Review Article

EUS-guided through-the-needle microbiopsy of pancreatic cysts: Technical aspects (with video)

Bojan Kovacevic[1], Evangelos Kalaitzakis[1], Pia Klausen[1], Lene Brink[1], Hazem Hassan[1], John G. Karstensen[2], Peter Vilmann[1]

[1]Gastroenterology Unit, Division of Endoscopy, Herlev and Gentofte Hospital, Copenhagen University, Herlev, Denmark; [2]Gastroenterology Unit, Pancreatitis Centre East, Hvidovre Hospital, Department of Clinical Medicine, Copenhagen University, Hvidovre, Denmark

ABSTRACT

Pancreatic cystic lesions are frequently encountered and diagnostically challenging as some of the cysts may have malignant potential (mucinous) while others are completely benign (serous). EUS-guided through-the-needle biopsy (EUS-TTNB) of the cyst wall has recently been introduced as an alternative to cyst fluid cytology. Several studies have shown that microbiopsies outperform cytology in terms of distinction between mucinous and nonmucinous lesions, but also in determining the specific cyst diagnosis. However, little is known about the technical aspects of tissue sampling with TTNB. Herein, we summarize our experience with the procedure in a tertiary referral center and discuss indications, technical aspects, and safety of the procedure. Most adverse events (AEs) associated with the procedure are mild, but there is emerging evidence that the rate of postprocedural pancreatitis is higher compared to standard fine-needle aspiration. The added diagnostic yield should therefore be placed in perspective with an increased risk of AEs. Prospective studies are warranted to fully identify which patient groups could benefit from EUS-TTNB.

Key words: EUS, intraductal papillary mucinous neoplasm, microbiopsy, pancreatic cyst, pancreatic cystic lesion, through-the-needle biopsy

INTRODUCTION

Management of pancreatic cystic lesions (PCLs) is challenging and relies on a combination of patient history, cross-sectional imaging and, in selected cases, EUS-FNA of the cyst fluid. Some of the PCLs are benign, such as serous cystic neoplasms (SCNs) or pseudocysts, whereas others are considered premalignant (intraductal papillary mucinous neoplasms [IPMNs] and mucinous cystic neoplasms). The clinical dilemma consists of correct differentiation between the two types, and, in case of premalignant (mucinous) cysts, determination of optimal timing of surgical resection. EUS-guided through-the-needle biopsy (EUS-TTNB) procedure was first described in 2016 and is predominantly used for diagnosing PCLs.[1] The forceps is introduced through a 19G FNA needle and has serrated jaws with an opening width of 4.3 mm,

Video Available on: www.eusjournal.com

Access this article online

Quick Response Code:

Website: www.eusjournal.com

DOI: 10.4103/eus.eus_12_20

How to cite this article: Kovacevic B, Kalaitzakis E, Klausen P, Brink L, Hassan H, Karstensen JG, *et al.* EUS-guided through-the-needle microbiopsy of pancreatic cysts: Technical aspects (with video). Endosc Ultrasound 2020;9:220-4.

Address for correspondence

Dr. Bojan Kovacevic, Herlev Hospital, Borgmester Ib Juuls Vej 1, Herlev 2730, Denmark. E-mail: bojan.k@dadlnet.dk

Received: 2019-11-22; **Accepted:** 2020-03-05; **Published online:** 2020-06-30

allowing for targeted biopsies of the cyst wall under EUS guidance [Figure 1]. Initial studies reported high technical success (85%–100%) and higher diagnostic yield of TTNBs compared to FNA cytology.[2-11] However, the technical aspects of the procedure to optimize the diagnostic yield have only been scarcely discussed. In the following, we describe our experience with the procedure in more than 150 patients, focusing on the technical aspects.

INDICATIONS

As the technique is relatively new, it has not yet been implemented in the current guidelines on the management of patients with PCLs.[12] Although the guidelines recommend further risk stratification by EUS in cysts with worrisome features, there are no clear recommendations on when to perform EUS-FNA.[12] Several studies have shown that EUS-FNA can provide additional diagnostic value, especially in small presumed branch duct (BD)-IPMNs without worrisome features.[13,14] Communication with the pancreatic duct (PD) observed on cross-sectional imaging is pathognomonic for a BD-IPMN [Figure 2c], whereas a microcystic or "honeycomb" configuration implies an SCN [Figure 2b]. However, unilocular or oligocystic lesions without clear connection to the PD [Figure 2a] represent a diagnostic challenge and are, in lack of additional diagnostic methods, usually classified as BD-IPMN. We have demonstrated that TTNBs can substantially change the clinical management of PCLs in 12%–19% of the cases,[4] mostly by providing the diagnosis of an SCN in an oligocystic or unilocular cyst and thus leading to discontinuation of follow-up. Large cysts (>3 cm) and cysts harboring features such as mural nodules [Figure 2d], thickened enhanced cyst walls, PD dilation, lymphadenopathy, an elevated serum level of carbohydrate antigen (CA) 19-9, or a rapid rate of cyst growth have an increased risk of malignancy,[12] and EUS-TTNB should be considered in these cases.

