Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of pancreatic cysts by combined cytopathology and cystic content analysis

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Abstract

Recent advances in imaging technology have resulted in an increase in incidental discoveries of pancreatic cystic lesions. Pancreatic cysts comprise a wide variety of lesions and include non-neoplastic cysts and neoplastic cysts. Because some pancreatic cysts have more of a malignant potential than others, it is absolutely essential that an accurate diagnosis is rendered so that effective care can be given to each patient. In many centers, endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) has emerged as the modality of choice that enables one to distinguish between mucinous and non-mucinous lesion, diagnose malignancy and collect cyst fluid for further diagnostic studies, such as pancreatic enzyme levels, molecular analysis and other tumor biomarkers. The current review will focus on EUS-guided FNA and the cytological diagnosis for pancreatic cysts.

Key words: Pancreatic cyst; Endoscopic ultrasound; Fine needle aspiration; Diagnosis; Cystic fluid analysis; Cytology

Core tip: Pancreatic cysts comprise non-neoplastic cysts and neoplastic cysts. It is absolutely essential that an accurate diagnosis is rendered so that effective care can be given to each patient. In many centers, endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) has emerged as the modality of choice that enables one to distinguish between mucinous and non-mucinous lesion, diagnose malignancy and collect cyst fluid for further diagnostic studies, such as pancreatic enzyme levels, molecular analysis, and other tumor biomarkers. The current review will focus on EUS-guided FNA and the cytological diagnosis and new classification for pancreatic cysts.

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INTRODUCTION

The overall prevalence for cystic lesions of the pancreas has been estimated to be no more than 1% of all pancreatic neoplasms[1]. However, the advent of high-resolution imaging modalities has led to the increased frequency of incidentally discovered pancreatic cysts. In the United States, a prevalence of incidental pancreatic cysts estimates about 2.6% to 13.5% of adults[2-4]. The increasing incidence of pancreatic cystic lesions has been directly linked to increasing age[5]. Pancreatic cystic lesions are also being detected sooner rather than later as reflected in the decreasing median sizes of pancreatic cysts both in the United States and in other countries[6]. Although a recent study suggests that only 2% of pancreatic cysts are malignant at diagnosis[7], the trend of increasing discovery of pancreatic cysts is significant because some types of pancreatic cystic lesions carry an augmented risk for malignant transformation.

Pancreatic cysts comprise a wide variety of lesions and include non-neoplastic cysts and neoplastic cysts. The classification and nomenclature of pancreatic cysts are very important for pathologic and clinical diagnosis. The non-neoplastic cysts include pseudocysts, retention cysts, lymphoepithelial cysts, benign epithelial cysts, and congenital cysts. Non-neoplastic cysts are believed to have low to no malignant potential. Neoplastic cysts are typically categorized as mucinous and non-mucinous based on the type of epithelium they possess[8]. The mucinous cysts consist of mucinous cystic neoplasms (MCN) and intraductal papillary mucinous neoplasms (IPMN). The non-mucinous cysts include serous cystadenomas, solid pseudopapillary neoplasms (SPN), cystic pancreatic neuroendocrine tumors (PNET), cystic pancreatic ductal adenocarcinomas (PDA) and its variants, cholangiocarcinoma, acinar cell carcinoma, high-grade neuroendocrine carcinoma (small cell and large cell), pancreatoblastoma, lymphomas, sarcomas, and metastatic tumors. The neoplastic cysts are categorized as being malignant (*i.e.*, PDA, PNET) or having malignant potential (*i.e.*, MCN, IPMN, SPN). Among mucinous subtypes of cysts, it has also been suggested that branch duct IPMN (BD-IPMN), while having malignant potential, may exhibit more indolent behavior compared to main duct-IPMN[9,10].

The management options for pancreatic cystic lesions are as varied as the lesions they are designed to diagnose and treat. Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) is a major technique used in many institutions to sample pancreatic cystic lesions. As will be described in this review, there are multiple ways available to further study these lesions, including cytologic diagnosis and cystic content analysis by chemical and molecular tests. A new histologic classification system for pancreatic lesions has also been introduced to help clinicians and patients understand the malignant potential of each type of pancreatic lesion. Based on new diagnosis and classification of the cystic lesions, most patients need no further treatment. However, many patients require surveillance or other more invasive therapies (*i.e.*, surgical resection) depending upon the risk of malignant transformation. Hence, obtaining an accurate differential diagnosis is of utmost importance in properly managing these patients in such a way that minimizes risk of complications[11].

EUS-GUIDED FNA SAMPLES FOR PANCREATIC CYSTS

Initial imaging studies

The initial clinical workup for incidentally discovered pancreatic cysts involves the use of radiologic imaging to further characterize the lesion[12]. MRI with magnetic resonance cholangiopancreatography (MRCP) is the preferred method over pancreatic protocol multidetector (MD) CT because MRCP is able to evaluate the presence of septa, nodules, main-duct involvement, and branch duct involvement. In many cases, it is also able to accurately distinguish between mucinous and non-mucinous cysts[13,14]. Studies have also found that, within the proper clinical context, MRI and CT are capable of determining which pancreatic cystic lesions are more likely to be malignant[15,16]. This is especially true if the features pathognomonic for a given lesion are present. However, in many instances, the combined clinical and radiologic picture is unable to elucidate the type of lesion or its likelihood of harboring malignancy, thus making definitive treatment difficult to achieve. Much of this has to do with the fact that morphologic features of many pancreatic cystic lesions frequently overlap and can appear similar on imaging studies[17]. In this regard, cytologic diagnosis with EUS-FNA is a good means to arriving at a more definitive diagnosis.

