Cystic Lesions of the Pancreas: Diagnostic Criteria with an Emphasis on Pitfalls

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INTRODUCTION

Cystic lesions of the pancreas include inflammatory processes, rare developmental cysts, cystic neoplasms, solid neoplasms that have undergone degeneration, and rare miscellaneous entities. The majority are pseudocysts, which account for approximately 75-90%. Cystic neoplasms as a group are relatively uncommon, accounting for approximately 5% of all pancreatic neoplasms. Cystic lesions of the pancreas are increasingly being detected with abdominal imaging studies. Cysts in the pancreas, in contrast to cysts in other organs, are more likely to be neoplastic rather than benign or insignificant, a very important point for the person reviewing the specimen from the cyst. In particular, there is a high incidence of Intraductal papillary mucinous neoplasm (IPMN), a pre-invasive precursor lesion, among incidental pancreatic cysts.

A pancreatic cyst aspirate should never be interpreted as consistent with cyst contents because of this. Instead, it is assessed with the goal of determining if a neoplasm is present or not.

Endoscopic ultrasound (EUS) has become the most frequently employed method to sample pancreatic cysts at most large academic centers and now at large community hospitals. EUS-FNA is performed by a gastroenterologist using an echoendoscope with a biopsy chamber through which the needle is passed. The mass is imaged with a radial scanner first and then a linear array scanner is introduced to guide the aspiration. Needles vary from 25 to 19 gauge. One of the manufacturers has developed a new prototype with a reverse bevel that on preliminary use appears to increase the cellular yield. A new echobrush has been developed to sample the lining of the cyst wall. Pilot studies using this device show increased cellularity in these samples compared to FNA samples. A pancreatic cyst aspirate should never be interpreted as consistent with cyst contents because of this. Instead, it is assessed with the goal of determining if a neoplasm is present or not.

The sensitivity for pancreatic FNAB for diagnosis of cystic lesions is much lower than that for solid lesions, although the specificity for the diagnosis of malignancy is higher. Particularly problematic is the diagnosis of serous cystadenoma.

ALGORITHMIC APPROACH

Assessment of the pancreatic cyst fluid FNA sample needs to begin before reviewing the slides. An integrative approach to the evaluation of pancreatic cyst aspirates incorporating the clinical history, radiological findings, cytological findings and ancillary studies yields the most clinically relevant interpretation of the aspirate material.

Age and gender are important as some neoplasms show specific gender and age predilections. As an example, mucinous cystic neoplasms typically occur in middle-aged females. Most crucial are the imaging findings. Masses may present as completely cystic lesions or as solid and cystic lesions, and may or may not show a connection to the main pancreatic duct. Solid and cystic lesions include any solid tumor that has undergone cystic degeneration, such as pancreatic neuroendocrine tumor, and also solid pseudo-papillary tumor. Purely cystic appearing lesions include mucinous cystic neoplasms, serous cystadenoma, side branch intraductal papillary mucinous neoplasms (IPMN) and pseudocysts. When a dilated main pancreatic duct is identified or a connection to the ductal system is demonstrated, a diagnosis of intraductal papillary mucinous neoplasms may be made. More specifics may be included by the person reporting the imaging findings, such as an impression of serous cystadenoma.

A stepwise analysis of the aspirate sample begins with an evaluation of the gross fluid
sample. At low power, assess cellularity, architectural features and background. At intermediate power, architectural details can be assessed in greater detail. At high power, the nuclear, cytoplasmic and mitotic features are appreciated.

CEA and amylase measured in the pancreatic cyst fluid are the most validated ancillary studies. Other ancillary studies may include mutational analysis of kRAS and other genes, and other types of molecular tests.

CYTOLOGY

Cytology of Benign
Normal Pancreas and Contaminants

The epithelium of the large pancreatic ducts is composed of columnar epithelium arranged in flat, honeycombed, two-dimensional sheets of cells. The nuclei are centrally located, or may be present in a palisaded “picket-fence” arrangement in which nuclei are basally located. In contrast to the epithelium of the large ducts, intralobular duct epithelium is composed of cuboidal shaped cells with scant basophilic cytoplasm, and are usually present as flat sheets, small clusters or tubular structures.

Aspirates of the normal pancreas consist predominantly of acinar type epithelium, typically arranged either singly or in small acinar shaped structures. The cells are pyramidal or triangular in shape and contain abundant, granular cytoplasm with numerous intracytoplasmic zymogen granules. The nuclei are round, have a granular chromatin pattern, contain prominent nucleoli, and are either central, or eccentrically located.

Islet cells are rarely detected in aspirates of the normal pancreas. When present, they occur as loose aggregates of cells that contain wispy, ill-defined amphophilic cytoplasm and oval nuclei with a stippled chromatin pattern.

