Endoscopic Ultrasound in the Diagnosis of Mucinous Pancreatic Neoplasms

Authors:

Tony S. Brar, MD[1]

Vikas Khullar, MD[2]

Yaseen Perbtani, DO[2]

Dennis Yang, MD[2]

[1] Department of Medicine, University of Florida, Gainesville, FL

[2] Division of Gastroenterology, Hepatology and Nutrition, University of Florida, Gainesville, FL

Corresponding Author:

Dennis Yang, MD

Division of Gastroenterology, Hepatology, and Nutrition

University of Florida College of Medicine

1329 SW 16th Street, Room #5251

Gainesville, Florida 32608, United States

Email:

Dennis.Yang@medicine.ufl.edu

TEL: (352) 273-9474

FAX: (352) 627-9002

Conflict of Interest: None

ABSTRACT

There has been an increased detection of incidental pancreatic cystic neoplasms (PCNs) over recent years. Accurate diagnosis and characterization of these PCNs are essential given their varying potential for malignant transformation. Endoscopic ultrasound (EUS) plays a key role in the evaluation of these lesions; yet, it is not without its limitations. Fluid cytological evaluation is often compromised by the acellular specimen whereas the diagnostic accuracy of chemical and molecular markers is not optimal. More recently, a novel EUS-guided through-the-needle biopsy forceps has been introduced. Potential advantages include targeted tissue sampling and acquisition of larger samples that may allow histological analysis. Further large prospective comparative studies are needed to determine the ideal modality for evaluation of PCNs.

1. INTRODUCTION

There has been an increase in the detection of pancreatic cystic neoplasms (PCNs) over recent years. The rising prevalence, which has been estimated to range from 3 to 14% (1, 2), can be in part attributed to the increased availability and utilization of improved high quality, abdominal imaging modalities (3). The increased detection rate of PCNs has posed a clinical dilemma as accurate diagnosis can be challenging yet of utmost importance given the potential for malignant transformation (4, 5).

PCNs encompass a broad heterogeneous group of pancreatic lesions with different morphological and histological features with varying clinical implications (6). The most common types of PCNs are intrapapillary mucinous neoplasms (IPMNs), mucinous neoplasms (MCNs), serous cystadenomas (SCNs) and solid pseudopapillary tumors (SPNs) (7). Distinguishing among these types of PCNs is important as management strategies can range from surveillance to surgical resection, depending on their potential for malignant transformation (7).

Initial evaluation of PCNs is generally based on radiologic imaging. Magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreato-graphy (MRCP) is generally recommended as it can provide important morphological information (i.e. presence/absence of septa, nodules, cyst contents and duct communication) and has been associated with 76-91% accuracy in detecting malignancy (8, 9). Alternatively, a pancreatic protocol multi-detector computed tomography (MDCT) can also be obtained as part of the diagnostic evaluation. The major drawback of imaging is that different PCNs with varied malignant potential may commonly share similar morphological features, thereby rendering radiological findings indeterminate (10) and requiring the need for sampling for accurate diagnosis.

2. EUS AND EUS-FNA IN THE EVALUATION OF PCNs

EUS is an additional modality that can be utilized for the categorization of PCNs and further identification of lesions that have an increased malignant potential (Table 1). EUS permits high-resolution diagnostic imaging of the pancreas parenchyma and its ductal system; allowing the identification of important diagnostic features and predictors of malignancy; including cyst characteristics (i.e. size, shape, septations, wall structure, communication with the main pancreatic duct), the presence/absence of solid lesions, nodules or lymphadenopathy (9). In a retrospective analysis of 50 patients, EUS was shown to have similar sensitivities to MRI for the identification of cyst septations (77.8%), main pancreatic duct dilatation (85.7%) and communication with the PD (88.9%) (11). Conversely, the diagnostic accuracy of EUS for nodules is limited and was estimated to be 57% in a pathology-based study of IPMNs (12). Indeed, mucus and not nodules accounted for most echogenic lesions detected by EUS in that study; highlighting the limitations of EUS imaging alone for the evaluation of PCNs. Furthermore, EUS performance has been shown to be highly operator dependent, with several studies demonstrating a high inter-observer variability and only fair to moderate agreement among endosonographers when evaluating PCNs (13).