EUS-FNA procedure for pancreatic cysts

EUS-guided FNA is a safe procedure that employs the use of an image guidance system and an endoscope that is passed through the esophagus and into the stomach and/or duodenum. Because the importance of obtaining a good sample as well as adequate sample preparation cannot be overemphasized, many centers perform EUS-guided FNA in conjunction with rapid on-site evaluation (ROSE) by a cytopathologist or cytotechnologist. ROSE has been shown to improve the diagnostic yield of specimens and turnaround time obtained by EUS-guided FNA[18-21]. During ROSE, a cytopathologist or cytotechnologist screens air-dried smears that are first stained with rapid-Romanowsky method, such as Diff-Quik® and Hemacolor®, in order to

determine specimen adequacy and to give a preliminary diagnosis, if possible. The rest smears can be alcohol-fixed and stained with the Papanicolaou stain. Additional FNA samples are collected in saline or other alcohol-rich preservative solutions for liquid-based preparations (*i.e.*, ThinPrep®, SurePath®), cytocentrifuge preparations, or cell blocks. Cell blocks are especially helpful in the event that immunohistochemistry is needed to differentiate between the different types of pancreatic lesions. Specimens obtained by EUS-guided FNA can also be used for further diagnostic studies, such as enzymatic testing and molecular testing (to be discussed below in detail in this review).

The advantages of EUS-guided FNA are numerous and include direct real-time visualization of the needle, identification of smaller lesions that can be missed by imaging studies, as well as identification of local metastases and invasion of structures[22]. One study recently showed that the incremental increase in diagnostic yield of EUS and fluid analysis over CT and MRI for prediction of a neoplastic cyst is 36% and 54%, respectively. Complication rates were also low, with pancreatitis being the most common complication (1.1%)[23]. One large prospective multicenter study revealed a complication rate of 6%; bleeding was the most common complication[24]. An extremely rare complication associated with EUS-guided FNA is tumor seeding, especially with IPMN[25-27].

Despite the high specificity of EUS-guided FNA, the main disadvantage that comes with EUS-guided FNA is that samples obtained are often hypocellular. The study by de Jong *et al*[28] showed that a cytopathologic diagnosis was only possible in one-third to one-half of all cases examined. However, it has also been suggested that the sensitivity, which can range from 60% to 100%, often depends upon an institution's experience with the technique[29]. One way to potentially overcome the low sensitivity of this procedure is to do cystic wall puncture (CWP), a procedure in which a targeted FNA of the cyst wall is performed after removal of cyst fluid. One study utilizing this method reported adequate cytologic material in 81% of all cases. Complication rate was minimal with only one patient developing mild pancreatitis post-CWP[30]. The study by Rogart *et al*[31] also showed that CWP may also be helpful in increasing the diagnostic yield of mucinous cystic lesions of the pancreas. Furthermore, there are some important diagnostic pitfalls. For example, GI contamination can cause one to interpret an inadequate specimen as adequate, thus leading to a false-negative diagnosis. Conversely, markedly reactive epithelial cells can be mistaken for malignancy[32,33]. Fortunately, it is possible to avoid these diagnostic pitfalls by making sure cytopathologists have a working knowledge of normal, reactive, and neoplastic pancreatic conditions as well as being sure to correlate all cytologic findings with each patient's clinical history and imaging studies.

DIAGNOSIS OF PANCREATIC CYSTS BY CYTOLOGY

Standardized terminology and classification of pancreatic cysts

Aspirates obtained from EUS-guided FNA are graded in much the same way as aspirates obtained for other non-gynecological specimens. Specimens that lack sufficient cytologic material to render a diagnosis are designated as "unsatisfactory". Specimens that have adequate cytologic material and that are helpful in explaining the presence of a radiologically detected lesion are designated as "satisfactory". Satisfactory specimens are further characterized as "negative for malignancy", "atypical", "benign neoplasm", "suspicious for malignancy" or "positive for malignancy" depending upon the degree of cytologic atypia, cellularity (or lack thereof) and other background features present. Wherever possible, more descriptive terms are also used if a specific pathologic diagnosis can be made. However, there is variable, if not conflicting terminology, used in different institutes and even by individual pathologists. Therefore, tremendous effort has been made to develop a standardized system of classification for pancreatic cytopathology. Recently, Pitman *et al*[34] published a "standardized terminology and nomenclature for pancreatobiliary cytology: The Papanicolaou Society of Cytopathology Guidelines" (see modified guideline in Table 1). In their categorization, "Non-Diagnostic" lesions are in Category I, lesions classified as "Negative for Malignancy" are in Category Ⅱ, "Atypical" lesions are in Category Ⅲ, lesions classified as "Suspicious for Malignancy" are in Category Ⅴ, and lesions that are "Malignant" are in Category Ⅵ. Category Ⅳ consists of Category ⅣA for "Neoplastic: Benign" and Category ⅣB for "Neoplastic: Other". Serous cystadenoma is the main neoplasm in Category ⅣA. In Category ⅣB, they include both mucinous neoplasms, such as IPMN and MCN, and non-mucinous neoplasms, such as pancreatic endocrine tumor. However, as detailed below, the morphologic, molecular, and immunohistochemical features are very different for these lesions, with mucinous neoplasms having a greater potential to become malignant than non-mucinous neoplasms. Therefore, we suggest that Category ⅣB should be further separated into Category ⅣB1 as "Neoplastic: Mucinous neoplasm" and Category ⅣB2 as "Neoplastic: Non-mucinous neoplasm". IPMN, MCN, and intraductal papillary neoplasm of the bile ducts should be included in Category ⅣB1, and pancreatic endocrine neoplasm, SPN, and the rare gastrointestinal stromal tumor should be in Category ⅣB2. Nevertheless, this classification system serves a significant step towards a much needed uniform categorization of these lesions. Ultimately, the authors hope that each category of pancreatic tumor will be further discussed with gastroenterologists, GI surgeons, and GI oncologists. The following section will

