Review Article

Endoscopic ultrasound guided - fine needle aspiration (EUS-FNA), in comparison with gross and histologic diagnoses of pancreatic lesions

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Received May 18, 2014; Accepted July 22, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: EUS-FNA of the pancreas is the preferred method of investigating and diagnosing pancreatic masses, both solid and cystic. Most solid tumors of the pancreas are conventional pancreatic ductal adenocarcinomas (PDAC). EUS-FNA has a variable sensitivity but high specificity in the preoperative diagnosis of pancreatic lesions, although the diagnosis for well differentiated PDAC is much more challenging than for poorly differentiated PDAC. The criteria for distinguishing other solid neoplastic lesions from PDAC are reviewed. Contamination by duodenal or gastric epithelium is a common pitfall in the diagnosis of the two most common cystic lesions, intraductal papillary mucinous neoplasm and mucinous cystic neoplasm. In this review, based on our experience, we highlight and discuss the cytologic criteria for the diagnosis of pancreatic lesions, correlate these with the gross and histologic appearance, and discuss ancillary tests including KRAS and GNAS mutations, diagnostic pitfalls and future perspectives. Future studies on identifying and validating more sensitive and specific molecular markers are necessary to improve the EUS-FNA diagnosis of pancreatic lesions.

Keywords: Pancreatic solid lesions, pancreatic cystic lesions, endoscopic ultrasound guided fine needle aspiration (EUS-FNA), preoperative diagnosis, whipple procedure

Introduction

Endoscopic ultrasound (EUS) is frequently employed in academic centers and large community hospitals. EUS-guided fine needle aspiration (EUS-FNA) of the pancreas has replaced transcutaneous FNA as the preferred method of investigating and diagnosing pancreatic solid and cystic lesions. EUS-FNA is performed by a gastroenterologist using an echoendoscope with a biopsy chamber through which the needle is passed. The tissue samples obtained allow diagnosis of lesions as small as 0.5 cm, and staging of patients by FNA of lymph nodes, during the same procedure [1-4].

The indication for EUS-FNA of the pancreas is the presence of a mass (solid or cystic), frequently found during work up for symptoms related to dilated pancreatic or bile ducts. Clinical and radiological information regarding the mass should always be integrated into the diagnostic process, and when combined with the cytological findings can greatly assist in producing diagnostic accuracy. However, sometimes imaging findings can be misleading, such as with mass-forming chronic or autoimmune pancreatitis [5, 6]. The reported sensitivity of EUS-FNA of the pancreas ranges from 64% to 96% in multiple studies, with most series reporting a specificity of 100% [3, 7-11]. At Cedars-Sinai Medical Center, based on a series of preoperative EUS-FNA and subsequent pancreatic resection in 74 patients, our sensitivity for the diagnosis of pancreatic neoplastic processes is 91.4% with a specificity of 92.3% and an accuracy of 91.6%. The sensitivity and specificity for diagnosis of PDAC are 96.5% and 100%, respectively [Lai et al, unpublished data].

Although the diagnostic cytologic criteria for pancreatic lesions do not change with respect to the method used to sample them, the technique used and approaches selected to obtain the tissue can impact the overall cytological appearance of the lesion and thus the accuracy
EUS-FNA in pancreas

Figure 1. Well differentiated pancreatic adenocarcinoma (PDAC). (A) EUS-FNA touch preparation shows honeycomb pattern of normal pancreatic ductal epithelium (left, x400) and “drunken” honeycomb pattern of well differentiated PDAC (right, x400); (B) Pap-stained smear shows nuclear parachromatin clearing, anisonucleosis and nuclear overlapping (left, x400); EUS-FNA core biopsy shows perineural invasion of the tumor (right, x200); (C, D) The pancreatic tail is bivalved through the pancreatic duct and shows a white to tan, ill-defined fleshy lesion (C), and histologically, tumor is composed of well differentiated glands with cribriforming architecture and desmoplasia (D left, x100; right, x400).

of interpretation [12]. For instance, the recognition of gastric and duodenal epithelial contamination is critical for the accurate interpretation of EUS-guided FNA biopsies [12, 13]. This article focuses on a review of the literature in light of our experience using of EUS-FNA in cytologic diagnosis of pancreatic solid and cystic lesions. Here we will review diagnostic criteria, pitfalls, ancillary tests and future perspectives.