Mesothelial cells and hepatocytes are occasional contaminants in FNAB of the pancreas. Mesothelial cells appear as polygonal cells arranged in flat sheets, and show round to oval shaped nuclei and intercellular cytoplasmic “windows”. Normal hepatocytes are large polygonal cells with dense, sharply defined cytoplasm and round, central or eccentric nuclei with prominent nucleoli.

Gastric or duodenal epithelium are the most problematic of the contaminants, since they may be abundant in aspirates performed under EUS guidance, and, therefore, may be misinterpreted as representative of a neoplastic process. Gastric and duodenal epithelium typically presents as flat, monolayered sheets of glandular epithelium with a uniform, honeycomb pattern similar to normal pancreatic ducts. Both are accompanied by extracellular mucin that is thin, but that may have degenerated debris or inflammation.

Duodenal epithelium is composed of enterocytes and goblet cells. Enterocytes are non-mucinous, columnar cells with a microvillous brush border. Goblet cells appear as sporadically placed mucinous cells that have the appearance of a fried egg. Intraepithelial lymphocytes may also be seen distributed among the sheets of duodenal epithelium.

Aspirates of gastric epithelium will be composed of foveolar cells and, sometimes, parietal and chief cells if the aspirate traverses the gastric glands. Foveolar cells have cytoplasmic mucin, most often contained in upper third of the cytoplasmic compartment, imparting a cup like appearance. The cells may exhibit degenerative grooves and inclusions. A feature of gastric epithelium is stripped naked nuclei with grooves in a background of abundant extracellular mucin. Parietal cells and chief cells may appear grouped together. Parietal cells have abundant, well defined cytoplasm with numerous granules that are deep blue on Diff-Quik smears. Chief cells will have dense, pink, well-defined cytoplasm on Diff Quik. Both have a low nuclear to cytoplasmic ratio and round nuclei.

Non-Neoplastic Cysts

Non-neoplastic cysts of the pancreas include inflammatory, infectious and congenital cysts. Most of these entities are rare. Pseudocysts are the most common, and thus, are the most clinically relevant. Aspiration of a pseudocyst typically reveals an abundant amount of turbid, brown fluid that contains inflammatory cells (mostly macrophages), fibrin, and debris and pigment. The fluid lacks lining epithelial cells, but may contain benign cells from adjacent pancreatic parenchyma. Yellow pigmented material serves as a surrogate marker of a pseudocyst.
**Lymphoepithelial Cyst of the Pancreas**

Lymphoepithelial Cyst of the Pancreas (LECP) is typically discovered incidentally. These lesions have a very typical CT scan appearance showing a well-circumscribed mass protruding from the anterior surface of the pancreas into the lesser sac. Aspirates of LECP will yield a variable amount of thick and white material that may resemble keratinous debris. The smears will show abundant anucleated squames, mature superficial squamous cells, lymphocytes, histiocytes, background debris and cholesterol clefts and crystals. The cellblock may show squamous cells with a preserved granular layer. This finding in an aspirate from a pancreatic cyst is diagnostic of LECP. The findings in this cyst are different from the lymphoepithelial cyst of the head and neck which typically have more lymphocytes and fewer squamous cells, which leads some observers to misdiagnose this particular cyst of the pancreas. The cyst fluid findings will be confusing since this cyst fluid will yield a very high CEA and amylase level.

The differential diagnosis includes pseudocyst, dermoid cyst, splenic epidermoid cyst, other cystic neoplasms, and squamous cell carcinoma. The presence of the squamous material excludes the diagnosis of pseudocyst and other cystic neoplasms. The lack of malignant epithelium will exclude squamous cell carcinoma. The distinction among the other squamous cysts that occur in this area may not be possible without clinical and radiological correlation.

**Cystic Neoplasms**

Inherently cystic neoplasms include serous cystadenomas and mucinous cystic neoplasms. Intraductal papillary-mucinous neoplasms and intraductal papillary oncocytic neoplasms cause dilatation of the pancreatic ducts that often appears cystic on radiological studies. Acinar cell cystadenocarcinoma is an inherently cystic variant of acinar cell carcinoma. Finally, solid neoplasms, such as pancreatic neuroendocrine tumors or solid pseudo-papillary tumors, may undergo cystic degeneration as well.

