Improving the yield of EUS-guided histology

Luca Barresi, Matteo Tacelli, Ilaria Tarantino, Fabio Cipolletta, Antonino Granata, Mario Traina
Endoscopy Service, Department of Diagnostic and Therapeutic Services, IRCCS-ISMETT (Mediterranean Institute for Transplantation and Highly Specialized Therapies), Palermo, Italy

INTRODUCTION

Tissue acquisition (TA) is one of the pieces, together with epidemiology, clinical data, radiology, EUS, and cystic fluid analysis, which forms the complicated puzzle of differential diagnosis in pancreatic cystic lesions (PCLs). TA is not only useful in discriminating between various types of pancreatic cysts, but also fundamental in assessing the grade of dysplasia.

All published guidelines[1-5] suggest using EUS-FNA of cystic fluid, not as a routine test, but only in cysts in which the results are likely to alter management, and in cysts in which the diagnosis is unclear. For example, in the International Association of Pancreatology's Fukuoka Guidelines,[1] the indication for EUS-FNA is the presence of these so-called "worrisome features" which are composed of several alerting clinical or morphological characteristics. Almost all the subsequent published guidelines have identified several similar characteristics of warning, which advise further investigation, particularly EUS-FNA of cystic fluid. For these reasons, in recent years, there have been many attempts to improve TA in PCLs with many different devices in order to obtain an adequate specimen to be analyzed.

Access this article online	
Quick Response Code:	Website: www.eusjournal.com
	DOI: 10.4103/eus.eus_45_18

CYTOLOGY

Cytology of cystic fluid

The first proposed technique, and currently the most widely performed for the diagnosis of PCLs, is cytology of cystic fluid (CCF). In 2004, Brugge *et al.*[6] found that CCF can individuate mucinous lesions with a 34.5% sensitivity, an 84% specificity, and a 51% diagnostic accuracy. Unfortunately, in this study, identification of malignant lesions was only 22%. More recently, similar discouraging results, in terms of adequacy, were obtained by the Dutch Group of de Jong *et al.*[7] in 2011, with cyst fluid sent for cytology providing adequate cellular yield in only 31% of cases.

Throughout the years, numerous studies of diagnostic accuracy, sensitivity, and specificity of CCF have been published.[8,9] These studies produced contrasting results, leading to two meta-analyses.[10,11] Thosani found that CCF had a 63% overall sensitivity and an 88% overall specificity in the identification of mucinous cysts. The authors concluded in their discussion that a 63% overall sensitivity is probably overestimated, likely because of verification bias. In the other meta-analysis, in 2014, two groups of patients were analyzed. In Group 1, the diagnostic accuracy of cytology in the

How to cite this article: Barresi L, Tacelli M, Tarantino I, Cipolletta F, Granata A, Traina M. Improving the yield of EUS-guided histology. Endosc Ultrasound 2018;7:301-5.

Address for correspondence
Dr. Luca Barresi, Endoscopy Service, Department of Diagnostic and Therapeutic Services, IRCCS-ISMETT (Mediterranean Institute for Transplantation and Highly Specialized Therapies), Palermo, Italy. E-mail: lbarresi@ismett.edu
Received: 2018-06-23; **Accepted:** 2018-07-06; **Published online:** 2018-10-15

identification of malignant lesions was evaluated, with sensitivity and specificity values of 51% and 94%, respectively. In Group 2, potentially malignant lesions (mucinous lesions, cystic islet cell tumor, and solid pseudopapillary tumor) were identified, with sensitivity and specificity of 52% and 97%, respectively.

From these studies, it appears clear that CCF obtained with EUS-FNA has median diagnostic accuracy and sensitivity values of less than 50%, and even if suggested by guidelines as the current gold standard when performing EUS-FNA in PCLs, it has a very low diagnostic accuracy both in discriminating mucinous from nonmucinous lesions and malignant/potentially malignant from benign lesions. Although one reason for these poor values is that only in a small number of centers are there very experienced cytopathologists in pancreatic disease, the most important reason is that very few neoplastic cells are usually dispersed in the cystic fluid.

To overcome the problem of the low adequacy of CCF, a better target for TA appears to be the cystic wall.