EUS-FNA in diagnosis of solid pancreatic lesions

The first step in approaching an FNA of a pancreatic lesion is to incorporate the imaging features, specifically whether it is solid or cystic. The common pancreatic solid masses include ductal adenocarcinoma, mass-like fibrosis in chronic sclerosing pancreatitis, especially autoimmune pancreatitis, pancreatic neuroendocrine tumor, and metastatic neoplasms. Other rare solid neoplastic entities such as solid-pseudopapillary neoplasm, acinar cell carcinoma, lymphoma and pancreatoblastoma should also be considered when evaluating an FNA of a solid pancreatic mass. EUS-FNA is a safe and highly accurate method for tissue diagnosis of patients with solid pancreatic masses [7, 8, 12]. With an understanding of the cytological features and the incorporation of clinical, radiological and ancillary studies, most solid lesions in the pancreas can be diagnosed to a sufficient degree for proper patient management.

Neoplastic solid lesions

Pancreatic ductal adenocarcinoma (PDAC): Ninety percent of primary pancreatic solid neo-
plasms are PDAC. On CT scan, PDACs appear as poorly defined hypodense masses with central attenuation distorting the normal pancreatic lobulations and are often associated with an abrupt stricture of the main pancreatic duct. For tumors located within the pancreatic head, the characteristic dually dilated main pancreatic and common bile duct, the so-called “double duct” sign, is a diagnostic clue [15]. Criteria for cytologic diagnosis of well-differentiated PDAC include irregular cellular distribution in a sheet (“drunken honeycomb”) (Figure 1A right), anisonucleosis with > 4 fold nuclear size variation in a group, parachromatin clearing (Figure 1B left), irregular nuclear membranes (often subtle) and abundant cytoplasm (often visibly mucinous) [17]. Lack of architecture is the major limitation of cytology compared to histology in diagnosis of well differentiated PDAC, because the cytopathologist cannot see: a) distribution of ductal structures; b) abnormal localization of the lesional cells; c) whether or not ducts are adjacent to medium sized vessels, wrapping around nerves (Figure 1B right) or isolated in fat; d) contours or angulation of ducts; e) luminal contents; f) stromal reaction/invasion; and g) setting of dysplasia (sometimes papillary). On cytologic examination it can be difficult to determine if strips of abnormal epithelial cells are invasive, or originate from pre-invasive lesions of intraductal papillary mucinous neoplasm (IPMN) or from high grade pancreatic intraepithelial neoplasia (PanIN). The addition of a trucut needle EUS-guided biopsy is often very helpful, even when only small tissue fragments are retrieved, particularly for well-differentiated PDAC [16]. The corresponding gross appearance in the pancreatic resection, with a white to tan, ill-defined and fleshy mass on cut surface, and histologic features of Figure 2. Poorly differentiated PDAC. (A) EUS-FNA touch preparation (left, x400) and core biopsy (right, x400) show nuclear pleomorphism and mitotic figure (arrow); (B) Pap-stained smear shows nuclear pleomorphism (x400); (C, D) The pancreatic head is bivalved through the pancreatic duct and common bile duct revealing a white to tan, ill-defined fleshy lesion causing dilatation of the ducts (arrows) (C), and histologically, tumor is composed of atypical epithelial cells with nuclear pleomorphism (D left, x100; right, x400).
well-differentiated PDAC are shown in Figure 1C, 1D.

Cytologic diagnosis for high grade PDAC is less challenging, with strikingly abnormal cytologic features, including marked nuclear atypia. The nuclei are often overlapping, with irregular nuclear membranes, hyperchromasia, pleomorphism and prominent nucleoli. Single atypical cells, mitoses and coagulative necrosis are helpful diagnostic clues (Figure 2A, 2B). The corresponding gross appearance on resection is the same as for well-differentiated PDAC; this example shows an ill-defined mass causing dilatation of the proximal common bile duct and distal pancreatic duct (Figure 2C). Histologically, the tumor has architectural atypia, with loss of glands and solid groups of tumor cells showing variation in nuclear size and shape with prominent nucleoli, in a desmoplastic background (Figure 2D).