PREPROCEDURE PREPARATION

When performing EUS-TTNB, usual preparation instructions for upper gastrointestinal endoscopy apply. The current ESGE guidelines on endoscopy in patients with antiplatelet or anticoagulant therapy should be followed.[15] EUS-TTNB is, similar to EUS-FNA, classified as a high-risk endoscopic procedure, and anticoagulant and/or antiplatelet therapy should be paused accordingly. The patients should be screened for any signs of bleeding disorders, and we advise against performing the procedure in case of uncorrected thrombocytopenia (platelet count $<50 \times 10^9$/L) or prolonged prothrombin time (international normalized ratio, INR >2). Adequate sedation is essential when performing TTNB, as the procedure requires delicate coordination and precise movements of the needle and the forceps. Type of sedation used is scarcely reported in the current literature on EUS-TTNB and ranges

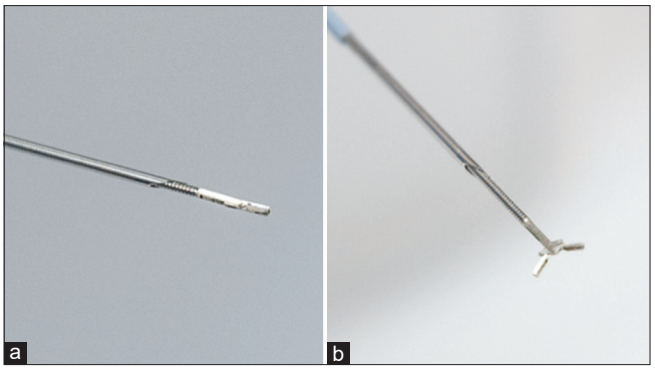

Figure 1. Microbiopsy forceps protruding through a tip of a 19G EUS-needle with closed (a) and open jaws (b)

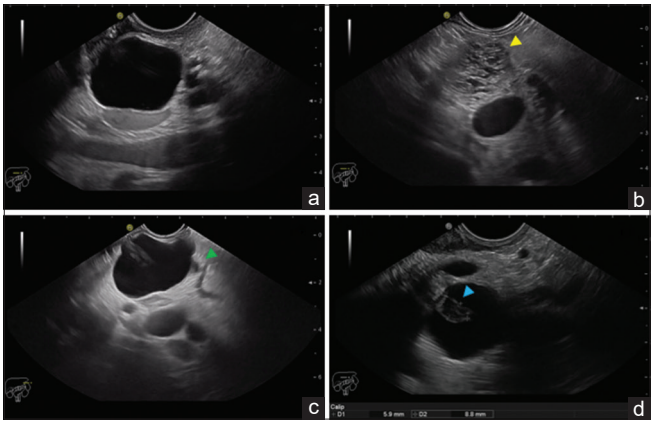

Figure 2. An unilocular cyst with no worrisome features is presented in the top left corner (a), whereas the right image (b, yellow arrowhead) showing a microcystic lesion with honeycomb configuration, consistent with a serous cystic neoplasm. In another unilocular cyst (c), a clear connection to the pancreatic duct is observed (green arrowhead). The finding is consistent with an intraductal papillary mucinous neoplasm. Last image (d) shows a cyst with an 8.8-mm large mural nodule (blue arrowhead)

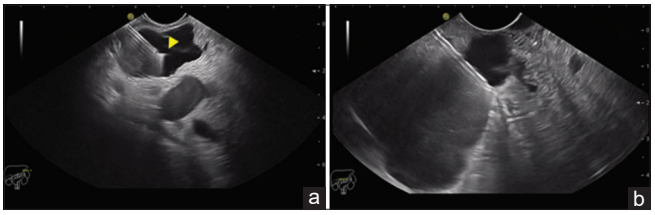

Figure 3. EUS image of the microforceps (a) protruding through an EUS-needle with open jaws (yellow arrowhead). Following closure of the forceps, the tenting effect is observed (b)

from conscious sedation to general anesthesia.[5] At our department, we utilize propofol sedation administered by a trained nurse in case of a relatively healthy patient (American Society of Anesthesiologists score I–II and body mass index <35 kg/m^2) or by an anesthesiologist in all other cases.

