The role of EUS-FNA in the evaluation of pancreatic cystic lesions

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INTRODUCTION

Pancreatic cystic lesions are a common, often incidental, finding that can be detected in up to 13.5% of cross-sectional imaging studies.[1] The inherent difficulty in their management stems from the fact that they can be generally classified into two main groups which differ greatly in their malignant potential: mucinous cysts and nonmucinous cysts. Mucinous cysts include intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms, which can be precursors of pancreatic adenocarcinoma. Even within this group, the rate of malignant transformation varies widely and can range from 10% to 70% depending on whether the lesions have high-risk features.[2-4] On the other hand, nonmucinous cysts include serous cystic neoplasms, which are often indolent and benign in nature.

This differentiation is critical to identify premalignant or malignant lesions that will benefit from appropriately timed surgical resection, and also to avoid unnecessary surveillance or surgery in benign cysts, which may lead to high costs and even harm to patients.[5] Determining the small minority of cysts that are at a higher risk of having or developing malignancy has been challenging – even official guidelines have not been able to come to an agreement as to which features are considered suspicious. The American Gastroenterology Association considers size (≥3 cm), a dilated main pancreatic duct, or the presence of an associated solid component as features associated with an increased risk of malignancy.[5] In addition to the above, the American College of Gastroenterology recommends that further evaluation should be pursued if cysts are associated with new or worsening diabetes, rapid increase in size (≥3 mm/year), obstructive jaundice, acute pancreatitis, or a significantly elevated CA 19-9.[6] On the other hand, the Fukuoka guidelines classify cysts into two categories: those with "worrisome" features which require further evaluation and those with "high-risk" features for which surgical resection should be considered. Lymphadenopathy, thickened enhanced cyst walls, and an abrupt change in the main pancreatic duct caliber with distal pancreatic atrophy are among the additional features evaluated.[4]

Commonly used diagnostic tools to evaluate pancreatic cysts include computed tomography, magnetic resonance imaging, and EUS-FNA. However, there is no single test that reliably distinguishes between the two groups. Instead, a combination of clinical history, imaging

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characteristics, and cyst fluid analysis is used to aid in further classification.

EUS-FNA

EUS is useful in the assessment of pancreatic cyst morphology. Based on EUS imaging, several cyst features are traditionally believed to be associated with malignant or premalignant cysts, namely, having a thick cyst wall; having a dilated main pancreatic duct; the presence of septation; or the presence of mural nodules or a mass within the cyst.[7] However, the diagnostic accuracy of using EUS alone to assess the risk of malignancy in lesions varies widely and can range from 40% to 93%.[8] It is also hampered by poor interobserver variability even among experienced endosonographers, and only has modest accuracy when used in isolation to differentiate between mucinous and nonmucinous cysts.[9-11] Unfortunately, given its invasive nature, EUS is not recommended as a first-line investigation for the evaluation of small benign cysts, and is generally considered insufficient for further characterization of cystic pancreatic lesions and their malignant potential.[12]

Fortunately, the utility of EUS lies not just in being able to provide morphological assessment of the cyst. It is also able to provide additional valuable diagnostic information via FNA and subsequent analysis of the cyst fluid, particularly for indeterminate cysts or those with high-risk features. Currently, the assessment of cyst fluid cytology and other intracystic markers such as cyst fluid tumor markers such as carcinoembryonic antigen (CEA) has been widely used to help differentiate between mucinous and nonmucinous cysts.

Fluid cytology

Fluid cytology should technically be able to provide an accurate diagnosis of pancreatic cystic lesions. Identification of glycogen-rich cells suggests benign serous cystadenomas, whereas identification of mucin-containing cells suggests the presence of mucinous lesions. Indeed, a meta-analysis including 18 studies and a total of 1438 patients demonstrated a high pooled specificity of 93% for differentiating mucinous and nonmucinous cysts.

