a spray-based fixative. An alternative to the aforementioned is the liquid-based cytology, where the sample is transported in a liquid medium (saline, alcohol, or Cytolyt). However, if no fixative is used (in case of saline), the sample should be promptly transferred to the cytology laboratory in order to minimize cellular degeneration. Creation of cell blocks involves utilization of the centrifuged pellet, usually by adding plasma and thrombin in order to form a clot (cell block), which is then processed as histology. This method is shown to be superior to the conventional smear cytology in several prospective, nonrandomized trials. Nonetheless, the difference between the two methods was not statistically significant in a recent meta-analysis including only subepithelial tumors. Micro-cores (visible tissue fragments) are usually processed separately and fixated in formalin and embedded in paraffin. Histological preparation methods, including the cell block technique and direct preparation of micro-cores, are optimized for subsequent immunohistochemistry and downstream molecular analyses, which are increasingly utilized.

ADVERSE EVENTS

EUS-TA is an established and safe procedure with an overall risk of adverse events as low as 0.29%. Adverse events described include hemorrhage (0.15%–3.7%), acute pancreatitis (0.29%–2.0%), and infection (0.4%–3.9%). Observed hemorrhage is in most cases self-limiting, and EUS-TA can be safely performed even in patients treated with aspirin and nonsteroidal anti-inflammatory drugs. However, P2Y12 receptor antagonists and oral anticoagulants should be discontinued prior to EUS-TA in order to minimize the risk of hemorrhage. Risk of infection is considered generally low when performing EUS-TA of solid lesions, even in transrectal and/or transcolonic approach, and current guidelines do not recommend routine prophylactic antibiotic treatment when biopsy of solid lesions is performed. In case of cystic lesions, however, the risk of infection is
higher, and administration of prophylactic antibiotics is recommended.\textsuperscript{[77]} The risk of tumor cell seeding along the needle-tract, otherwise seen in percutaneous biopsy, is considered negligible in the setting of EUS-TA of solid lesions.\textsuperscript{[69]} Similarly, in a meta-analysis of 5124 cases of EUS-FNA in pancreatic cystic lesions, no peritoneal seeding was observed.\textsuperscript{[60]}

**NOVEL APPLICATIONS AND FUTURE ASPECTS**

Advances in DNA sequencing techniques in recent years have led to significantly lower costs and higher availability of genetic analyses. Mutations in the KRAS gene are frequently observed in case of pancreatic cancer (up to 90\%) and have been proposed as a biomarker. Combination of cytology, and KRAS mutation, has been shown to increase sensitivity, especially in indeterminate cases.\textsuperscript{[70]-[72]} Furthermore, the expression of several different biomarkers in FNA samples has been shown to correlate with survival. Itoi et al. reported correlation between mRNA expression of ribonucleotide reductase subunit M2 (RRM2) and survival in 35 patients receiving gemcitabine.\textsuperscript{[73]} Similarly, Ma et al. found that S100A4 mRNA levels in FNA samples correlate with survival.\textsuperscript{[74]} Levels of hENT1 and HSP27 in FNA tissue also seem to predict sensitivity to gemcitabine in patients with unresectable pancreatic cancer.\textsuperscript{[75,76]} Only a few studies addressed the question whether FNA or FNB is superior in terms of sample adequacy for next-generation sequencing (NGS). Larson et al. observed no difference between the two needle types,\textsuperscript{[77]} but in a larger trial by Elhanafi et al. including 167 patients, the proportion of samples sufficient for NGS was higher in the FNB group.\textsuperscript{[78]} However, both the abovementioned studies are retrospective in design, and prospective comparative studies are lacking.

Recently, novel tumor culture models were developed, where tumor cells are embedded into a three-dimensional matrix with which the cells can interact (organoids). These models are superior to conventional expensive and time-consuming monolayer cultures or xenograft models that have been extensively used for in-depth insight and understanding of the carcinogenesis and cancer–environment interactions of different tumors. In case of pancreatic cancer, it was recently shown that organoids can be successfully and rapidly generated from tumor cells, not only from resected specimens, but also from EUS-FNB samples obtained at the time of initial diagnosis and before the initiation of oncological treatment.\textsuperscript{[79]} In a second study, patient-derived pancreatic cancer organoids were exposed to different chemotherapeutic agents, and their response to chemotherapy was measured (“pharmacotyping”).\textsuperscript{[80]} The study showed a strong correlation between patient response and organoid susceptibility to chemotherapeutics. Although still in development, EUS-guided organoid creation and propagation is a new and fascinating application of EUS-TA and may open new ways to precision medicine.

**CONCLUSION**

EUS-TA is invaluable for staging and diagnosis of a variety of upper gastrointestinal and mediastinal lesions. Overall, FNA and FNB needles perform seemingly equally in terms of diagnostic accuracy, however, the second-generation FNB needles require less passes. The third-generation FNB needles (crown-cut needle types) seem to be preferable to FNA needles as well as to the second-generation FNB needles, when larger histological specimens and preserved tissue architecture are required. All future studies comparing histological procurement between different needles types should preferably be randomized and as a minimum also evaluate the size and quality of the specimen. EUS-TA is constantly under development, and new applications of this technique include tumor risk stratification according to its genetic profile as well as minimally invasive creation of patient-derived organoids, hallmarks of personized medicine. It remains yet to be shown, whether these applications will lead to a decisive shift from aspiration to biopsy, \textit{i.e.}, from A to B.

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**Conflicts of interest**

Peter Vilmann is member of Advisory Board for Medi-Globe.
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