Cytology of cystic wall

Because of the scarcity of neoplastic cells in cystic fluid, two different techniques used to collect more cells were developed: brushing and FNA of cystic wall. Brushing has yielded variable results in published studies. Although better results with respect to CCF have been observed in some studies, brushing has resulted in high rates of adverse events (approximately 10%),[12,13] such as acute pancreatitis and postbrushing bleeding (in one case, a retroperitoneal bleeding, with subsequent death).

In 2012, a study[14] was published in which the difference between cytology + carcinoembryonic antigen (CEA) of cystic fluid and FNA of the cystic wall with a 22G FNA needle (Cook Medical, Winston-Salem, NC) was prospectively evaluated. Cellular material adequate for cytological evaluation was reported in 81% of cases. The difference in terms of diagnostic performance was about 29%. The adverse events reported with this technique were consistently low (1.45%). Unfortunately, this is the only available study of this technique.

HISTOLOGY

After cystic walls became the preferred target, the following step was to try to obtain histological samples (or better microhistological samples) that could improve the diagnostic yield and make the diagnosis easier, even for nonexpert cytopathologists.

Several EUS devices and techniques have been tried in the quest for obtaining microhistological specimens instead of cytologic samples from the cystic walls.

Tru-Cut needle

The first study of sampling of the cystic walls was made using a 19G needle, the Tru-Cut biopsy device (Quick-Core; Wilson-Cook, Winston-Salem, NC, USA). There is only one published study, with 10 patients.[15] Fine-needle biopsy (FNB) with the Tru-Cut needle was diagnostic in 7/10 patients, and there were no complications. However, the Tru-Cut needle was very stiff and hard to use, especially in angled positions, and furthermore, it is no longer available.

ProCore needle

The 22G ProCore needle with side fenestration (EchoTip Ultra FNB, Cook Medical, Ireland) was used in another study.[16] The diagnostic adequacy was about 65%, but adequacy increased to 94.4% and 100% in the subgroups of patients with a solid component or with a malignant lesion, respectively. Moreover, 46.1% of samples were considered adequate for a microhistological examination (defined as a specimen composed of stroma covered with surface epithelium). Unfortunately, in the subgroup of patients without solid components in the PCL, diagnostic adequacy was about 37%, very similar to the low diagnostic yield of CCF. Mild complications were observed in 3.3% of patients, though there were no severe complications. Even if the ProCore needle led to better results with respect to EUS-FNA of cystic fluid, with the advantage of obtaining a microhistological specimen in about half of the cases, the results in PCLs without solid components were quite disappointing. Furthermore, also for this type of needle, no further studies have been published.

EUS-guided biopsy using a micro forceps

In 2010, Aparicio *et al.*[17] carried out a pilot study utilizing a 0.8-mm micro forceps designed for PolyScope® (Lumenis Surgical, USA) through a 19G EUS needle in two patients with PCLs. Since then, several case reports have been published, using different micro forceps, for a total of 14 cases described.[18-24] In most of these studies, a specifically developed micro forceps to use in cystic lesions was employed: The

Moray micro forceps (Moray™ micro forceps, US Endoscopy, Mentor, Ohio, USA).[20-24]

This technique was called by different authors either EUS-guided micro forceps biopsy (MFB) or EUS-through-the-needle biopsy [TTNB, Figure 1].

Recently, two retrospective studies using the Moray micro forceps have been published.[25,26] The studies reported a high technical success (90.4%–100%) and a very high diagnostic yield of 88.9% in one, in which MFB dramatically changed the diagnosis in 26% of patients, providing diagnoses otherwise not suggested by traditional cytology or cyst fluid CEA.