**Serous Cystadenoma**

Aspiration of a serous cystadenoma typically obtains only a scant amount of clear, serous fluid. The cells are arranged in flat, monolayered sheets, as dispersed single cells or stripped nuclei. The background is usually proteinaceous, watery or bloody, and may also contain stromal cells composed of fibrous tissue and blood vessels. The cells are cuboidal in shape and possess clear cytoplasm. The nuclei are round and uniform in shape, but may vary in size. The PAS stain with and without diastase, may be performed on cytology samples to demonstrate the presence of cytoplasmic glycogen, a characteristic feature of serous cystadenomas. Since the stroma is vascular, these cysts may hemorrhage, in which case, the aspirate will yield blood and hemosiderin laden macrophages. The cyst aspirates may yield only small, cuboidal macrophage like cells. Cytology lacks sensitivity for the diagnosis of serous cystic neoplasms because of the difficulty in obtaining cells from these neoplasms. This is an example where knowledge of the radiological findings is crucial. If the imaging studies are interpreted as characteristic of serous cystadenoma, then the pathologist needs to evaluate the sample carefully for even a few diagnostic cells. Smears from a serous cystic neoplasm will not contain many diagnostic cells. Only a few characteristic groups associated with a solitary cystic mass with characteristic findings of serous cystic neoplasm are diagnostic. However, oligocystic and solid variants of serous cystadenomas occur. These will not be readily diagnosed on imaging studies since their features will overlap with those of other neoplasms.

**Mucinous Cystic Neoplasms (MCN)**

Mucinous cystic neoplasm occurs predominantly in female, middle aged patients in the distal pancreas. On imaging studies it appears as a solitary cystic mass that does not connect to the ductal system. Gross exam will show a unilocular or multilocular cyst filled with viscid, white mucin. Histology will show columnar, mucin containing neoplastic cyst lining cells surrounded by ovarian type stroma that will express estrogen receptor and inhibin if assessed using immunohistochemistry. These neoplasms are considered mass forming pre-invasive precursor...
lesions, and therefore, it is imperative to correctly diagnose them before they progress to invasive adenocarcinoma. Resection is curative. The cytology of this neoplasm is similar to that of intraductal papillary mucinous neoplasm, since both are considered cystic mucinous neoplasms.

**Acinar cell cystadenocarcinoma**

A cystic variant of acinar cell carcinoma has been reported\(^\text{17}\). The aspiration features have not been described, but it could be assumed that they will be similar to the solid variant.

**Intraductal Papillary Mucinous Neoplasms (IPMN)**

Intraductal papillary mucinous neoplasm is a neoplastic process composed of ductal type cells with cytoplasmic mucinous metaplasia that replace the normal ductal epithelium, and form complex papillary structures. These neoplasms connect to the ductal system and lack ovarian type stroma. The process may involve the main pancreatic duct, side branches of the main pancreatic duct or both. They are also mass forming cystic pre-invasive precursor lesions that can give rise to either invasive tubular type ductal adenocarcinoma or mucinous noncystic carcinoma (colloid carcinoma).

**Cytology of Cystic Mucinous Neoplasms: MCN and IPMN**

Since both MCN and IPMN have similar cytomorphological features, their cytological features will be considered together.

Diagnosis may begin at the time of aspiration, since aspiration of fluid may be difficult because the fluid is often extremely viscous, and, thus, does not readily pass into the needle, providing an immediate clue to the diagnosis. Gross examination of the fluid will reveal highly viscid clear to white fluid or clear fluid with strands of floating mucus plugs. The material may be expressed from the needle as a plug of mucin. This is diagnostic, regardless of the cellularity.

The classic cytomorphological appearance is the presence of thick background mucin and neoplastic glandular mucinous epithelium. The characteristic mucin pattern is abundant, thick background mucin, spread out over the entire slide forming a film, similar to colloid in thyroid aspirates or it may be clumped. On Papanicolaou stains, it will have a characteristic pink hue. Sometimes with basophilia. On Diff Quik, it will appear pink. A characteristic feature is the presence of degenerated cellular material, called oncotic cells\(^\text{18}\), with debris and macrophages. The mucin may be scant in quantity. Background material with ferning may help one identify background mucin. Longstanding mucin may appear as dense material with cracks, or as clumps bearing a resemblance to a handheld fan. Psammomatous calcifications are a feature of IPMN. The background may also contain acute inflammation and necrosis.

Neoplastic glandular epithelium with cytoplasmic mucin is the diagnostic feature. The cells may be arranged singly, on edge, in sheets, papillary clusters, or small papillary tufts. The sheets or groups do not form a honeycomb pattern, but rather, are subtly hypercellular and crowded.

The appearance of the cytoplasm may vary. The characteristic cell type is a columnar cell with abundant cytoplasmic mucin, the appearance of mucinous metaplasia or the cells may be rounder, with vacuolated cytoplasm. The vacuoles may not be as easily recognized on Diff-Quik stained smears. Signet ring cells occur, but these are seen more often in high-grade dysplasia or invasive carcinoma.

The cellularity is variable. The higher the grade of dysplasia of the lining epithelium, the more cellular the samples tend to be, with invasive carcinomas typically being the most cellular.

