Review Article

Role of endoscopic ultrasound-guided fine-needle aspiration cytology, viscosity, and carcinoembryonic antigen in pancreatic cyst fluid

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ABSTRACT

Due to the advances and increased utility of abdominal cross-sectional imaging, the diagnosis of pancreatic cysts continues to increase. Many endosonographers, pancreatologists, and surgeons consider endoscopic ultrasound (EUS) to be an essential tool in the management of pancreatic cystic lesions (PCLs). EUS can help distinguish between mucinous and nonmucinous lesions and may identify the specific cyst type. EUS achieves these goals by delineating the cyst morphology, identifying high risk stigmata and worrisome features, and through image-guided fine-needle aspiration (FNA) and cyst fluid analysis. However, recent consensus statements have called to question the utility and diminished the role of EUS in this setting. The aim of this review is to assess the role and advances of EUS-FNA in pancreatic cyst fluid analysis, specifically in terms of fluid cytology, viscosity, and carcinoembryonic antigen (CEA) analysis.

Key words: Carcinoembryonic antigen (CEA), cytology, endoscopic ultrasound (EUS), fine-needle aspiration (FNA), pancreatic cyst fluid analysis, viscosity

INTRODUCTION

Pancreatic cystic lesions (PCLs) are increasingly being detected largely due to the growing use of and technological improvements in noninvasive cross-sectional abdominal imaging and because of an aging population.[1-4] The estimated prevalence of PCL among persons between 40 years and 84 years of age within the United States is 2.5%.[5] However, they are identified in about 3% of the patients undergoing abdominal computed tomography (CT) and approximately 20% of the patients undergoing magnetic resonance imaging (MRI).[6,7] In addition, autopsy studies demonstrate pancreatic cystic lesions (PCLs) in 25% or more patients.[8] Contrary to most cysts located in other organs, several cysts types that comprise the majority of PCLs have malignant

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potential. The differential diagnosis of PCLs is broad and includes:

1. Nonneoplastic cysts with no malignant potential [e.g., serous cystadenomas (SCAs), pseudocysts, retention cysts, lymphoepithelial cysts, and benign epithelial cysts];
2. Cystic neoplasms [e.g., mucinous lesions: Mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN)]; and
3. Nonmucinous cysts with varying malignant potential [e.g., solid pseudopapillary epithelial neoplasm (SPEN)].[9]

Accurate distinction is necessary to determine prognosis and guide clinical decision-making. However, distinguishing the cyst type is notably challenging and prone to error. The goal is to prevent patients with benign lesions who have nil or only nominal malignant potential from undergoing unnecessary investigations with the attendant risk and cost. Likewise, there is a need to identify premalignant lesions that warrant initial investigation and surveillance in order to provide early intervention when indicated. Due to the wide array of management strategies, the need for accurate diagnosis is of utmost importance.

While PCLs are often first detected by CT, the diagnostic sensitivity of CT is less than 70% for determining a malignant lesion with a specificity of 87-100%.[10] T2-weighted MRI provides superior soft tissue contrast and is more useful in identifying PCLs.[7] The utility of MRI largely lies in its ability to recognize a communication between cysts and the main pancreatic duct, and when compared to CT it is beneficial through the avoidance of ionizing radiation.[11] Despite recent technological advances, the diagnostic accuracy for PCLs remains low and in the range of 20-80%.[12] EUS further defines the cyst morphology but also suffers from inadequate diagnostic accuracy when based on imaging alone. The ability to obtain cyst fluid and cytology from worrisome areas offers incremental diagnostic sensitivity.[13] The aim of this review is to discuss the role of EUS-guided cyst fluid analysis, focusing on the fluid cytology, viscosity, and carcinoembryonic antigen (CEA).