An advantage of EUS over cross-sectional imaging is that it allows sampling of cyst fluid when the diagnosis by imaging alone cannot be ascertained. EUS-FNA can be safely performed under Doppler guidance (to avoid intervening vasculature in the path of the needle) with a 19-, 22-, or 25-gauge needle. To reduce the risk of infection from cyst aspiration, effort should be placed in collapsing the cyst with a single needle pass and the administration of prophylactic antibiotics (14).

Table 1. Features of Pancreatic Cystic Neoplasms

	Age at presentation	Gender Distribution	Imaging Characteristics	Aspirate Characteristics	Cytology Findings	Malignant Potential
Intrapapillary Mucinous Neoplasms (IPMNs)	Typically 50 - 70 years of age	Males = Females	Dilated pancreatic duct +/- parenchymal atrophy Solid component, may suggest malignancy	Viscous or Thin	Columnar cells with variable atypia Stains positive for mucin; yield <50% High yield from solid component for malignancy	Moderate to High
Mucinous Neoplasms (MCNs)	Typically 50 - 70 years of age	Mostly Females	Unilocular or septated cyst +/- wall calcifications Solid component, may suggest malignancy	Viscous	Columnar cells with variable atypia Stains positive for mucin; yield <50% High yield from solid component for malignancy	Moderate
Serous Cystadenomas (SCNs)	Typically 50 - 70 years of age	Males < Females	Microcystic/ honeycomb appearance Oligocystic appearance less common	Thin, often bloody	Cuboidal cells that stain positive for glycogen; yield <50%	Negligible
Solid Pseudopa-pillary Tumors (SPNs)	Typically 20 - 30 years of age	Males < Females	Solid and cystic mass +/- calcifications	Bloody	Characteristic branching papillae with myxoid stroma High yield from solid component	Moderate to High

Modified from:

Khalid A, Brugge WR. ACG practice guidelines for the diagnosis and management of neoplastic pancreatic cysts. Am J Gastroenterol 2007; 102:2339.

Wu J, Jiao Y, Dal Molin M, et al. Whole-exome sequencing of neoplastic cysts of the pancreas reveals recurrent mutations in components of ubiquitin-dependent pathways. Proc Natl Acad S

3. Cyst Fluid Evaluation

Initial visual inspection of the fluid aspirate can sometimes provide useful diagnostic information. Cyst fluid from mucinous PCNs is generally grossly thick, transparent and highly viscous (15). The fluid viscosity can be assessed by the commonly known “string sign” which is performed by

placing a drop of the aspirate between the thumb and index finger and slowly pulling apart. A positive "string sign" (string of fluid > 1 cm for > 1 second between fingers) has been shown to be highly specific (95%) for mucinous PCNs (16). Conversely, fluid from non-mucinous PCNs, such as serous cystadenomas (SCAs), tend to be thin and serosanguinous given the high vascularity of these lesions.

3.1. Cytology

Cytologic evaluation of cyst fluid depends on the detection of cells in the aspirate. Malignant cells are seen in malignant PCNs whereas mucin-containing cells can be seen with IPMNs and MCNs. Periodic acid-Schiff (PAS) glycogen-containing cells are pathogmonic of SCAs (17). While the presence of cells in the cytologic sample can provide a specific diagnosis, the overall diagnostic utility of this approach is significantly hampered by the often paucicellular fluid. In a multicenter prospective study of 341 patients who underwent EUS-FNA of pancreatic cystic lesions with surgical resection, the diagnostic sensitivity and specificity of cytology for PCN was 35% and 83% respectively. The sensitivity of cytology for diagnosis malignancy was even lower at 22% (18).