Pancreatic neuroendocrine tumor (PanNET): PanNETs comprise up to 5% of all pancreatic malignancies. It is estimated that these neoplasms have an estimated incidence of 4 to 5 per 100,000 individuals per year in the United States [17]. 77% of PanNET cases can be correctly diagnosed by preoperative FNA [18]. The World Health Organization classifies PanNETs similarly to other extrapulmonary NETs, into well differentiated (grade 1 or 2) or poorly differentiated (grade 3), equivalent to small cell carcinoma. The majority of PanNETs are well differentiated [19]. Cytologic features of well differentiated PanNET include predominantly single cells with a monotonous appearance, plasmacytoid features, coarse and stippled chromatin (“salt and pepper”), with or without small nucleoli (Figure 3A). Grossly, the tumor frequently shows a homogeneous solid cut surface (Figure 3B). Histologically, these tumors show characteristic features of NETs (Figure 3C, 3D).
Solid pseudopapillary neoplasm of pancreas (SPN): SPN is rare, however, the number of SPNs reported in the literature has seen a 7-fold increase since 2000 compared with prior years [21]. SPNs are found primarily in young women who present with nonspecific symptoms. Surgery remains the mainstay of treatment with an excellent long-term prognosis [22]. In cytology, there is papillary branching, myxoid stroma, clinging cells and single cells, euchromatin, oval, indented, grooved nuclei, and perinuclear vacuoles/globules (Figure 4A, 4B). Gross appearance of the tumor shows solid and cystic components on the cut surface (Figure 4C) although some tumors are predominantly cystic. Histologically, the tumor shows pseudopapillary architecture, bland nuclear features and admixed foamy macrophages (Figure 4D). Positive immunostains of CD10 and nuclear expression of β-catenin are helpful.

Acinar cell carcinoma (ACC): ACC is a rare malignant epithelial neoplasm with exocrine acinar differentiation. Cytologically, the tumor may show several thick, small to large clusters of tumor cells present singly or loosely cohesive. Singly dispersed naked nuclei, occasionally with crush artifact, are frequently observed. The nuclear contour is smooth and the chromatin finely clumped. The cytoplasm contains many coarse D-PAS-positive granules [23].

Pancreatoblastoma: Pancreatoblastoma is a rare epithelial malignancy with bimodal peak incidences in childhood and in adulthood. It is characterized by multiple lines of differentiation including acinar, endocrine and ductal, with characteristic squamous corpuscles. FNA will show cells with small to medium sized, crowded, oval nuclei with fine chromatin, small inconspicuous nucleoli, rare nuclear grooves, molding and scant cytoplasm. The squamous corpuscles commonly seen in tissue section may not be identified in the FNA specimens. The cytologic differential diagnosis between pancreatoblastoma and SPN can be challenging.
Nuclear molding and occasional mitoses are more often seen in pancreatoblastoma especially when the primitive components are sampled, and these findings are not observed in SPN [24].

**Metastatic pancreatic solid lesions:** Since the majority of patients with pancreatic metastases have a history of an extra-pancreatic malignancy [25], a comprehensive clinical history is extremely helpful. Renal cell carcinoma (RCC) is particularly prone to metastasize to the pancreas as a solitary nodule, even decades after resection of the primary neoplasm [26, 27]. The cytological features of metastatic clear cell RCC include large polygonal cells singly, in clusters and especially tethered to vascular structures. The uniform “punched out holes” of fat and glycogen in the cytoplasm are only appreciated on air-dried smears (Figure 5A left) whereas the often prominent nucleoli are best appreciated on fixed smears. Stripped naked nuclei are also common. Cell block and EUS-guided core biopsy show the histologic features of clear cytoplasm (Figure 5A right and 5B upper) and architecturally the tumor cells are arranged in nests with intervening blood vessels (Figure 5C, 5D). Positive immunochemical staining for RCC, CD10 and Pax8 is helpful in confirming the diagnosis of metastatic RCC (Figure 5B lower). Other metastases include carcinomas of the lung, breast, stomach, colon, and ovary, and malignant melanoma. The cytological features are similar to those of the corresponding primary tumors. Immunostains on cell block combined with the clinical history should be diagnostic.