Table 1 Pancreatic cytology terminology (modified from pitman *et al*[34], 2014)

Terminology category	Definition	Example interpretations
Category Ⅰ: Non-diagnostic	No diagnostic or useful information about the solid or cystic lesion sampled	Gastrointestinal contamination only; Non-specific cyst contents with insufficient cyst fluid volume for ancillary testing; Evaluation limited by scant cellularity
Category Ⅱ: Negative (for malignancy)	Adequate cellular and/or extracellular tissue to evaluate	Benign pancreatobiliary tissue in the setting of vague fullness and no discrete mass; Acute pancreatitis; Chronic pancreatitis; Autoimmune pancreatitis; Pseudocysts; Lymph epithelial cyst; Spleenful/accessory spleen
Category Ⅲ: Atypical	Cells present with cytoplasmic, nuclear, or architectural features that are not consistent with normal or reactive cellular changes of the pancreas or bile ducts and are insufficient to classify them as a neoplasm or suspicious for a high-grade malignancy	Atypical ductal cells obscured by crush artifact; Scant population of small monomorphic polygonal cells of unclear origin: Normal cigar cells *vs* endocrine proliferation; Atypical bile duct epithelium with nuclear features suggestive of repair in a background of acute inflammation; Atypical bile duct epithelium with mucinous metaplasia and mild nuclear atypia
Category ⅣA: Neoplastic: Benign	The presence of a cytological specimen sufficiently cellular and representative, with or without the context of clinical, imaging and ancillary studies, to be diagnostic of a benign neoplasm	Scant non-mucinous cuboidal epithelium and scant hemosiderin-laden macrophages in a non-mucinous cyst fluid consistent with the clinical impression of a serous cystadenoma
Category ⅣB1: Neoplastic: Mucinous neoplasm	Premalignant such as intraductal papillary neoplasm of the bile ducts (IPN-B), IPMN or MCN with low, intermediate or high-grade dysplasia by cytological criteria	MCN: Typically a multiloculated, mucin-producing epithelial neoplasm with sub epithelial ovarian-type stroma that in almost all cases does not communicate with the pancreatic ductal system and in almost all cases occurs in women; located in the body or tail; easily removed comparing life-long surveillance; IPMN: Primarily intraductal proliferations of ductal epithelium creating a macroscopic lesion resulting in ductal dilatation, cyst formation and/or a mass lesion; 1 Main-duct IPMN: Associated with diffuse dilatation of any portion of the main pancreatic duct or the entire pancreas; 2 BD-IPMN: Cysts adjacent to a non-dilated main pancreatic duct; IPN-B: A papillary proliferation of mucin containing neoplastic cells that may occur anywhere in the ductal system; similar to IPMN
Category ⅣB2: Neoplastic: Non-mucinous neoplasm	A low-grade malignant neoplasm such as well-differentiated PanNET, SPN or rare GIST	PanNET (pancreatic endocrine tumor and pancreatic endocrine neoplasm): A well-differentiated proliferation of the pancreatic endocrine cells creating a mass lesion greater than 0.5 cm that may or may not be functional by producing inappropriate levels of various hormones and that may or may not demonstrate aggressive features on histological examination; SPN: A solid, secondarily cystic low-grade epithelial neoplasm with established clonal mutations in cancer-associated genes and an ability to metastasize; GIST: Spindle cell and/or epithelioid mesenchymal neoplasms with differentiation along the lines of the interstitial cell of Cajal that usually expression c-kit protein (CD117), DOG1 and CD34 by immunohistochemistry; located in a peripanreatic location
Category Ⅴ: Suspicious (for malignancy)	when some, but an insufficient number of the typical features of a specific malignant neoplasm are present, mainly pancreatic adenocarcinoma	Rare markedly atypical epithelial cells suspicious for adenocarcinoma; Mucinous cyst with high-grade epithelial atypia and abundant coagulate necrosis suspicious for invasive carcinoma; Solid cellular neoplasm with features suspicious for acinar cell carcinoma. Tissue for confirmatory ancillary studies is not available
Category ⅥA: PDAC and variants	A group of neoplasms that unequivocally display malignant cytological characteristics and include PDAC and its variants, cholangiocarcinoma, acinar cell carcinoma, high-grade neuroendocrine carcinoma (small cell and large cell), pancreatoblastoma, lymphomas, sarcomas and metastases to the pancreas	PDAC: A malignant invasive gland (duct) forming epithelial neoplasm typically composed of classic tubular glands; 85%-90% of all pancreatic malignancies; Colloid carcinoma (mucinous, non-cystic): Abundant extracellular mucin production, with at least 80% of the tumor on histology demonstrating large pools of extracellular mucin and cuboidal epithelial cells "floating" in the mucin

Category VIA:	A group of neoplasms that unequivocally display malignant cytologic characteristics excluding PDAC and its variants; including acinar cell carcinoma, high-grade neuroendocrine carcinoma (small cell and large cell), cholangiocarcinoma, pancreatoblastoma, lymphomas, sarcomas and metastases to the pancreas	Medullary carcinoma: Poor histologic differentiation, syncytial growth pattern, pushing borders and an intense lymphoplasmacytic response
Malignancy:		Undifferentiated carcinoma with osteoclast-like giant cells: Distinctive type of sarcomatoid carcinoma with the striking and unique cytohistologic features characterized by a prominent component of reactive osteoclast-like giant cells in a background of spindle cells.
Others		Undifferentiated carcinoma: A high-grade carcinoma composed of large, undifferentiated, markedly pleomorphic cells; 2%-7% of PDAC
		Cholangiocarcinoma: The diagnostic criteria for invasive cholangiocarcinoma are the same as for ductal adenocarcinoma; usually diagnosis by bile duct brushings with high false negative rate due to overlying benign epithelium, insufficient sampling, reactive change with stent; degeneration due to bile
		Acinar cell carcinoma: A rare malignant epithelial neoplasm with exocrine acinar differentiation
		Poorly-differentiated neuroendocrine carcinoma (small cell carcinoma or large cell neuroendocrine carcinoma): Rare high-grade neuroendocrine tumor with < 1% of pancreatic tumor and 2%-3% of PanNETs
		Pancreatoblastoma: A rare neoplasm, primarily of childhood, characterized by acinar differentiation, endocrine differentiation and distinctive squamoid nests
		Non-Hodgkin lymphoma: Rare and usually involve the pancreas secondarily
		Metastatic tumors: Secondary neoplasms involving the pancreas are rare; most common: Renal cell carcinoma