Technical variations with EUS needle sampling have been introduced to improve the cytological diagnostic yield. A single center prospective study of 66 patients with PCNs demonstrated that puncturing the cyst wall for sampling following fluid aspiration may potentially increase the diagnostic yield by 29% when compared to fluid cytology alone (19). In a separate study, Barresi et al reported a 65% specimen adequacy rate for cyto-histologic assessment in pancreatic cystic lesions by using a fenestrated core EUS needle (20). Overall, further studies are still needed to determine the most optimal approach for fluid sampling that may provide the highest diagnostic yield with an acceptable safety profile.

3.2. Chemical Analysis

Chemical analysis of cyst fluid aspirate is commonly performed to assist with PCN characterization. Traditionally, carcinogenic embryonic antigen (CEA) has been the main fluid biomarker used to distinguish if a cyst is mucinous. This practice is primarily based on a landmark study by Brugge et al demonstrating that CEA >192ng/ml was associated with a 73% sensitivity and 84% specificity for mucinous lesions. The overall diagnostic accuracy of CEA for mucinous cysts was 79%, which was significantly greater than the accuracy of either EUS (51%) or cytology (59% ($p<0.05$) (21). In contrast, a low CEA of less than 5ng/ml has 95% specificity for non-mucinous lesions, such as pseudocysts, SCA, and neuroendocrine tumors (18). Currently there is no reliable data to support the use of cyst fluid CEA levels to distinguish between benign and malignant PCNs.

The use of other chemical biomarkers has not been shown to have significant clinical utility in the evaluation of PCNs. While typically amylase levels are more elevated in IPMN than MCN, this is neither specific nor sensitive. Conversely, a cyst fluid amylase less than 250U/L is 98% specific in ruling out a pseudocyst (18).

3.3. Molecular Analysis

There has been an increasing effort over recent years to identify molecular biomarkers that may assist in the evaluation of PCNs. Recently, a study of 142 surgically resected pancreas cysts revealed that KRAS mutation was 54% sensitive and 100% specific for mucinous cysts (22). The authors demonstrated that a combination of CEA and KRAS was associated with an improved sensitivity of 83% for mucinous lesions with a decrease in specificity to 85%. Mutation in GNAS has been another diagnostic marker that has been studied for the evaluation of PCNs. GNAS mutations have been detected in 64% of surgically resected IPMNs, with 100% prevalence in intestinal type IPMNs (23). In a

separate study, the presence of both KRAS and GNAS mutations had 65% sensitivity and 100% specificity for mucinous lesions (24). The presence of KRAS or GNAS mutations has not been shown to reliably differentiate IPMN vs MCN nor predict malignancy. While the role and clinical utility of molecular biomarkers remains to be determined, they may represent an additional means to improve diagnostic accuracy, especially when only scant cyst fluid is available.

4. EUS-GUIDED THROUGH-THE-NEEDLE BIOPSY

EUS-FNA allows biochemical, cytological and DNA molecular analysis for the diagnosis and differentiation of PCNs. This approach can be limited by small cyst fluid volume and/or scant cellularity seen in the specimen. More recently, a novel EUS-guided microforceps (Moray^{TM} microforceps; US Endoscopy, Mentor, OH, USA) has become available. The Moray^{TM} EUS microforceps are 0.8mm in diameter, toothed, forceps that fit through a 19-guage EUS-FNA needle. The forceps can be visualized under EUS and permits targeted tissue sampling of the cyst wall and/or solid components within the PCN (Figure 1). Shakhatreh and colleagues reported the feasibility of the microforceps in two patients with pancreas cysts (25). In both cases, the microbiopsy specimens yielded fragments of mucinous columnar epithelium and mucoid material, which assisted in diagnosis of IPMN. More recently, we also reported the successful use of the microforceps in a patient with recurrent episodes of acute pancreatitis and a 22mm cyst in the body of the pancreas (26). In this case, an EUS-guided FNA was initially attempted but no fluid could be aspirated for analysis. The microforceps permitted targeted biopsies from the cyst wall with the specimen revealing mucinous epithelium with pleomorphism. The patient underwent distal pancreatectomy with surgical specimen confirming an IPMN with carcinoma in-situ. The potential advantage of this new device is the possibility of procuring satisfactory

specimens that may permit histopathologic analysis (Figure 2). Future large prospective studies are necessary to further corroborate these very early yet promising findings.