**Non-neoplastic solid lesion**

**Sclerosing chronic pancreatitis:** The clinical presentation and imaging appearance of chronic
pancreatitis are characteristic and only rarely raise the possibility of malignancy. Classic radiological images of chronic pancreatitis demonstrate irregular ductal dilatation often associated with stricture formation, obstruction and calcifications. However expansile or mass-like fibrosis in chronic pancreatitis and the focal form of autoimmune pancreatitis frequently simulate malignancy. Cytologic features of sclerosing chronic pancreatitis include scant cellu-

Figure 6. Autoimmune chronic pancreatitis. A. EUS-FNA touch preparation shows cytologically bland ductal epithelium, some lymphocytes and plasma cells (x400); B. EUS-FNA core biopsy shows fibrotic tissue with chronic inflammation (x100); C-F. The pancreatic head is bivalved through the pancreatic duct and common bile duct revealing a fibrotic cut surface (C left), parenchyma atrophy and calcification with a stone in the pancreatic duct better seen after fixation (arrow) (C right); In histology, pancreatic parenchyma shows atrophy with fibrosis and chronic inflammation (D, x40) with germinal centers or lymphoid aggregates (E, x400). Immunohistochemistry shows dense IgG4 positive plasma cells in the lymphoid aggregates (F, x400).
EUS-FNA in pancreas

![Figure 7](image)

**Figure 7.** Intraductal papillary mucinous neoplasm (IPMN) of the pancreas. A. EUS-FNA cell block with PASD stain shows PASD positive mucous cells in the cyst lining epithelium (x200); B. Core biopsy shows moderate dysplasia of the papillary epithelium (x100); C. Resection of the central part of the pancreas shows pancreatic duct and a side branch type cyst; D. A histologic section shows moderate dysplasia of the cyst lining epithelium (x40, inset, x400).

Polarity, with an admixture of ductal cells, islet cells, and inflammatory cells. The ductal cells are in cohesive, monolayered sheets, with only few single cells, and maintained polarity. There is minimal nuclear overlap, mild anisonucleosis, smooth nuclear membranes, rare/normal mitoses, and no coagulative necrosis. Some plasma cells may be present in the touch preparation (Figure 6A). EUS-guided core biopsy shows chronic inflammation and fibrotic stroma (Figure 6B), that must be carefully examined to rule out the presence of malignant glands. The corresponding gross appearance shows atrophic pancreatic parenchyma with fibrosis, causing loss of the normal pancreatic parenchymal lobulation; calcification and a stone in the pancreatic duct are also seen (Figure 6C). Histologically, there is pancreatic atrophy, parenchymal or periductal fibrosis, and chronic inflammation (Figure 6D). A high density of IgG4 plasma cells highlighted by immunohistochemistry is suggestive of IgG4 autoimmune pancreatitis (Figure 6E, 6F).

EUS-FNA in diagnosis of pancreatic cystic lesions

Cystic lesions of the pancreas are common; in fact, small pancreatic cysts were found in 73 of 300 patients in an autopsy study, with the prevalence increasing with age [28]. There is increasing detection of these cystic pancreatic lesions by high-resolution abdominal imaging techniques. The majority of these lesions are identified in the elderly, many of whom have co-morbidities with higher operative risk. There are two essential questions for any pancreatic cystic lesion identified: 1) Is the cyst lesion mucinous or non-mucinous? 2) Is the cystic lesion benign or malignant?

EUS-FNA is a safe method of pre-operative evaluation of pancreatic cysts with no increase...
EUS-FNA in pancreas

Figure 8. Mucinous cystic neoplasm of the pancreas. A. EUS-FNA touch preparation shows abundant mucinous material (left, x100) and cell block shows some mucous cells in the cyst lining epithelium (right, x400); B. PASD stain highlights extracellular mucin and intracytoplasmic mucin in some epithelial cells (x200); C. The mucinous cyst is present in pancreatic parenchyma, not communicating with the pancreatic duct; D. Ovarian type stroma is present beneath the mucinous lining epithelium (x200, inset, x400).

in adverse events compared to EUS-FNA of solid lesions [4]. The common pancreatic cystic lesions include intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN), serous cystadenoma and pseudocysts. Some solid lesions with predominant cystic changes and other rare cystic lesions such as lymphoepithelial cyst may also be encountered. In most cases, the purpose of pre-operative investigations by EUS-FNA is primarily to distinguish mucinous neoplasms from pseudocysts and serous cystadenomas, as the latter two do not necessarily require surgical intervention. Although cytologic analysis may under-diagnose the grade of neoplasia in a mucinous cyst, the negative predictive value (NPV) for carcinoma in an FNA without atypical epithelial cells is reported to be approximately 85% [29]. In small branch duct IPMN with no high risk features on imaging, the NPV of cytology is 96% [30].