MCN: Mucinous cystic neoplasms; IPN-B: Intraductal papillary neoplasm of the bile ducts; IPMN: Intraductal papillary mucinous neoplasm; cPanNET: Pancreatic neuroendocrine tumor; SPN: Solid pseudopapillary neoplasms; PDAC: Pancreatic ductal adenocarcinomas.

now describe the cytologic features of some of the more common pancreatic cystic lesions in accordance with the current classification described by Pitman *et al*[34].

Pseudo cyst (category II: Negative): Pseudocysts are the most common type of pancreatic cysts, accounting for at least 75% of all pancreatic cystic lesions. They generally arise in the setting of acute pancreatitis and are due to autodigestion of the pancreatic parenchyma. By definition, pseudocysts lack an epithelial lining and are instead composed of an inflammatory, fibrous capsule surrounding a region of necrosis. Aspirates are typically paucicellular and consist of granular debris, hemosiderin-laden macrophages, and bile (Figure 1A).

Lymphangiomas and lymphoepithelial cysts (category II: Negative): Lymphangiomas and lymphoepithelial cysts are both very rare benign lesions of the pancreas. The former is characterized cytologically by a uniform population of small, round lymphocytes accompanied by histiocytes, plasma cells, centrocytes, and centroblasts, whereas the latter is characterized by numerous anucleated squamous cells and amorphous debris with rare to no lymphocytes present. Aspirates from lymphangiomas tend to be very cellular[35] (Figure 1B); however, aspirates from lymphoepithelial cysts are largely acellular. Although EUS-guided FNA may have a limited role in identifying lymphoepithelial cysts, it has been proposed that paying attention to signal intensity on MRI may be helpful in identifying these lesions preoperatively[36].

Serous cystadenoma (category IVA: Neoplastic: benign): Serous cystadenomas comprise 1% to 2% of all pancreatic neoplasms. There are two types that are named based on the number and size of its cysts. Serous microcystic adenomas, which are the more common of the two types, have numerous small cysts, whereas serous oligocystic adenomas have fewer but larger cysts. Serous cystadenomas occur most frequently in older women, with the preferred sites being the body and tail of the pancreas. Aspirates of serous cystadenomas are sparsely cellular and may contain rare fragments of flat sheets and/or loose clusters of cuboidal cells with glycogenated cytoplasm and indistinct cytoplasmic borders (Figure 1C).