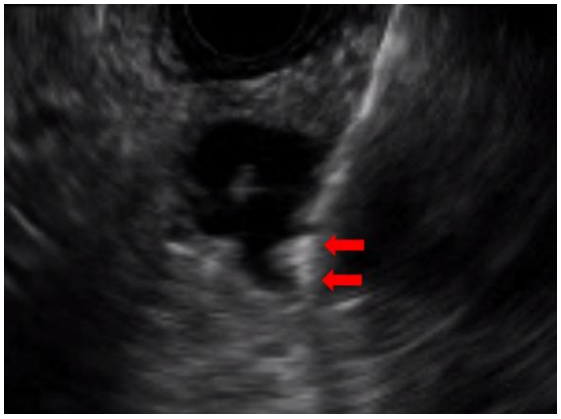

Figure 1. EUS targeted cyst wall tissue sampling of the PCN

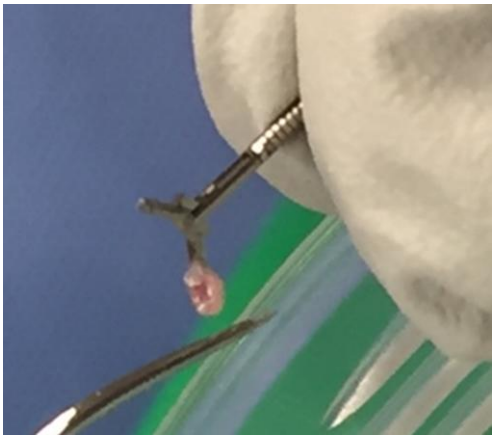

Figure 2. Procuring a satisfactory sample of the PCN for histopathologic analysis

5. CONCLUSION

EUS has proven to be a critical component in the evaluation of PCNs. As the number of incidentally discovered lesions continues to rise, EUS-FNA will hold high value to help better delineate which PCNs carry increased risk for malignant transformation. While the ideal diagnostic modality remains to be determined, novel cyst markers and tissue sampling techniques are promising and further underscore the importance of future large comparative trials.

References

1. Lee KS, Sekhar A, Rofsky NM, Pedrosa I. Prevalence of incidental pancreatic cysts in the adult population on MR imaging. Am J Gastroenterol. 2010;105(9):2079-84.
2. Laffan TA, Horton KM, Klein AP, Berlanstein B, Siegelman SS, Kawamoto S, et al. Prevalence of unsuspected pancreatic cysts on MDCT. AJR Am J Roentgenol. 2008;191(3):802-7.
3. Kawaguchi Y, Mine T. Endoscopic approach to the diagnosis of pancreatic cystic tumor. World J Gastrointest Oncol. 2016;8(2):159-64.
4. Chung JW, Chung MJ, Park JY, Bang S, Song SY, Chung JB, et al. Clinicopathologic features and outcomes of pancreatic cysts during a 12-year period. Pancreas. 2013;42(2):230-8.
5. Klibansky DA, Reid-Lombardo KM, Gordon SR, Gardner TB. The clinical relevance of the increasing incidence of intraductal papillary mucinous neoplasm. Clin Gastroenterol Hepatol. 2012;10(5):555-8.
6. Lekkerkerker SJ, Besselink MG, Busch OR, Dijk F, Engelbrecht MR, Rauws EA, et al. Long-term follow-up of neoplastic pancreatic cysts without high-risk stigmata: how often do we change treatment strategy because of malignant transformation? Scand J Gastroenterol. 2016:1-6.
7. Jacobson BC, Baron TH, Adler DG, Davila RE, Egan J, Hirota WK, et al. ASGE guideline: The role of endoscopy in the diagnosis and the management of cystic lesions and inflammatory fluid collections of the pancreas. Gastrointest Endosc. 2005;61(3):363-70.
8. Lee LS. Incidental Cystic Lesions in the Pancreas: Resect? EUS? Follow? Curr Treat Options Gastroenterol. 2014;12(3):333-49.
9. Kadiyala V, Lee LS. Endosonography in the diagnosis and management of pancreatic cysts. World J Gastrointest Endosc. 2015;7(3):213-23.
10. Chai SM, Herba K, Kumarasinghe MP, de Boer WB, Amanuel B, Grieu-Iacopetta F, et al. Optimizing the multimodal approach to pancreatic cyst fluid diagnosis: developing a volume-based triage protocol. Cancer Cytopathol. 2013;121(2):86-100.
11. Kim YC, Choi JY, Chung YE, Bang S, Kim MJ, Park MS, et al. Comparison of MRI and endoscopic ultrasound in the characterization of pancreatic cystic lesions. AJR Am J Roentgenol. 2010;195(4):947-52.
12. Zhong N, Zhang L, Takahashi N, Shalmiyev V, Canto MI, Clain JE, et al.