However, fluid cytology only has a moderate pooled sensitivity of 54%.[13] A negative result cannot reliably exclude a mucinous cystic neoplasm, making its usefulness in clinical practice limited. This is due to several factors: first, cyst fluid often lacks sufficient cellular contents for diagnosis.[14] Second, cyst fluid aspirates often contain gastrointestinal contaminants from within the needle track. Mucin-secreting cells can be found in both mucinous pancreatic neoplasms and normal pancreatic duct lining. It can be difficult to distinguish cystic cellular contents from gastrointestinal contaminants, which can lead to misinterpretation of results.[15]

In addition, some countries such as Japan do not routinely practice EUS-FNA of suspected mucinous pancreatic cysts because of concerns over the possibility of peritoneal seeding following EUS-FNA, leading to pseudomyxoma peritonei.[16] However, in a retrospective study of 175 patients who had IPMNs resected, preoperative EUS-FNA was shown not to increase the risk of peritoneal seeding compared with patients who did not undergo preoperative tissue sampling.[17]

Fluid carcinoembryonic antigen

Fluid CEA is a marker of mucin production and is secreted from the luminal surface of glandular cells of mucinous cysts. It is the most reliable marker to distinguish mucinous cysts from nonmucinous cysts, with mucinous cysts having significantly greater fluid CEA values.[18] However, the challenge lies in determining the optimal cutoff value, with studies reporting different levels. Cizginer *et al.* showed that a value of ≥109.9 ng/mL achieved an overall accuracy of 86%, whereas Brugge *et al.* reported that the optimal cutoff was ≥192 ng/mL, with an overall accuracy of 79%.[11,18] van der Waaij *et al.* also found that a cutoff of ≥800 ng/mL had a positive predictive value of 94% for mucinous cysts, but at the cost of having a lower negative predictive value.[19] The converse is also true, with a low fluid CEA of ≤5 ng/mL being indicative of nonmucinous cysts and being useful in excluding mucinous lesions. Most guidelines continue to recommend a cutoff value of ≥192 ng/mL, but in general, using a higher cutoff value results in a higher specificity but a lower sensitivity for differentiating mucinous from nonmucinous cysts.

Although fluid CEA as a diagnostic marker is superior to both EUS and fluid cytology for identifying mucinous cysts, its overall accuracy is not high enough for it to be used in isolation. It is also unable to predict the presence of malignancy or the histologic grade. In addition, 1 ml of cyst fluid is needed before analysis

can be accurately carried out, which is a relatively large amount of cyst fluid. On the whole, fluid CEA is still best used in combination with other diagnostic tools to most accurately delineate the nature of pancreatic cysts.[20]

METHODS TO IMPROVE THE ACCURACY OF EUS-FNA IN DIAGNOSING MALIGNANT PANCREATIC CYSTS

A significant number of pancreatic cystic lesions are still wrongly classified even when using a combination of both EUS and cyst fluid analysis for assessment. Preoperative diagnosis can be incorrect in up to a third of patients when compared with postoperative surgical histology.[21,22] There is a pressing need to improve our diagnostic accuracy for pancreatic cysts. Two recent methods have shown great promise: analysis of molecular DNA markers and the use of microforceps biopsy devices for sampling.

Molecular DNA markers

DNA sequencing has revealed the genetic profiles and molecular DNA markers for specific pancreatic cyst types. In IPMNs, the most frequent genetic alteration is an oncogenic KRAS mutation, with a prevalence of >80%. Somatic mutations in the oncogene GNAS are also commonly seen, particularly in IPMNs involving the main pancreatic duct. Other potential gene mutations involve the tumor suppressor gene RNF43, CTNNB1, β-catenin, TP53, PIK3CA, PTEN, CDKN2A, and SMAD4, though activating mutations in KRAS and GNAS account for the bulk of all alterations. The genetic profile seen in mucinous cystic neoplasms is similar to those found in IPMNs, with the main difference being an absence of GNAS mutations. Nonmucinous cysts have not been found to have KRAS or GNAS mutations.[23]

Studies have sought to use these differences as a means of diagnostic classification between pancreatic cyst types. The multicentric prospective PANDA study showed that the presence of mutant KRAS had a sensitivity of 45% and a specificity of 96% for identifying a mucinous cyst.[20] A meta-analysis confirmed the ability of KRAS to differentiate mucinous and nonmucinous cysts, with a pooled specificity of 0.98. However, the low pooled sensitivity of 0.47 suggests that it is not suitable to be used alone for diagnosis.[24] Including GNAS mutation testing together with KRAS analysis increased the sensitivity and the specificity of being able to diagnose mucinous cysts – in particular, IPMNs.[25]

Molecular marker analysis was traditionally done with the use of Sanger sequencing. However, there are limitations associated with the conventional assay involving the inherent sensitivity and specimen requirements. The use of next-generation sequencing has allowed for a lower limit of detection, higher specificity and sensitivity for mucinous differentiation, and the added benefit of being able to assay multiple genes simultaneously by using only minute amounts of cyst fluid and DNA.[26] Unfortunately, next-generation sequencing is technically complex and costly, requiring dedicated infrastructure and personnel, making it difficult to implement into clinical practice.