Mucinous neoplasm (category IVB: Neoplastic: others): There are two distinctive types of mucinous tumors, namely MCN and IPMN. Because both of these entities share many morphologic features, it is almost impossible to tell the difference between the two based on cytomorphologic features alone. In these cases, direct correlation with clinical and imaging studies is required. In general, MCNs occur almost exclusively in middle-aged women, with most being located in the body or tail of the pancreas. Of note, these lesions are closed cysts that do not communicate with the ductal system. A defining histologic feature of these lesions is the presence of ovarian-type stroma directly beneath mucinous epithelium that is positive for estrogen and progesterone receptors. On the other hand, IPMN is seen more commonly in men and are typically seen in the head of the pancreas. Unlike MCN, IPMN is radiologically shown to communicate with the ductal system (typically involving the main pancreatic duct)
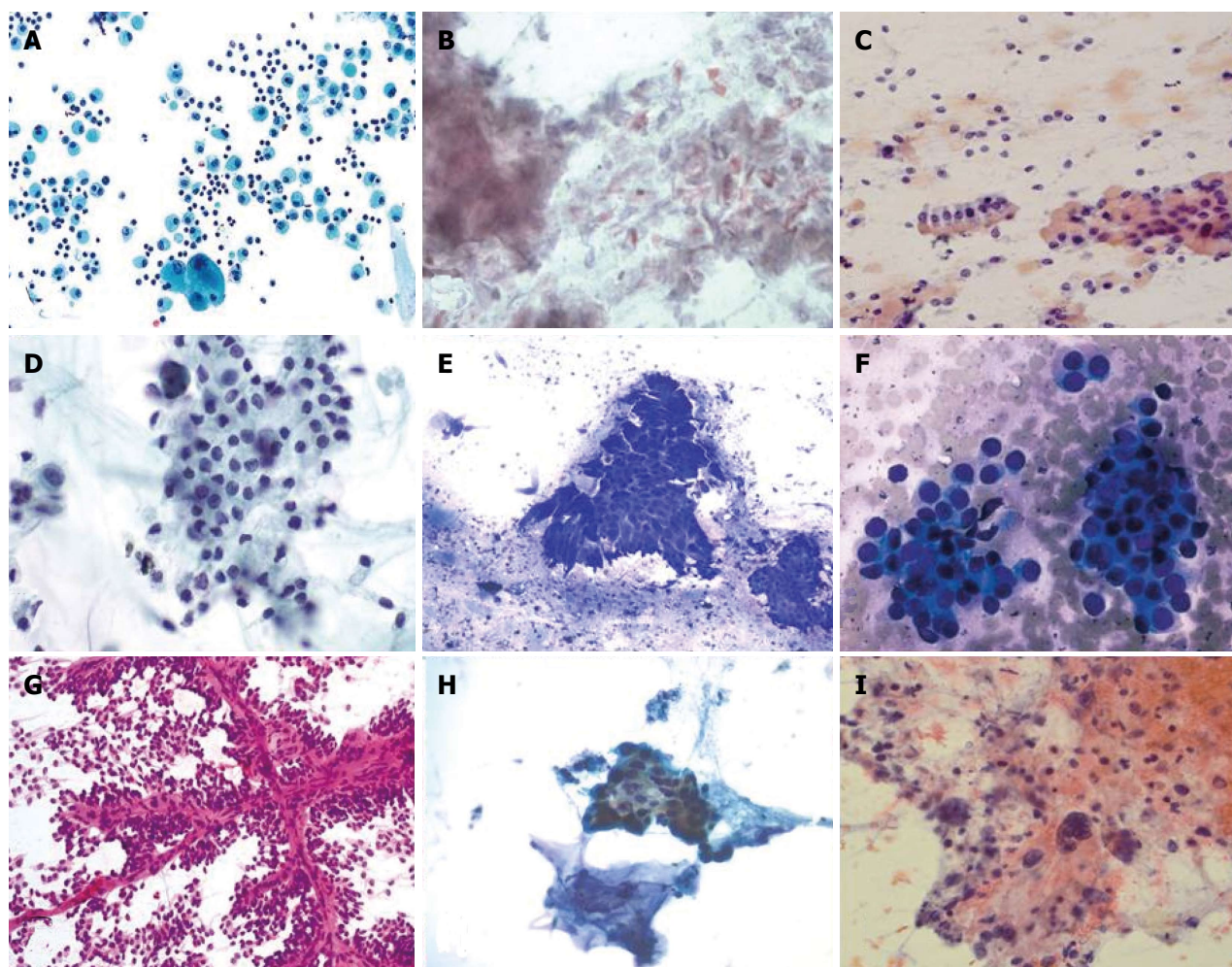
Figure 1 Cytopathologic features of pancreatic cysts. A: Pseudocyst: Notice the macrophages and inflammatory cells; epithelial cells are not seen; B: Lymphoepithelial cyst: Numerous anucleated squamous cells and keratinized debris are seen; C: Serous cystadenoma: One group of bland, monomorphic epithelial cells is present along with background histiocytes; D: Mucinous cystic neoplasm: A sheet of columnar cells with low-grad dysplasia in the background of mucin; E: Intraductal papillary neoplasm: Large papillary clusters are lined by tall, columnar cells containing intracytoplasmic mucin in the background extracellular mucin; F: Pancreatic neuroendocrine neoplasm: Bland, monomorphic epithelial cells with eccentrically placed nuclei arranged singly and in clusters characterizes this lesion; G: Solid pseudopapillary neoplasm: Notice the delicate, branching vessels and the poorly cohesive, bland epithelial cells; H: Pancreatic ductal adenocarcinoma with mucinous cyst: The ductal cells are arranged haphazardly and are characterized by hyperchromasia, nuclear pleomorphism and irregular nuclear contour; I: Undifferentiated carcinoma with osteoclast-like giant cells and mucinous cystic neoplasm: Multiple osteoclast-like giant cells and large haphazardly nucleus in the background of mucin.

and has the ability to grow along the entire length of the pancreatic duct and its branches. Aspirates of MCN and IPMN are hypocellular specimens that contain thick mucin and, if present, columnar mucinous sheets (Figure 1D and E). Cytologic interpretation is somewhat less problematic if nuclear and architectural atypia indicative of dysplasia or malignancy is identified. The WHO uses a three-tier classification based on degree of dysplasia present: benign, borderline, or malignant. Recent years, molecular tests such as KRAS and GNAS mutation are developed for differentiating MCN and IPMN. Nevertheless, given the malignant potential of these lesions, surgical resection is the most often utilized treatment of choice. Since cystic mucinous neoplasms have unique cytopathologic, molecular features and high risk for malignancy compared to non-mucinous neoplasms, we suggest that the Category Ⅳ

B should be separated into two subcategories: Category ⅣB1: Neoplastic: Mucinous and ⅣB2: Neoplastic: Non-mucinous.

Cystic PNET (category ⅣB: Neoplastic: others): PNET represent approximately 1% to 2% of pancreatic neoplasms. Most PNET are small, functional solid tumors, but cystic pancreatic neuroendocrine tumors (cPanNETs) account for 13% to 17% of PanNETs[37]. PNET can secrete a variety of hormones, including insulin, glucagon, and somatostatin, and adrenocorticotrophic hormone. Although they can occur in any age group, they most commonly occur in adults. Aspirates of well-differentiated PNETs can range from sparsely cellular to highly cellular specimens consisting predominantly of abundant isolated cells and numerous bare nuclei. However, loosely cohesive clusters of cells and pseu-

dorosette formation are not uncommon. The cells are characterized by uniformly round, eccentrically placed nuclei with "salt-and-pepper" chromatin and moderate amounts of cytoplasm (Figure 1F). More poorly differentiated PNETs display more nuclear pleomorphic and higher mitotic activity. In doubtful cases, immunohistochemical stains for chromogranin and synaptophysin can be extremely helpful in confirming the diagnosis if the cell block is available. The surgical resection is the first line treatment. Enucleation or cytoreductive surgery is also recommended for patients with locoregional recurrences or hepatic metastases. Regional adjuvants such as radiofrequency ablation, transarterial chemoembolization, and others are often employed in an attempt to palliate symptoms and prolong survival[38]. Again, because cystic PNETs can be both functional and non-functional tumors with special morphological and immunohistochemical features, it should be separated from MCN into category IVB2: Neoplastic: Non-mucinous.