Neoplastic cystic lesions: mucinous

Intraductal papillary mucinous neoplasms (IPMN): IPMN is a neoplastic mucinous cyst measuring more than 5 mm involving the main duct and/or side branch duct of the pancreatic ductal system. The 5 mm size cutoff is used to distinguish IPMN from PanIN. IPMN is lined by often papillary and variably atypical mucinous epithelium. An estimated incidence from recent reports places IPMN at approximately 20% of all neoplastic pancreatic cysts and 5% of all pancreatic neoplasms. IPMNs occur at a peak age of around 65 years [31]. Complete surgical resection is currently the treatment of choice for main duct neoplasms. Non-invasive IPMNs have a 94% 5 year survival rate [32]. This rate drops to 36% in IPMN with invasive carcinoma, but this rate is still significantly better than conventional PDAC [33]. Small side branch IPMNs are usually benign whereas main duct and com-
EUS-FNA in pancreas

Figure 9. Serous cystadenoma of the pancreas. (A) Pap-stained smear of the cyst fluid shows a low cellularity with some hemosiderin-laden macrophages (left, x200) and EUS-FNA core biopsy shows a group of cytologically bland epithelial cells (right, x400); (B) The cytologically bland epithelial cells are positive for PAS (left, x400) but negative in PASD stain (right, x400), suggestive of serous epithelium; (C, D) The tumor is resected and shows a well circumscribed and thick-walled cystic mass (C), which is lined by cuboidal cytologically bland epithelial cells (D, x40, inset, x400).

Bined type IPMN have a higher risk of malignancy, although side branch IPMNs greater than 2 cm have a high incidence of malignancy as well as high-grade dysplasia [34]. On EUS-FNA cytology, there are highly variable amounts of extracellular mucin and cyst lining epithelium [29, 35, 36] (Figure 7A, 7B) and the degree of epithelial atypia may be variable and not representative of the highest degree of dysplasia, owing to the heterogeneity of the cyst lining. The corresponding gross appearance shows a cystic lesion arising from the main duct and/or branch duct (Figure 7C). Histologically, IPMN is further classified as no dysplasia or low, moderate or high grade dysplasia (Figure 7D).

Mucinous cystic neoplasm (MCN): MCN is a benign or potentially low grade malignant cystic epithelial neoplasm composed of cells which contain intracytoplasmic mucin [31]. Like IPMN, the prognosis of MCN is directly related to the presence or absence of an invasive carcinoma and the cytologic features on EUS-FNA specimens are similar to that seen in the IPMN. There are variable amounts of extracellular mucin and cyst lining epithelium (Figure 8A, 8B). Different than IPMN, it is seen almost exclusively in women with a mean age of 45 years. Grossly, MCN does not communicate with the pancreatic duct (Figure 8C). Histologically, there is a characteristic ovarian type stroma beneath the mucinous lining epithelium (Figure 8D). In our experience, the subepithelial ovarian type stroma required for the diagnosis of a MCN is not apparent on EUS-FNA.

Pitfall of gastrointestinal contamination of EUS-FNA in diagnosis of mucinous pancreatic cystic lesions: Generally, selecting the endoscopic approach to obtain specimen for EUS-FNA cytology and/core needle biopsy is dependent on the location of the lesion. If the lesion
is at the body or tail, then a trans-gastric approach will be used. If the lesion is at the head, a trans-duodenal approach will be employed. Therefore, with respect to EUS-FNA, the introduction of gastrointestinal (GI) contamination into the cytological specimen by the very nature of the technique produces a diagnostic challenge and pitfall for the cytopathologist [9-11]. An educated and experienced cytopathologist is critical for accurate interpretation. Duodenal epithelium is recognized by the large, folded sheet-like arrangement of evenly spaced cells studded with goblet cells. Duodenal nuclei are generally uniformly small, round and regularly spaced in a group or sheet, and, except for the occasional goblet cell, the cytoplasm does not appear clear or vacuolated. Sometimes, papillary-like groups of duodenal villi are apparent and this raises the differential diagnosis of intraductal papillary neoplasm. Recognition of goblet cells scattered among bland epithelial cells should deter the interpretation of a neoplasm. In our experience, contamination by gastric epithelium is frequently more problematic, particularly when evaluating a cystic lesion of the pancreatic tail. This is not surprising since the majority of low grade IPMNs are lined by gastric foveolar type epithelium with little or no atypia [47]. Gastric epithelial cells may also occur as large sheets, but more commonly occur as smaller, flat monolayered sheets. Luminal edges may be seen, but a brush border is absent. Foveolar cells display cytoplasmic mucin that is typically confined to the upper third of the cytoplasmic compartment forming a mucin-cup. Keep in mind, however, that most IPMNs arise in the head of the pancreas, a location that generally utilizes a trans-duodenal approach.