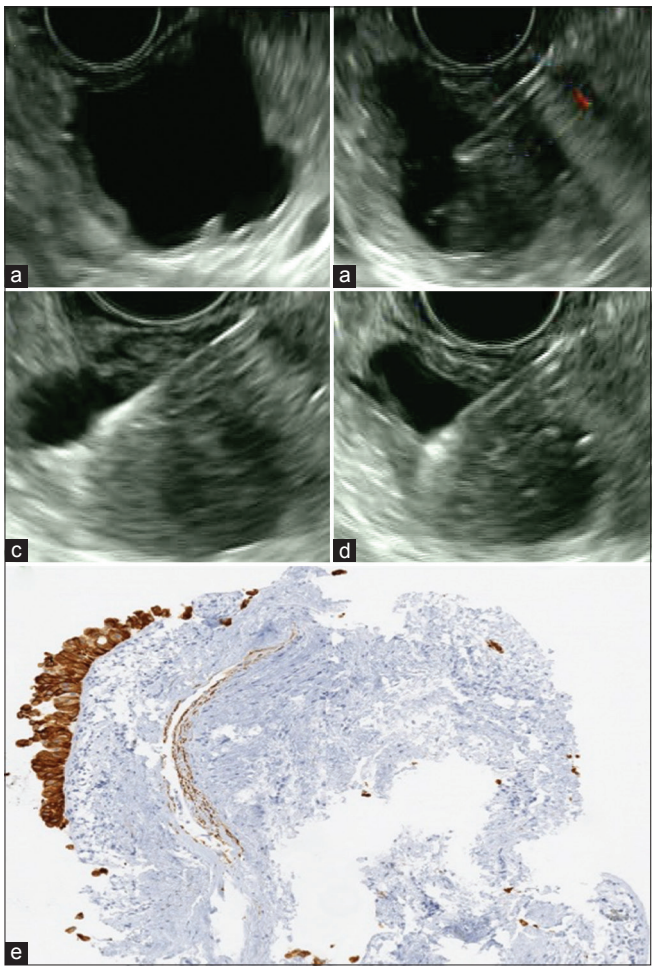

Figure 1. Example of EUS-through-the-needle biopsy. Pancreatic cystic lesion with a thickened cyst wall (a). Insertion of a 19-gauge FNA needle inside the pancreatic cystic lesion (b). Opening of the valves of micro forceps inside the pancreatic cystic lesion (c). Sampling of the cyst wall with micro forceps determining the so-called "tent sign" (d). Microhistological specimen of a branch duct intraductal papillary mucinous neoplasm obtained with EUS-through-the-needle biopsy. It can be seen the presence of both epithelial lining and stroma of cystic wall. In this specimen, it was possible to perform immunohistochemistry for cytokeratin-7, a protein used to highlight (brown-colored cells) epithelial line (e)

The aim of the other study was to compare the TA and diagnostic tissue yield of MFB with cyst fluid cytology. The diagnostic tissue yield was evaluated at three levels: the ability of differentiation between mucinous/nonmucinous cysts, which showed no difference (P = 0.188) between diagnostic yield of cytology from cystic fluid (20/42, 47.6%), and histological yield from MFB (26/42, 61.9%). No difference (P = 0.113) was seen also in detection of high risk for malignancy (cytology from cystic fluid in 23/42, 54.7% *vs.* MFB 30/42, 71.5%). However, the ability of MFB to provide a specific cyst-type diagnosis was 35.7% (15/42), and cytology was 4.8% (2/42, P = 0.001). Furthermore, surgical histology was concordant with MFB in 6 of 7 (85%) patients and with cytology in 1 of 7 (15%) patients. Therefore, the diagnostic yield of the MFB for a specific type of cyst was significantly higher than the diagnostic cytology yield, and concordance with surgical histology was very high for EUS-TTNB and low for cytology.

With regard to adverse events, the first study reported no complications, while the Basar study reported only two adverse events: an aspecific abdominal pain and an intracystic hemorrhage.

We have retrospectively collected information in a multicenter study of 56 patients who underwent EUS-TTNB of PCLs.[27] This study confirms the results reported by Mittal concerning feasibility and diagnostic yield, which were 100% and 83.9% (47/56), respectively. This study also showed better performance of EUS-TTNB with respect to CCF, which had a diagnostic yield of 36.1%. Concordance of EUS-TTNB with surgical histology regarding the type of lesions was 11/12 (91.6%), while concordance for histologic severity of lesion was 9/12 (75%). However, adverse events occurred in 9/56 (16%) patients, with self-limiting intracystic hemorrhage the most common (7/56, 12.5%). However, all adverse events were considered mild because they resolved spontaneously without any treatment. Intracystic hemorrhage is common even when performing a standard FNA in PCLs and, to date, only one case requiring transfusion has been reported.[28,29] Further studies are needed to verify whether this complication could have a clinical significance or should be considered simply a side effect of EUS-TTNB in PCLs.