SPN (category IVB: Neoplastic: others): SPN are uncommon tumors of unknown malignant potential that predominantly occur in young women. Aspirates of these lesions are highly cellular, with the most characteristic features being myxoid or hyalinized vascular stalks lined by single or multiple layers of cells exhibiting round to oval nuclei, nuclear grooves, and indistinct cell borders (Figure 1G). Immunostain for β-catenin with nuclear positivity has emerged as a helpful attribute in diagnosing SPN. Other immunohistochemical stains that are helpful in confirming the diagnosis include CD10, CD56, vimentin and SMAD4. Surgical resection of these tumors leads to a good prognosis. With the special morphological features and immunohistochemical features of these lesions, SPN should be classified as Category IVB2: Neoplastic: non-mucinous.

Pancreatic ductal adenocarcinoma with cystic neoplasm (Category VI: Malignant): Pancreatic ductal adenocarcinomas (PDAC) with cystic neoplasm is the most common malignant cystic neoplasm of the pancreas and usually arises from MCN and IPMN. PDAC typically occur in older individuals, with smoking and alcohol abuse being major risk factors. Despite being able to detect these lesions at earlier stages, long term survival remains abysmal, with 90% of all patients dying within one year of diagnosis. Cytologically, aspirates are usually very cellular and consist of atypical ductal cells with irregular nuclear contours and prominent, centrally placed nucleoli arranged singly or in clusters and sheets (Figure 1H). Mitotic figures can also be seen.

Sensitivity and specificity of cytology based EUS-guided FNA

Although it has been established that EUS-guided FNA has a valuable role in the multidisciplinary approach to the management of pancreatic cystic lesions, much controversy remains in regards to its ability to accurately triage patients with incidentally discovered lesions that appear benign on imaging. In one of the early studies performed by Frossard *et al*[39] in 2003, it was determined that EUS-guided FNA successfully identified the lesion of interest in 65 cases (97%). The overall sensitivity, specificity, positive predictive value, and negative predictive value for EUS-guided FNA in this study were 97%, 100%, 100%, and 95%, respectively[39]. The cytologic diagnosis of cystic lesions with EUS-FNA has been studied extensively with widely variable sensitivity[40-44]. The sensitivity has been reported to range from 23% to 100% and specificity has been reported to range from 71% to 100%[40,45,46]. One meta-analysis showed that the pooled sensitivity and specificity in diagnosing mucinous cystic lesions were 63% and 88%, respectively, in 11 studies and 54% and 92%, respectively, in 4 prospective studies[45]. In one recently published meta-analysis study, the sensitivity and specificity of cytology was 0.42 and 0.99; the sensitivity and specificity of K-RAS was 0.39 and 0.95; and the sensitivity and specificity of the combined test of cytology and K-RAS was 0.71 and 0.88, respectively[47]. The sensitivity in our study (47%) was between two meta-analysis results[46]. We further studied the false negative rate of EUS-FNA, and we found that the false-negative rate (3%) caused by an interpretative error was significantly lower than that caused by a sampling error (23%) (*P* = 0.003). This finding suggests that sampling error, rather than interpretative error by cytology, is a major cause of high false-negative rates. We further examined the false-negative rate for solid lesions and cystic lesions. The false-negative rate for cystic lesions was significantly higher than that for solid lesions (53% *vs* 15%; *P* = 0.005). Recently, Rogart *et al*[31] reported that cyst wall puncture performed during FNA improved the diagnostic yield for mucinous cysts[31]. In addition, cytologic classification with high-grade epithelial atypia in cystic lesion FNA specimens demonstrated a higher prediction for malignancy and added value for the clinical evaluation of cystic lesions[42,48]. One study also found that certain factors, such as the identification of a solid component and performing more than one pass, resulted in significant increases in sensitivity (as high as 78%)[49]. In light of these issues with sensitivity, a newer series has suggested that EUS-guided FNA, when used in conjunction with other "screening" tests, contributes to a triple-negative screening test (*i.e.*, no high-risk stigmata, no worrisome features, and no high grade atypia on cytology) that has a negative predictive value for malignancy of 99%[50]. In general, EUS-guided FNA has a low sensitivity, but good specificity[45]. More sensitive and specific techniques are needed and should be developed as new technologies emerge, such as cystic fluid analysis by chemical or molecular tests and confocal laser endomicroscopy.

Recently, cystic PNET diagnosis and management received a lot of attention. In one study, cytology made a specific diagnosis of a cystic PNET in 71% of the biopsies

compared with a specific diagnosis by EUS in 38% of cases[37]. All cysts but one revealed low carcinoembryonic antigen (CEA) levels (range, 0.2 to > 500 ng/mL; mean, 29.5 ng/mL), and amylase levels were < 500 U/L in all but 2 cases (range, 16-1493 U/L; mean, 205 U/L). In another study, cystic PNETs were found to be larger than solid PNETs (mean 26.8 mm *vs* 20.1 mm, *P* = 0.05) and more frequently nonfunctional (96% *vs* 80%, *P* = 0.03). With histology as the reference standard, EUS-FNA accuracies for malignancy of cystic and solid PNETs were 89.3% and 90%, respectively; cystic PNETs were less associated with metastatic adenopathy (22% *vs* 42%, *P* = 0.03) and liver metastasis (0% *vs* 26%, *P* < 0.001). Cystic fluid analysis (*n* = 13), showed benign cystic PNETs had low CEA, Ki-67 ⩽ 2%, and no loss of heterozygosity (LOH). Patients with cystic and solid PNETs had similar recurrence risk up to 5 years after complete resection[51]. In one review which compared EUS and EUS-FNA for cystic PNET, they found that EUS-FNA cytology and cyst fluid analysis is a useful adjunct to abdominal imaging in the diagnosis of pancreatic cystic lesions. They hypothesize that cyst fluid characteristics, including cytomorphological features, is the most accurate test to achieve a preoperative diagnosis and to provide a basis for prognostic prediction[52].