It has been demonstrated that using molecular marker analysis in combination with clinical features, cyst fluid analysis, and EUS morphology can increase the sensitivity and specificity of pancreatic cyst classification.[27] However, most guidelines feel that the use of molecular DNA markers is still investigational and not ready for routine clinical use, given the diagnostic performance limitations and significant costs involved. The American College of Gastroenterology does recommend that it can be considered in cases where the diagnosis is indeterminate and where the results are likely to change management.[5] Large multicentric validation studies are still pending.

Microforceps biopsy

The ideal tissue specimen for examination would be a histological core specimen, as this would allow for improved tissue architecture interpretation, the ability to perform immunostaining or advanced molecular diagnostic testing, and subsequent increased diagnostic accuracy, However, the diagnostic yield obtained has always been suboptimal due to the limited tissue samples that can be obtained, even when using larger 19G FNA needles. Alternative sampling techniques have been studied previously – FNA needles specially designed to obtain histological specimens (*e.g.*, ProCore, SharkCore, and TruCut) only resulted in a marginal increase in tissue acquisition, whereas cytology brushes had high rates of technical failure and intracystic bleeding.[28,29]

The microforceps, or through-the-needle biopsy device, was developed to address this need. It is designed to

pass through a standard 19G EUS-FNA needle, and has an open jaw width of 4.3 mm under constant EUS visualization; the device is slowly advanced through the needle and then manipulated to capture the desired tissue. It can obtain the targeted tissue samples from the cyst wall, septa, or mural nodules. This sampling method has been consistently demonstrated to have a high technical success rate, and is feasible even in lesions as small as 1.5 cm, irrespective of the location of the cyst within the pancreas. It allows for simultaneous tissue sampling and pancreatic cyst fluid acquisition. An adequate histological specimen was also able to be obtained in a significantly higher proportion of cases using the microforceps biopsy as compared to the standard EUS-FNA needle.[30]

When compared with FNA cytology, it appears that histology obtained from microforceps biopsy helps to improve the diagnostic accuracy of cyst subtypes. Reports from case studies and case series have shown a higher concordance of histology to surgical pathology of mucinous cysts, with one study demonstrating a diagnostic accuracy of 100% compared with 21% with FNA cytology.[30] Tissue from the microforceps biopsy was also diagnostic of the degree of dysplasia in 80% of cysts. This is critical as it altered the treatment course in these patients, as the final histology was discordant with the initial benign EUS morphology findings. A meta-analysis involving 203 cysts compared the use of molecular marker analysis against microforceps biopsy for the evaluation of pancreatic cystic lesions. A surgical pathology specimen was used as a reference standard for diagnosis. The use of microforceps biopsies led to a high diagnostic yield (73%) and a high rate of correctly identifying cyst subtypes (70.7%).[31] However, the studies were all retrospective and had a small sample size.

The overall rate of adverse events is low, ranging from 0% to 12.5%. Intracystic bleeding (6.1%) was the most commonly reported event, but the bleeding was self-limited and not significant in all cases. The other common adverse event was postprocedural acute pancreatitis, occurring in up to 5.3% of patients. Again, most of these episodes were mild, but one patient developed a symptomatic pseudocyst requiring endoscopic drainage.[30]

CONCLUSION

Accurate diagnosis and classification of pancreatic cystic lesion subtype continues to remain a challenge. We need this to confidently risk stratify patients into those who can be monitored with surveillance imaging and those who should be considered for surgical removal. Evaluation of a newly diagnosed pancreatic cyst routinely involves EUS and FNA for assessment of cyst morphology and analysis of cyst fluid for cytology and CEA. However, while these are useful, they do not yet have sufficient diagnostic capability to consistently differentiate mucinous and nonmucinous cysts, or malignant from benign cysts.

Molecular analysis of cyst fluid and exploring better sampling techniques with the microforceps biopsy are promising methods to increase the diagnostic yield. However, these will need large prospective trials and validation before they can be used in routine practice. For now, they can only be considered as complementary investigations to be used when first-line investigations prove unrevealing.

Conflicts of interest

There are no conflicts of interest.

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