Neoplastic cysts: non-mucinous

Serous cystadenoma (SCA): SCA is a benign, slow growing neoplasm of the pancreas. It is
commonly seen in women, at a mean age in the 7th decade, often asymptomatic, but can hemorrhage and cause pain. It is associated with von Hippel-Lindau (VHL) disease with deletion of 3p25 even in sporadic cases [37]. Imaging studies typically show a circumscribed, multilobulated, microcystic lesion with fibrous septae, central scar, and calcifications. EUS-FNA may reveal a clear or bloody fluid. Cytologically, there is a low cellularity with hemosiderin-laden macrophages in a clean or bloody background (Figure 9A). In some cases, one or multiple groups of small cuboidal cells with glycogen rich cytoplasm on PAS stain may be seen (Figure 9B). Grossly, SCN may appear as a thin-walled or thick-walled cystic and well-circumscribed lesion with fibrosis (Figure 9C). Histologically, the cysts are lined by cuboidal, bland epithelial cells (Figure 9D).

Lymphoepithelial cyst (LEC) of the pancreas: LEC is a rare benign cyst lined by squamous epithelium with subepithelial non-neoplastic lymphoid tissue. Pancreatic LEC is typically discovered incidentally. CT scan of LEC frequently shows a well-circumscribed cystic mass with extrapancreatic location [40]. EUS-FNA will yield a variable amount of material that will be thick, and white, and may resemble keratinous debris. Cytologically, the smears show abundant anucleated squamous, mature superficial squamous cells, lymphocytes, histiocytes, background debris and cholesterol clefts and crystals. The cell block may show squamous cells with large amount of granular layer mixed with mature B and T lymphocytes.

Non-neoplastic cystic lesion

Pseudocyst of the pancreas: Pseudocyst is the most common and clinically relevant non-neoplastic cystic lesion of the pancreas. It is most commonly seen in patients with alcohol abuse. Approximately 10% of patients with acute pancreatitis will develop a pseudocyst. It occurs as a consequence of damage to the pancreatic parenchyma that results in necrosis and autodigestion of pancreatic tissue from the release and activation of pancreatic enzymes [38]. Pseudocysts may be medically managed, but some require drainage or resection [39]. Radiographically, they are usually solitary, small to very large (up to 20 cm), well-demarcated, thin walled, unicocular, non-septated, mostly peripancreatic cysts that can occur anywhere in the pancreas, but are most commonly seen in the pancreatic tail. No mural nodule is identified. On EUS-FNA cytology specimens, there are cyst debris with blood, proteinaceous material and hematoxidin-like pigmented macrophages, variable inflammation with no cyst lining epithelium (Figure 10A). Grossly, pseudocyst shows a cavity filled with dirty fluid or necrotic material (Figure 10B). Histologically, there are necrotic tissue and cellular debris in the cystic space, that is lined by macrophages and other inflammatory cells (Figure 10C, 10D).