ENDOSCOPIC ULTRASOUND-GUIDED FINE-NEEDLE ASPIRATION

EUS-guided FNA plays a central role in the differentiation and diagnosis of PCLs.[14] EUS typically provides a detailed evaluation of the overall cyst morphology, cyst wall, and internal contents. While the appearance of different cyst types often overlap, some cysts possess certain morphologic features that may provide greater diagnostic specificity.[15] The EUS morphologic appearance provides a diagnostic accuracy of only 50% in differentiating mucinous from nonmucinous cystic lesions.[16] In addition to providing an initial imaging diagnosis, EUS offers the ability to aspirate fluid and perform biopsy on a solid component if present.[17] Studies have demonstrated that the addition of cyst fluid analysis increases the diagnostic accuracy over EUS imaging alone.[18] Cyst fluid aspiration allows cytological evaluation, determination of fluid viscosity, and assessment of tumor markers, pancreatic enzymes, and DNA analysis.[19]

CYST FLUID CYTOLOGY

Cytological diagnosis of cystic fluid relies on the detection of malignant cells, mucin-containing cells, and glycogen-containing cells. Malignant cells are seen in malignant lesions. In a retrospective study from Massachusetts General Hospital, cytology detected 30% more cancers with high risk imaging features.[20] In another study, cytology was a better predictor of malignancy than the symptoms.[21] Mucin-containing cells are seen in IPMNs and mucinous cystic neoplasia but cannot distinguish between these two cysts types and the absence of mucinous cells does not exclude either entity. The presence of glycogen-containing Periodic acid-Schiff (PAS) cells confirms the diagnosis of a SCA.[22] While some suggest that the lack of epithelial cells on cytologic evaluation suggests a pseudocyst, especially in the presence of an inflammatory smear, as noted it is common for the specimen to contain no epithelial cells, which makes their absence an unreliable criterion for pseudocysts. As a result, diagnosing pseudocyst by cytology remains a diagnosis by exclusion.[23]

While cytological analysis of cyst fluid may provide a specific diagnosis, reliance on cytology is severely hampered by poor diagnostic sensitivity that largely results from the paucicellular sample commonly collected. The poor sample adequacy largely results from the limited shedding of cells from the cyst wall and presence of denuded areas of the epithelium. In addition, certain diagnostic features such as ovarian stroma within MCNs is located deep in the epithelium and never identified from cyst fluid analysis.

In a multicenter prospective study involving 341 patients who underwent EUS-FNA of PCL, 112 patients underwent surgical resection with a gold standard for comparison. This study demonstrated a diagnostic sensitivity of cytology for detecting mucinous lesions of 35% and specificity of 83% patients. The overall accuracy was 59%, which was similar to the overall accuracy of EUS morphology alone. Furthermore, the sensitivity of cytology for diagnosing malignancy in a malignant mucinous cystic lesion was only 22%.[22] Another study by Chebib *et al.* showed the cytology to be more specific than imaging for detecting malignancy in cysts >3 cm in size.[24]

Other measures may be taken to enhance the cytological yield. Adding cytology brushings may improve the diagnostic accuracy for mucinous lesions of 2 cm or larger in size. In this study, cytobrushing samples from PCLs were significantly more likely to detect intracellular mucin than was FNA alone (62% versus 23%, $P = 0.001$).[25] Others have shown that direct cyst wall aspiration can improve the cytological diagnostic sensitivity by 29% for mucinous lesions when compared to standard fluid cytology alone.[26] This is typically performed after aspirating the cystic fluid and allowing the cyst wall to collapse for easier sampling.[27] Despite promising preliminary data, the use of intracystic brush cytology and direct cyst wall aspiration are seldom performed. It is the author's opinion that the lack of use largely reflects some uncertainty regarding the added diagnostic sensitivity and technical issues and the potential for adverse events. The diagnostic accuracy is increased up to 90% when cystic fluid tumor marker level, amylase level, and mucin stain are added to cytology.[16]

CYST FLUID VISCOSITY

Gross evaluation and inspection of the pancreatic cystic fluid may assist in the diagnosis.[28] The subjective impression that the cystic fluid is thick and viscous and difficult to aspirate into the FNA needle often indicates a high mucin content. Fluid is typically assessed using the "string sign." This clinical test is performed by placing a drop of fluid between the thumb and index finger and determining the length of stretch before disruption of the string. It is simple, free of charge, and can be performed by the endosonographer directly after cyst aspiration.