Histologic and imaging features of mural nodules in mucinous pancreatic cysts. Clin Gastroenterol Hepatol. 2012;10(2):192-8, 8.e1-2.

13. Tanaka M, Fernández-del Castillo C, Adsay V, Chari S, Falconi M, Jang JY, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. Pancreatology. 2012;12(3):183-97.

14. Tarantino I, Fabbri C, Di Mitri R, Pagano N, Barresi L, Mocciaro F, 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(1):41-4.

15. Layfield LJ, Ehya H, Filie AC, Hruban RH, Jhala N, Joseph L, et al. Utilization of ancillary studies in the cytologic diagnosis of biliary and pancreatic lesions: the Papanicolaou Society of Cytopathology guidelines for pancreatobiliary cytology. Diagn Cytopathol. 2014;42(4):351-62.

16. Bick BL, Enders FT, Levy MJ, Zhang L, Henry MR, Abu Dayyeh BK, et al. The string sign for diagnosis of mucinous pancreatic cysts. Endoscopy. 2015;47(7):626-31.

17. Brandone N, Poizat F, Thomassin-Piana J, Turrini O, Popovici C, Terris B. [A solid variant of a serous cystadenocarcinoma of the pancreas]. Ann Pathol. 2016;36(2):125-9.

18. van der Waaij LA, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. Gastrointest Endosc. 2005;62(3):383-9.

19. Hong SK, Loren DE, Rogart JN, Siddiqui AA, Sendecki JA, Bibbo M, 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(4):775-82.

20. Barresi L, Tarantino I, Traina M, Granata A, Curcio G, Azzopardi N, 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(1):45-50.

21. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, et al. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. Gastroenterology. 2004;126(5):1330-6.

22. Nikiforova MN, Khalid A, Fasanella KE, McGrath KM, Brand RE, Chennat JS, et al. Integration of KRAS testing in the diagnosis of pancreatic cystic lesions: a clinical experience of 618 pancreatic cysts. Mod Pathol. 2013;26(11):1478-87.

23. Dal Molin M, Matthaei H, Wu J, Blackford A, Debeljak M, Rezaee N, et al. Clinicopathological correlates of activating GNAS mutations in intraductal papillary mucinous neoplasm (IPMN) of the pancreas. Ann Surg Oncol. 2013;20(12):3802-8.

24. Singhi AD, Nikiforova MN, Fasanella KE, McGrath KM, Pai RK, Ohori NP, et al. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. Clin Cancer Res. 2014;20(16):4381-9.

25. Shakhatreh MH, Naini SR, Brijbassie AA, Grider DJ, Shen P, Yeaton P. Use of a novel through-the-needle biopsy forceps in endoscopic ultrasound. Endosc Int Open. 2016;4(4):E439-42.

26. Coman RM, Schlachterman A, Esnakula AK, Draganov PV, Yang D. EUS-guided, through-the-needle forceps: clenching down the diagnosis. Gastrointest Endosc. 2016.