From the available studies, it seems that EUS-TTNB is a feasible technique, with a high diagnostic yield and mild adverse events. Furthermore,

this technique seems to have a better diagnostic yield compared with traditional EUS-FNA CCF. Another advantage of EUS-TTNB is that the specimens obtained are microhistological (defined as a specimen composed of a stroma covered with an epithelial lining). In one study, pathologists considered the specimens from EUS-TTNB adequate to reach a histological diagnosis in 47 of 56 (83.9%) of cases.[27] This makes the diagnosis easier for the pathologist and facilitates ancillary techniques such as immunohistochemistry of epithelium but also of the stroma.

Regarding the disadvantages of EUS-TTNB, it is possible to sample only part of the cystic walls, namely, the wall opposite the PCL with respect to the point of entrance of the 19G needle used to pass the micro forceps. Furthermore, the inhomogeneous distribution of dysplasia inside the PCLs and the not infrequent possibility that some PCLs have a denuded epithelium[30] make it more difficult to obtain adequate specimens. Performing several passages on the cystic walls augment the possibility of obtaining a diagnostic sample, representative of the real dysplasia inside the lesions.

CONCLUSIONS

Many steps forward have been made in the diagnosis and risk stratification for malignancy of PCLs. However, a significant percentage of benign PCLs are still wrongly sent to surgery, with all the related risks of a high number of complications and surgery-related mortality. The cystic walls have become the target of several devices aimed at augmenting the probability of diagnosis in PCLs. Moreover, to further improve diagnostic adequacy and facilitate the diagnosis, even in the absence of pathologists particularly expert in cytology and pancreatic pathology, the attempt is to obtain microhistological samples.

At the moment, EUS-TTNB seems to be one promising technique of TA for these challenging lesions. We think that the available studies justify new prospective ones to verify the efficacy and safety of this new technique.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. 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.
2. Italian Association of Hospital Gastroenterologists and Endoscopists, Italian Association for the Study of the Pancreas, Buscarini E, *et al.* Italian consensus guidelines for the diagnostic work-up and follow-up of cystic pancreatic neoplasms. *Dig Liver Dis* 2014;46:479-93.
3. Del Chiaro M, Verbeke C, Salvia R, *et al.* European experts consensus statement on cystic tumours of the pancreas. *Dig Liver Dis* 2013;45:703-11.
4. Vege SS, Ziring B, Jain R, *et al.* American Gastroenterological Association Institute Guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. *Gastroenterology* 2015;148:819-22.
5. Elta GH, Enestvedt BK, Sauer BG, *et al.* ACG clinical guideline: Diagnosis and management of pancreatic cysts. *Am J Gastroenterol* 2018;113:464-79.
6. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, *et al.* Diagnosis of pancreatic cystic neoplasms: A report of the cooperative pancreatic cyst study. *Gastroenterology* 2004;126:1330-6.
7. de Jong K, Poley JW, van Hooft JE, *et al.* Endoscopic ultrasound-guided fine-needle aspiration of pancreatic cystic lesions provides inadequate material for cytology and laboratory analysis: Initial results from a prospective study. *Endoscopy* 2011;43:585-90.
8. Maker AV, Carrara S, Jamieson NB, *et al.* Cyst fluid biomarkers for intraductal papillary mucinous neoplasms of the pancreas: A critical review from the international expert meeting on pancreatic branch-duct-intraductal papillary mucinous neoplasms. *J Am Coll Surg* 2015;220:243-53.
9. Oguz D, Öztaş E, Kalkan IH, *et al.* Accuracy of endoscopic ultrasound-guided fine needle aspiration cytology on the differentiation of malignant and benign pancreatic cystic lesions: A single-center experience. *J Dig Dis* 2013;14:132-9.
10. Thosani N, Thosani S, Qiao W, *et al.* Role of EUS-FNA-based cytology in the diagnosis of mucinous pancreatic cystic lesions: A systematic review and meta-analysis. *Dig Dis Sci* 2010;55:2756-66.
11. Wang QX, Xiao J, Orange M, *et al.* EUS-guided FNA for diagnosis of pancreatic cystic lesions: A meta-analysis. *Cell Physiol Biochem* 2015;36:1197-209.
12. Sendino O, Fernández-Esparrach G, Solé M, *et al.* Endoscopic ultrasonography-guided brushing increases cellular diagnosis of pancreatic cysts: A prospective study. *Dig Liver Dis* 2010;42:877-81.
13. Al-Haddad M, Gill KR, Raimondo M, *et al.* Safety and efficacy of cytology brushings versus standard fine-needle aspiration in evaluating cystic pancreatic lesions: A controlled study. *Endoscopy* 2010;42:127-32.
14. Hong SK, Loren DE, Rogart JN, *et al.* Targeted cyst wall puncture and aspiration during EUS-FNA increases the diagnostic yield of premalignant and malignant pancreatic cysts. *Gastrointest Endosc* 2012;75:775-82.
15. Levy MJ, Smyrk TC, Reddy RP, *et al.* Endoscopic ultrasound-guided trucut biopsy of the cyst wall for diagnosing cystic pancreatic tumors. *Clin Gastroenterol Hepatol* 2005;3:974-9.
16. Barresi L, Tarantino I, Traina M, *et al.* Endoscopic ultrasound-guided fine needle aspiration and biopsy using a 22-gauge needle with side fenestration in pancreatic cystic lesions. *Dig Liver Dis* 2014;46:45-50.
17. Aparicio JR, Martínez J, Niveiro M, *et al.* Direct intracystic biopsy and pancreatic cystoscopy through a 19-gauge needle EUS (with videos). *Gastrointest Endosc* 2010;72:1285-8.
18. Samarasena JB, Nakai Y, Shinoura S, *et al.* EUS-guided, through-the-needle forceps biopsy: A novel tissue acquisition technique. *Gastrointest Endosc* 2015;81:225-6.
19. Barresi L, Tarantino I, Ligresti D, *et al.* A new tissue acquisition technique in pancreatic cystic neoplasm: Endoscopic ultrasound-guided through-the-needle forceps biopsy. *Endoscopy* 2015;47 Suppl 1:E297-8.
20. 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.
21. Shakhatreh MH, Naini SR, Brijbassie AA, *et al.* Use of a novel