TECHNICAL ASPECTS OF TISSUE SAMPLING

Following the inspection of the cystic lesion and decision on an indication for biopsy, Doppler examination should be utilized to exclude any large vessels between the echoendoscope and the cystic lesion. If possible, the puncture route with the least pancreatic parenchymal tissue interposed between the transducer and the cyst lumen should be chosen; however, this may not always be possible due to interposed vessels or difficult locations. After determining the optimal route of puncture, a 19G EUS needle is introduced [Video 1]. Usually, the microbiopsy forceps may be preloaded in the needle and retracted a few millimeters behind the needle tip, or alternatively, the needle with the stylet is introduced into the cyst, and subsequently, the stylet is exchanged for the microbiopsy forceps. The former maneuver will diminish the procedural time and the time the needle is kept within the cyst. This is especially important when puncturing lesions from the duodenum, as the needle shaft becomes slightly bent when the echoendoscope is fully flexed. This can prevent successful passage of the forceps through the needle, due to its stiffness. To prevent this, we recommend preloading the forceps before insertion of the needle. However, the problem remains when the forceps has to be reintroduced to obtain a second or a third biopsy. In this case, slight repetitive, gentle opening and closing of the forceps during introduction through the needle reduces the friction and can help overcome this problem. Following successful puncture of the lesion, targeted biopsies should be attempted in case of mural nodules of thickening of the septations or the cyst wall. EUS-TTNB is associated with a high overall technical success rate as reported in several studies.[2-11] In our experience, failures in targeting the cyst lumen with the needle particularly occur in case of small cysts and in lesions located deep in the uncinate process. In the latter case, a more flexible nitinol needle may be utilized.

To obtain useful specimens for histological analysis, several different techniques can be used. The flexibility of the forceps when pushed far out distally to the needle tip prevents optimal transfer of the force, especially when the cyst wall is hard. In this case, applying too much force causes the forceps shaft to bend within the cyst and the jaws of the forceps only scrape the surface of the cyst wall, usually yielding no visible specimen. To overcome this, we recommend retracting the forceps to the needle tip with the jaws open [Figure 3a]. Subsequently, the needle within the cyst can be pushed together with the open forceps creating a more stable and improved force transfer, increasing the chance of a successful biopsy. This is especially important in case of large cysts (>4 cm), where the biopsy instruments are advanced to reach the opposing cyst wall. Partial and controlled aspiration of the cystic fluid can be useful in these cases, as the lesion will gradually collapse, bringing the opposing wall closer to the biopsy instruments. Smaller cysts (<2 cm) or multicystic lesions with relatively small compartments are similarly technically challenging, but in these cases, the maneuverability is greatly restricted requiring delicate coordination and movement of the forceps and the EUS-needle. Another issue in smaller lesions is that the cystic fluid is gradually lost during TTNB procedure, which is due to a combination of fluid loss along the needle tract and a vacuum effect during removal of the stylet or the forceps.

Continuous EUS monitoring is essential during tissue procurement. Following closure of the forceps jaws and retraction, observed "tenting" of the wall lining on EUS, is usually a good predictor of an adequate sample [Figure 3b]. If this tenting effect is not observed, the procedure should be repeated until the desired effect is experienced or alternatively until a clear resistance is felt when the biopsy forceps is retracted. To determine the optimal number of TTNBs needed to reach a histological diagnosis, Crino *et al.* performed a retrospective, single-center study.[3] The study concluded that the histological adequacy plateau was reached after obtaining two macroscopically visible TTNBs and that the addition of a third sample did not provide any additional information. An endoscopist should be aware of the risks associated with the procedure and try to minimize the intracystic needle time after obtaining an adequate number of samples. Following the retraction of the instruments, the lesion and the surrounding area should be reexamined to exclude any adverse events (AEs), such as intracystic hemorrhage.