Another technique that shows promise in improving the sensitivity and specificity of detecting and diagnosing pancreatic cystic lesions is confocal laser endomicroscopy. Confocal laser endomicroscopy is a novel imaging technology in which a low-power laser illuminates and scans a single focal plane of the tissue[53-56]. This technique allows for the detection of the microscopic detail of the surface epithelium in pancreatic cysts. Needle-based confocal laser endomicroscopy (nCLE) utilizes a sub-millimeter probe that is compatible with an EUS needle and enables real-time imaging with microscopic detail of pancreatic cystic lesions[56]. The presence of epithelial villous structures based on nCLE was associated with pancreatic cystic neoplasm (*P* = 0.004) and provided a sensitivity of 59%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 50%. This technique is rather new for evaluating the pancreatic cystic lesions. However, we believe that the development of this new technique may facilitate sampling the most suspicious area of a cyst in the future.

CYST FLUID ANALYSIS

As mentioned previously, pancreatic cystic neoplasms represent a diagnostic challenge for EUS-guided FNA because lining cells may or may not be adequately sampled, thus precluding further classification. The current way of solving this dilemma relies upon a combination of methods and includes visual cyst fluid evaluation at the time of immediate assessment, chemical analysis of cyst fluid, and molecular testing. All of these tests can be utilized to help to differentiate between mucinous and non-mucinous cysts.

Non-molecular methods

The possibility of a mucinous cyst can be strongly suggested by looking for the "string sign". This can be assessed by stretching out a drop of cyst fluid between the thumb and index finger and subsequently measuring the length of the string of cyst fluid. A length of at least 3.5 mm is believed to be consistent with that of a mucinous cyst. Studies have also shown that mucinous cysts consistently have a higher relative viscosity compared to serum, whereas the opposite is true for non-mucinous cysts, which a lower relative viscosity compared to serum[57].

Amylase: The chemical analysis of cyst fluid relies upon examining pancreatic enzyme levels as well as the presence of tumor markers. Pancreatic enzyme levels are typically used to differentiate between pseudocysts and neoplastic cysts. One of the most important enzymes studied in making this distinction is amylase. Pseudocysts and other non-neoplastic cysts consistently show elevated levels of amylase. In fact, one study showed that an amylase level below 250 U/L virtually excludes pseudocysts from the differential diagnosis[58]. Conversely, amylase is low in neoplastic cysts.

CEA: A variety of tumor markers have been studied for their ability to discriminate between mucinous and non-mucinous cysts[57-60]. According to many studies, CEA levels are the most accurate[60,61]. Although CEA cutoff values of > 192 ng/mL have been shown to have an accuracy of 79%[60], levels > 800 ng/mL have been shown to be highly predictive of mucinous neoplasms with a specificity of 98%. Unfortunately, the sensitivity, even at these markedly elevated levels, is still less than 50%[58]. It is also important to note that CEA cannot be used to distinguish between benign and malignant lesions[62,63]. Amylase, however, may be helpful in this regard[64]. In contrast, a very low CEA level ⩽ 5 is 95% specific for pseudocysts, neuroendocrine tumors, and serous cystadenoma[58].

Other markers: Multiple biomarkers have also been studied to identify pancreatic ductal carcinoma (PDAC). Plectin-1, a marker related to PDAC, was found to be a potentially promising biomarker for the detection of malignancy in IPMNs[65]. Plectin-1 expression was assayed using immunohistochemistry in cyst fluid and tissue sample from benign and malignant IPMN, as well as lymph node metastasis from carcinoma arising from IPMN. The sensitivity and specificity were 84% and 83%, respectively. In animal models, Cathepsin E is specifically and highly expressed in PDAC and pancreatic intraepithelial neoplasias (PanINs), A prospective double-blind control study was performed to evaluate the accuracy of this method in diagnosing PDAC and PanINs of all grades (> 82.7%)[66].

Molecular methods
K-RAS mutation: Molecular analysis of cyst fluid shows

Table 2 Criteria for integrated molecular pathology diagnostic categories

Diagnostic category	Molecular criteria[1]	Co-existing concerning clinical features[2]
Benign	DNA lacks molecular criteria	Not considered for this diagnosis
Statistically indolent	DNA meets 1 molecular criterion	None
SHR	DNA meets 1 molecular criterion	1 or more
Aggressive	DNA meets at least 2 molecular criteria	Not considered for this diagnosis

[1]Four molecular criteria that have been independently correlated with pancreatic malignancy or high-grade dysplasia are used to make an integrated molecular pathology diagnosis: (1) a single high-clonality mutation; (2) elevated level of high-quality DNA; (3) multiple low-clonality mutations; and (4) a single low-clonality oncogene mutation; [2]Include any of the following: cyst size > 3 cm, growth rate > 3 mm/year, duct dilation > 1 cm, carcinoembryonic antigen level > 1000 ng/mL, cytologic evidence of high-grade dysplasia. (Table 2 from Al-Haddad *et al*[75] 2015 was permitted by publisher). SHR: Statistically higher risk.

promise in distinguishing not only between mucinous and non-mucinous cysts, but also in the diagnosis of malignant cysts. One study that examined surgically resected specimens showed that the identification of the K-RAS mutation had a sensitivity of 54% and a specificity of 100% for mucinous cysts. The combined used of CEA and K-RAS improved the sensitivity to 83% while decreasing the specificity to 85%[67]. However, a smaller study found that there was no increase in sensitivity when combining CEA and K-RAS[68]. LOH and increased DNA quantity have also been tried as a means of accurately predicting the presence of a mucinous lesion, but the sensitivity for each method is less than 11%. However, the detection of any molecular changes (*i.e.*, K-RAS mutation, LOH, or increased DNA quantity) has been found to be 90% specific for mucinous cysts[12]. Recently, one meta-analysis study found that the sensitivity and specificity of K-RAS was 0.39 and 0.95; and the sensitivity and specificity of the combined test of cytology and K-RAS was 0.71 and 0.88, respectively[47]. The K-RAS mutation combined with cytology test greatly increases the sensitivity of EUS-FNA. K-RAS mutation analysis may also prove to be a powerful ancillary for testing cystic samples with scant cellularity.