A recently published study assessed the utility of the string sign for the diagnosis of mucinous pancreatic cysts. It revealed that the string sign was highly specific and improved diagnostic accuracy of pancreatic cyst fluid analysis. In this particular study, a test was considered positive when the string was ≥1 cm in length and lasted for ≥1 s. Combining the string sign with cytology, mucin staining, and CEA levels has led to improvement in the specificity (96%) and sensitivity (90%) for diagnosing mucinous cysts.[29] A study from 2009 suggested that an increased cyst fluid viscosity was associated with malignant or potentially malignant cysts. The median string sign was reported to be 0 mm for benign cysts and 3.5 mm for malignant cysts or cysts harboring malignant potential. A longer stretch implies a higher viscosity and higher mucin content and a higher malignancy potential.[30]

Despite being a promising tool and easy-to-perform test, the string sign remains subjective and prone to variations in interpretation. While we do support the use of the string sign as a soft indicator of a mucinous lesion, we do not feel that this finding alone can be considered diagnostic and we do not favor its use as a potential indicator of malignancy.

CARCINOEMBRYONIC ANTIGEN

Given the limitations in cyst fluid cytological analysis and viscosity assessment, cyst fluid tumor markers have emerged to potentially improve the diagnostic accuracy for the detection of malignant or premalignant pancreatic cysts. There are a number of purported tumor markers that have been extensively studied for the aforementioned reason.[15] Most studies pertain to the use of CEA, carbohydrate antigens (CAs) 19-9, CA 15-3, and CA72-4.

The literature has made it clear that markers other than CEA are of very limited utility and for this reason, our focus is on the use of CEA as a tool for potentially discriminating between mucinous and nonmucinous lesions and possibly even identifying malignant lesions.[16,31] The role of CEA as a potential marker to differentiate between PCLs was first reported in the 1980s.[32,33] Different cutoffs for CEA ranging from 5 ng/mL to 800 ng/mL have been utilized in multiple studies with varying ranges of sensitivity, specificity, and accuracy.[22,30,34-36] The value of different tumor markers was investigated in the Cooperative Pancreatic Cyst Study. In this study, various diagnostic modalities including tumor markers were evaluated with respect to histology as the gold standard. The accuracy of CEA

was calculated at 79% using a cutoff of 192 ng/mL. When comparing CEA cyst fluid measurement to other diagnostic modalities, it was concluded that it was more accurate than EUS and cytology in the diagnosis of mucinous cystic lesions. In addition, CEA alone yielded greater accuracy than any other combination of tests.[16] A subsequent study in which 442 pancreatic cyst samples were initially tested for CEA yielded a CEA cutoff of 30 ng/mL to differentiate between mucinous and nonmucinous cysts.[34] This study however, suffered from less than ideal measures to define the diagnostic gold standard and we do not favor its use or suggested CEA threshold. Currently, a cutoff of 192 ng/mL is typically referenced as the standard and approximately 0.2-1.0 mL of cyst fluid is required to perform this test.[37]

Cyst fluid CEA values are typically low in serous cystadenomas and cystic neuroendocrine tumors, and elevated for mucinous lesions. Data do not support the use of CEA for distinguishing premalignant mucinous lesions from those that have undergone malignant transformation.[38] A recent study by Nagashio *et al.* revealed a potential role for combining CEA and CA125 in an attempt to segregate MCNs from IPMNs but we regard these findings still as preliminary and of uncertain utility.[39]

CONCLUSIONS

The detection rate of pancreatic cysts has increased in recent years. Many of these cysts are asymptomatic and discovered incidentally. Pancreatic cyst fluid analysis plays a pivotal role in the workup of pancreatic cysts. Cyst fluid analysis is particularly important when clinical and imaging data are indeterminate. Apart from its diagnostic role as an imaging technique, EUS has the added advantage of allowing aspiration of the cyst contents for analysis. Biochemical analysis of the cystic fluid is an important part of the investigation process in conjunction with a variety of other tests. Measurement of cyst fluid CEA concentration has proven to be of the highest clinical utility. Lately, few studies have focused on cyst fluid DNA analysis and its potential role in the investigation of pancreatic cysts. These emerging markers remain under investigation and are not widely available. There remains a need to do further research to define appropriate diagnostic markers that are cost-effective and have a high yield when investigating PCLs. Various societies and committees advocate often disparate and conflicting recommendations regarding the clinical evaluation of PCLs. The value of any recommendation has been hampered by the paucity of well-designed studies. Nevertheless, the lack of sound data has not prevented some from making sweeping reforms to longstanding and broadly accepted dogma in terms of PCL evaluation. We encourage a maintained role for EUS and cystic fluid analysis in accordance with prior recommendations.

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Conflicts of interest

There are no conflicts of interest.

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