through-the-needle biopsy forceps in endoscopic ultrasound. *Endosc Int Open* 2016;4:E439-42.
22. Coman RM, Schlachterman A, Esnakula AK, *et al*. EUS-guided, through-the-needle forceps: Clenching down the diagnosis. *Gastrointest Endosc* 2016;84:372-3.
23. Attili F, Pagliari D, Rimbaș M, *et al*. Endoscopic ultrasound-guided histological diagnosis of a mucinous non-neoplastic pancreatic cyst using a specially designed through-the-needle microforceps. *Endoscopy* 2016;48 Suppl 1:E188-9.
24. Huelsen A, Cooper C, Saad N, *et al*. Endoscopic ultrasound-guided, through-the-needle forceps biopsy in the assessment of an incidental large pancreatic cystic lesion with prior inconclusive fine-needle aspiration. *Endoscopy* 2017;49:E109-10.
25. 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.
26. 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.
27. Barresi L, Crinò SF, Fabbri C, *et al*. Endoscopic ultrasound-through-the-needle biopsy in pancreatic cystic lesions: A multicenter study. Dig Endosc 2018. doi: 10.1111/den.13197. [Epub ahead of print].
28. Varadarajulu S, Eloubeidi MA. Frequency and significance of acute intracystic hemorrhage during EUS-FNA of cystic lesions of the pancreas. *Gastrointest Endosc* 2004;60:631-5.
29. Tarantino I, Fabbri C, Di Mitri R, *et al*. Complications of endoscopic ultrasound fine needle aspiration on pancreatic cystic lesions: Final results from a large prospective multicenter study. *Dig Liver Dis* 2014;46:41-4.
30. Gómez V, Majumder S, Smyrk TC, *et al*. Pancreatic cyst epithelial denudation: A natural phenomenon in the absence of treatment. *Gastrointest Endosc* 2016;84:788-93.