Ancillary tests for EUS-FNA diagnosis of pancreatic lesions

Pancreatic solid masses are reliably distinguished by FNA cytologic features. However, in pancreatic cystic lesions, cytology alone often lacks adequate sensitivity for the diagnosis due to the difficulty in obtaining sufficient cellular samples. Therefore, ancillary tests are often required and are critical for the evaluation of the pancreatic cystic lesions. Analysis of cyst fluid for mucin, amylase, and tumor markers has been shown to improve the sensitivity and specificity of EUS-FNA for the diagnosis of pancreatic cystic lesions [1]. We routinely stain one smear of the FNA from a cyst for PAS-D to demonstrate the presence or absence of extracellular mucin (Figures 7, 8). Depending on lab preference, mucicarmine stain may also be used. An elevated CEA level correlates with the presence of a mucinous neoplasm. A cutoff level of 192 ng/ml was established as being indicative of a mucinous neoplasm, either IPMN or MCN, by a prospective and multi-institutional study series [41, 42]. Further study showed a cutoff of 110 ng/ml may be more accurate in predicting a mucinous cyst [41]. However, CEA levels are not predictive of the presence of high grade dysplasia or invasive carcinoma in mucinous cystic lesions [43]. CEA less than 5 ng/ml supports a serous cystadenoma. Pseudocysts also have a low level of fluid CEA. An elevated amylase level is associated with a pseudocyst, and indeed, cyst fluid without a high amylase (< 250 u/l) is very unlikely to be from a pseudocyst. However, elevated amylase level may also be encountered in IPMN and lymphoepithelial cyst. Increase of both amylase and CEA has been reported in a squamoid cyst of the pancreatic duct [44]. MCN and serous cystadenoma usually do not have elevated amylase levels [3]. Molecular tests of KRAS and GNAS mutations support mucinous etiology as they are
identified in both MCNs and IPMNs [45]. GNAS mutations are present in up to 60% of IPMNs but not in MCNs. KRAS and GNAS mutations do not predict malignancy and neither of these mutations identifies high grade dysplasia [46].

**Future perspectives on EUS-FNA diagnosis of pancreatic lesions**

**Standardized terminology and nomenclature**

The Papanicolaou Society of Cytopathology has developed a set of guidelines for pancreatobiliary cytology including indications for EUS-FNA biopsy, techniques of EUS-FNA, terminology and nomenclature of pancreatobiliary disease, ancillary testing, and postbiopsy treatment and management [48]. The proposed terminology scheme recommends a six-tiered system: Non-diagnostic, Negative, Atypical, Neoplastic (benign or other), Suspicious and Positive. Unique to this scheme is that the “Neoplastic” category is separated into “benign” (serous cystadenoma), or “Other” (premalignant mucinous cysts, neuroendocrine tumors, and solid-pseudopapillary neoplasms). The suspicious or positive category is reserved for high-grade, aggressive malignancies including PDAC, ACC, poorly differentiated neuroendocrine carcinomas, pancreaticoblastoma, lymphoma, and metastases [48].

**EUS-FNA with rapid on-site cytologic evaluation**

The diagnosis of pancreatic cysts requires a multidisciplinary approach [45]. The introduction of EUS-FNA with rapid on-site cytologic evaluation gives the cytologist a great role in the management of pancreatic cysts [2].

**Future directions in ancillary tests**

A variety of additional tests are listed here, as they may prove to be useful in the future [50]. Use of UroVysion fluorescence in situ hybridization (UFISH) was recently demonstrated to improve the diagnosis of pancreatic malignancy in FNA material [49]. Loss of heterozygosity in >2 loci is reported to be associated with mucinous cysts and may help predict malignancy. Loss of immunohistochemical staining for the protein product of the SMAD4 gene and positive staining for mesothelin support a diagnosis of PDAC. Novel gene mutations (VHL, RNF43, and CTNNB1) may be of aid in the diagnosis of cystic neoplasms.

In summary, EUS-FNA of the pancreas is the preferred method of investigating and sampling pancreatic solid and cystic lesions. Most solid tumors of the pancreas are conventional ductal adenocarcinomas (PDAC). EUS-FNA cytology diagnosis for well-differentiated PDAC is much more challenging than for poorly differentiated PDAC. The sensitivity of EUS-FNA of the pancreas is reported to be 64-96% by multiple studies, with most series reporting a specificity of 100%. Contamination of duodenal or gastric epithelium is a common pitfall in diagnosis of intraductal papillary mucinous neoplasm and mucinous cystic neoplasms. Ancillary tests are often required, particularly in distinction between the various cystic lesions. Molecular analysis of KRAS and GNAS mutations may provide helpful diagnostic values for some cystic aspirates, in particularly for aspirates that yield very scant fluid where the cellular yield is not likely to be sufficient. Future studies to identify more sensitive and specific molecular markers are necessary to further improve the EUS-FNA diagnosis in pancreatic lesions.

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EUS-FNA in pancreas


EUS-FNA in pancreas


EUS-FNA in pancreas