GNAS mutation: Another diagnostic marker that has received considerable interest is the presence of GNAS mutations. Recent studies have shown that GNAS mutations can be detected in IPMNs[69,70]. It has also been shown that the combination of GNAS and KRAS mutations in cyst fluid is very specific for IPMNs. One study found GNAS mutations to be significantly more prevalent in IPMNs (42%) than in SCAs (10%), adenocarcinomas (0%), and MCNs (0%). This same study also showed that double GNAS and KRAS mutations only occur in IPMNs (P = 0.006) and that mutations in either gene equated to a sensitivity of 98% and a specificity of 84%[71]. GNAS mutations are rare to absent in MCN, SCA, PNET, or PDAC.

MicroRNA change: MicroRNA (miRNA) expression profiles have also received considerable interest and are currently being studied as another way to characterize pancreatic lesions. miRNA is nineteen to twenty-four nucleotide long single-stranded, non-coding regions of RNA that are highly stable and which may be useful in diagnosing various malignancies as well as pancreatic cystic neoplasms. In a recent study, together with IPMN surgical specimens, 65 cyst fluid samples were examined for differential selective miRNA candidate expression. A subset of 18 miRNAs separated high-grade from low-grade lesions. A logistic regression model using nine miRNAs allowed prediction of high-grade IPMNs, PNET and SPN *vs* low-grade IPMNs and SCA with a sensitivity of 89%, a specificity of 100% and area under ROC curve of 1[72]. Another study evaluated miRNA in 69 pathology specimens and identified several miRNA panels that enabled them to differentiate SCA from MCN and IPMN, and MCN from BD-IPMN with a sensitivity ranging from 85%-100% and a specificity of 100%[73].

Integrated molecular pathology: Perhaps the greatest dilemma in managing pancreatic cysts is the fact that none of the currently recommended guidelines can accurately predict the malignant potential of pancreatic cysts. For example, the current IAP 2012 criteria risk stratifies patients into two categories: "surveillance" criteria (low malignant potential) and "surgery" criteria (high malignant potential). Symptomatic patients with mucinous cysts and at least one other "high-risk stigmata" (*i.e.*, obstructive jaundice with a cyst located in the pancreatic head, a post-contrast enhancing solid component, a main pancreatic duct diameter ≥ 1 cm, abrupt change in duct caliber, cyst size ≥ 3 cm, presumptive diagnosis of MCN, and "suspicious" cytology) as detailed by the 2012 International Association of Pancreatology (IAP) guidelines should be referred for surgery[74]. Patients with cysts less than 1 cm and no concerning radiologic features can be monitored with periodic imaging studies. If more worrisome features are detected, the patients are subsequently referred for EUS-guided FNA to help determine the nature of the cyst (*i.e.*, mucinous versus non-mucinous) and whether malignancy is present. Nevertheless, given the high mortality rate for pancreatic cancer, the IAP ultimately recommends that any patient with "worrisome" features associated with malignancy undergo surgery. However, it has

been shown that approximately 60%-80% of patients undergoing surgery often have non-malignant disease. Therefore, other methods that prevent overtreatment of benign disease while providing early detection of cancer are needed.

Integrated molecular pathology (IMP) testing addresses this need it that incorporates all of the testing methods mentioned above (*i.e.*, cytology, imaging studies, fluid chemistry, and molecular analysis). Unlike other guidelines, it utilizes four different diagnostic categories of "benign", "statistically indolent", "statistically higher risk", or SHR, and "aggressive" based on both the number of molecular criterion met and other clinical features, if applicable (Table 2)[75]. In one study, 492 patients were categorized using IMP. Follow up for at least three years was available for 46% of patients. The overall accuracy was found to be 90%, and the specificity and negative predictive value were 91% and 97%, respectively. The sensitivity for malignant outcome with this cohort of patients was 83%, and the positive predictive value was 58%. When compared to the 2012 IAP criteria, it was found that the IAP criteria and IMP showed similar sensitivity and negative likelihood ratios. However, there was a statistically significant difference between the IAP guidelines and the IMP in that the specificity and positive likelihood ratios were higher using IMP criteria. These findings suggest that IMP is very useful in not only risk stratifying patients, but also in preventing patients with indolent disease from undergoing unnecessary surgeries[75].

CONCLUSION

EUS-guided FNA serves a pivotal role in the accurate diagnosis of incidentally discovered pancreatic cysts. Its advantages over imaging alone include the ability to confirm the presence or absence of suspicious features identified on radiologic imaging, determine whether a lesion is malignant, and monitor for changes in cystic lesions. The new classification schema, while not perfect, goes hand-in-hand with the role of EUS-guided FNA in that it helps clinicians and patients to have a better understanding of which lesions need to be treated as opposed to those which do not, thus sparing patients from undergoing procedures that may result in increased morbidity and/or mortality. Despite these advantages, arriving at a proper diagnosis still requires the integrated use of clinical, radiologic, and cytological findings. Newer chemical and molecular studies show promise in improving the ability of clinicians to effectively diagnose and treat these lesions.

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