ADVERSE EVENTS

AEs associated with the procedure range from 2% to 23%,[2-9] and the rate seems higher compared to a standard EUS-FNA procedure.[16] There is substantial heterogeneity regarding definitions of AEs in the current literature. Hence, the most common AE reported is intracystic hemorrhage, which is defined as hyperechoic changes in the cystic lumen following the tissue acquisition. However, all published cases were mild and in most cases without any clinical implication.[2-9] At our department, the patients are observed for an hour following EUS-TTNB, kept nil *per os*, and discharged upon uneventful recovery. All the observed cases of intracystic bleeding were, in our experience, self-limiting. Apart from intracystic hemorrhage, TTNB can cause acute pancreatitis, probably due to destruction of the cyst wall, disruption of the adjacent normal pancreatic tissue architecture, and leakage of the pancreatic juice. In a recent meta-analysis by Tacelli *et al.*, the rate of pancreatitis is reported as higher compared to EUS-FNA (8.6%, 95% confidence interval 4.0%–13.1%), with all cases being classified as mild.[17] However, our latest data show that the severity of AEs may be underestimated. In a prospective single-center study of 101 patients, we have observed a similar high overall rate of acute pancreatitis (9.0%) and a 3% risk of severe pancreatitis, leading to death of one patient due to multiorgan failure (unpublished data). We have examined whether procedural variables such as number of biopsies, forceps, and needle passes, as well as procedural and intracystic needle time could be associated to AEs, but none of the variables reached statistical significance. We speculated whether aggressive perioperative hydration with Ringer lactate and rectal nonsteroidal anti-inflammatory drugs (NSAIDs) plays a role in lowering the AE rate, as shown in studies on the prevention of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis.[18,19] Despite the fact that overall AE rate halved following introduction of these measures, the difference was statistically insignificant (17.6% *vs.* 8.3%, $P = 0.37$) (unpublished data). It remains yet to be determined whether aggressive perioperative hydration together with rectal NSAIDs can lower the rate of acute pancreatitis in patients undergoing TTNB.

In case of cystic lesions, the risk of infection when performing EUS-FNA is considered higher compared to solid lesions and administration of prophylactic antibiotics is recommended.[20] As for EUS-TTNB, published studies report prophylactic antibiotic treatment, either as a single administration or in combination with a prolonged 3–5 days oral treatment.[2,3,5,7,10,11,21] However, evidence considering antibiotic prophylaxis is scarce and of low level.

SAMPLE HANDLING AND PREPARATION

Implementation of the TTNB technique should be performed in close dialog with the local pathology department to ensure the highest possible diagnostic yield. Care should be taken when transferring the sample from the forceps jaws onto a biopsy paper with the accompanying needle tool, as haphazard handling of the biopsy material can disrupt the epithelial lining causing difficulties in interpretation. A thorough description of the sampling process has been provided by Crino *et al.*[3] Following extraction, the samples are inserted in a tube or a cassette (one per sample), fixated in formalin overnight, and embedded in paraffin. Subsequently, the tissue is sectioned and mounted on glass slides, stained conventionally and immunohistochemically, and can also be utilized for molecular analyses.[22]

CONCLUSION

EUS-guided microbiopsy (TTNB) is a novel diagnostic adjunctive for pancreatic cysts, providing histological samples with higher diagnostic yield compared to cyst fluid cytology. The technique offers targeted tissue sampling under EUS guidance but seems associated with a higher AE rate compared to EUS-FNA. The method requires some procedural considerations but can, following a short training period, be performed with high technical success. Further studies are needed to define proper indications for EUS-TTNB.

Acknowledgment

We wish to thank the Danish Cancer Society, Arvid Nilsson Foundation, Ingrid Munkholm Foundation, Toyota Foundation, Novo Nordisk Foundation, and Herlev Hospital Research Foundation for their financial support.

Financial support and sponsorship

We received financial support from the Danish Cancer Society, Arvid Nilsson Foundation, Ingrid Munkholm Foundation, Toyota Foundation, Novo Nordisk Foundation, and Herlev Hospital Research Foundation.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Pham KD, Engjom T, Gjelberg Kollesete H, *et al*. Diagnosis of a mucinous pancreatic cyst and resection of an intracystic nodule using a novel through-the-needle micro forceps. *Endoscopy* 2016;48 Suppl 1:E125-6.
2. Barresi L, Crinò SF, Fabbri C, *et al*. Endoscopic ultrasound-through-the-needle biopsy in pancreatic cystic lesions: A multicenter study. *Dig Endosc* 2018;30:760-70.
3. Crinò SF, Bernardoni L, Brozzi L, *et al*. Association between macroscopically visible tissue samples and diagnostic accuracy of EUS-guided through-the-needle microforceps biopsy sampling of pancreatic cystic lesions. *Gastrointest Endosc* 2019;90:933-43.
4. Kovacevic B, Klausen P, Hasselby JP, *et al*. A novel endoscopic ultrasound-guided through-the-needle microbiopsy procedure improves diagnosis of pancreatic cystic lesions. *Endoscopy* 2018;50:1105-11.
5. Yang D, Samarasena JB, Jamil LH, *et al*. Endoscopic ultrasound-guided through-the-needle microforceps biopsy in the evaluation of pancreatic cystic lesions: A multicenter study. *Endosc Int Open* 2018;6:E1423-30.
6. Yang D, Trindade AJ, Yachimski P, *et al*. Histologic analysis of endoscopic ultrasound-guided through the needle microforceps biopsies accurately identifies mucinous pancreas cysts. *Clin Gastroenterol Hepatol* 2019;17:1587-96.
7. Mittal C, Obuch JC, Hammad H, *et al*. Technical feasibility, diagnostic yield, and safety of microforceps biopsies during EUS evaluation of pancreatic cystic lesions (with video). *Gastrointest Endosc* 2018;87:1263-9.
8. Zhang ML, Arpin RN, Brugge WR, *et al*. Moray micro forceps biopsy improves the diagnosis of specific pancreatic cysts. *Cancer Cytopathol* 2018;126:414-20.
9. Basar O. Pancreatic cyst biopsy: Improvement in diagnosis with micro forceps biopsy. *Cancer Cytopathol* 2018;126:227-8.
10. Hashimoto R, Lee JG, Chang KJ, *et al*. Endoscopic ultrasound-through-the-needle biopsy in pancreatic cystic lesions: A large single center experience. *World J Gastrointest Endosc* 2019;11:531-40.
11. Basar O, Yuksel O, Yang DJ, *et al*. Feasibility and safety of microforceps biopsy in the diagnosis of pancreatic cysts. *Gastrointest Endosc* 2018;88:79-86.
12. Tanaka M, Fernández-Del Castillo C, Kamisawa T, *et al*. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. *Pancreatology* 2017;17:738-53.
13. Ridtitid W, DeWitt JM, Schmidt CM, *et al*. Management of branch-duct intraductal papillary mucinous neoplasms: A large single-center study to assess predictors of malignancy and long-term outcomes. *Gastrointest Endosc* 2016;84:436-45.
14. Genevay M, Mino-Kenudson M, Yaeger K, *et al*. Cytology adds value to imaging studies for risk assessment of malignancy in pancreatic mucinous cysts. *Ann Surg* 2011;254:977-83.
15. Veitch AM, Vanbiervliet G, Gershlick AH, *et al*. Endoscopy in patients on antiplatelet or anticoagulant therapy, including direct oral anticoagulants: British Society of Gastroenterology (BSG) and European Society of Gastrointestinal Endoscopy (ESGE) guidelines. *Endoscopy* 2016;48:385-402.
16. Zhu H, Jiang F, Zhu J, *et al*. Assessment of morbidity and mortality associated with endoscopic ultrasound-guided fine-needle aspiration for pancreatic cystic lesions: A systematic review and meta-analysis. *Dig Endosc* 2017;29:667-75.
17. Tacelli M, Celsa C, Magro B, *et al*. Diagnostic performance of endoscopic ultrasound through-the-needle microforceps biopsy of pancreatic cystic lesions: Systematic review with meta-analysis. Dig Endosc 2020. doi: 10.1111/den.13626. [Epub ahead of print].
18. Wu D, Wan J, Xia L, *et al*. The efficiency of aggressive hydration with lactated ringer solution for the prevention of post-ERCP pancreatitis: A systematic review and meta-analysis. *J Clin Gastroenterol* 2017;51:e68-76.
19. Park CH, Paik WH, Park ET, *et al*. Aggressive intravenous hydration with lactated Ringer's solution for prevention of post-ERCP pancreatitis: A prospective randomized multicenter clinical trial. *Endoscopy* 2018;50:378-85.
20. Polkowski M, Jenssen C, Kaye P, *et al*. Technical aspects of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline-March 2017. *Endoscopy* 2017;49:989-1006.
21. Larghi A, Manfrin E, Fabbri C, *et al*. Interobserver agreement among expert pathologists on through-the-needle microforceps biopsy samples for evaluation of pancreatic cystic lesions. *Gastrointest Endosc* 2019;90:784-92.
22. Vestrup Rift C, Melchior LC, Kovacevic B, *et al*. Next-generation sequencing of endoscopic ultrasound guided microbiopsies from pancreatic cystic neoplasms. *Histopathology* 2019;75:767-71.