The nuclei show varying degrees of abnormalities. The subtlest are mild nuclear enlargement and hypochromasia with subtle nuclear membrane foldings. The hypochromatic nuclei may have small, peripherally located nucleoli, similar to those seen in papillary carcinoma. The nuclear atypia becomes more evident as the degree of dysplasia increases. Nuclear grooves and pseudo inclusions become more evident and are diagnostic of cystic mucinous neoplasms. The chromatin will become coarser, with abnormal paranuclear clearing and micronucleoli. Mitotic figures will be evident in high-grade dysplasia. The single dysplastic cell, which is a small cell with a high nuclear to cytoplasmic ration and nuclear convolutions, is diagnostic of a neoplastic process, and it is typically associated with higher-grade dysplasias. These cells may
be obscured by the cystic debris; therefore careful examination of the specimen, particularly in the areas of thick mucin, is needed.

The presence of background mucin with the features described should be considered indicative of the presence of neoplastic mucinous cyst even if diagnostic cells are not identified. Since aspirates are frequently scanty cellular, even a few cells should be considered diagnostic of a cystic mucinous neoplasm. Correlation with the imaging studies may lead to a specific diagnosis of IPMN vs. MCN.

**Invasive Carcinoma Associated with Mucinous Cystic Neoplasm or Intraductal Papillary Mucinous Neoplasm**

Aspiration of a solid area of an MCN/IPMN, when visualized by radiological guidance, may prove diagnostic of invasive adenocarcinoma. Invasion typically cannot be determined on the basis of evaluation of cystic or ductal fluid only. The presence of coagulative tumor necrosis and cells with significant anisonucleosis, and mitotic features, and other criteria of adenocarcinoma may indicate the presence of an invasive ductal carcinoma component. The intestinal type of intraductal papillary mucinous neoplasm is associated with non-cystic mucinous carcinoma.

**Intraductal Oncocytic Papillary Neoplasms (IOPN) and Invasive Oncocytic Carcinomas**

Intraductal oncocytic papillary neoplasm is an intraductal neoplasm which has morphologic and clinical features that overlap with those of IPMN. Aspirates from these may produce viscous fluid. Cytology smears show a pattern similar to that of IPMN. The cells are usually arranged in papillary clusters with background mucin that contains macrophages and cell debris. However, the main distinction is that the majority of the cells have dense oncocytic cytoplasm. Invasive oncocytic carcinomas develop from intraductal oncocytic papillary neoplasms. Aspirate smears will show features of malignancy. The cells will have abundant oncocytic cytoplasm; others will have glandular type cytoplasm. The nuclei will show grooves and inclusions.

The differential diagnosis includes pancreatic neuroendocrine tumors with oncocytic cytoplasm and hepatoid pancreatic ductal adenocarcinoma. Immunohistochemistry will assist with these differential diagnoses. PanNET will express neuroendocrine markers and hepatoid carcinomas will show expression for HepPar and AFP. Oncocytic carcinomas will not express either of these markers. Furthermore, the oncocytic epithelium will be intermixed with glandular, mucinous containing epithelium.

**Solid-Pseudopapillary Tumor (SPPT)**

Solid-pseudopapillary tumor of the pancreas has a characteristic cytological picture. Smears are typically quite cellular and contain numerous branching papillary-like groups. The papillary fronds have three layers: a central vessel, a middle mucinous stromal layer and an outer neoplastic layer. The layer of neoplastic epithelium is composed of monomorphic epithelial cells with round to oval nuclei, finely dispersed chromatin, nuclear indentations or grooves and small nucleoli. The cytoplasm of these cells is basophilic, varies in shape and quantity, may be vacuolated, or may contain PAS-positive granules. These cells may have cytoplasmic tails. The pathognomonic feature is “balls” of mucoid stroma that may or may not contain a rim of epithelial cells. SPPT strongly and diffusely express vimentin, NSE, alpha 1-anti-trypsin, CD10, CD56 and progesterone receptors. They are variably positive for pancytokeratins and synaptophysin. These tumors will show nuclear beta-catenin expression.

**Cystic Pancreatic Neuroendocrine Tumors**

Cystic pancreatic neuroendocrine tumors are uncommon, accounting for 3.4% of all cystic neoplasms and 4.3% of all pancreatic neuroendocrine tumors in one series. However, cystic degeneration in large, nonfunctional tumors is a relatively common occurrence. The cytological features are similar to those of their solid counterpart.

**PITFALLS**
Neoplastic Mucinous epithelium vs Gastrointestinal Contaminant

The main pitfall in the evaluation of pancreatic cysts is distinguishing benign gastric or duodenal epithelium from cystic mucinous neoplasms, MCN or IPMN. Gastrointestinal epithelium mimics the epithelium derived from these neoplasms because it consists of glandular, mucin containing epithelium and background mucin.

An approach to differentiating gastrointestinal epithelium from neoplastic mucinous epithelium will incorporate assessment of the following:

1. gross appearance of the fluid
2. appearance of mucin on smears
3. cytornorphological features of the cells

It is also critical to know where the mass is located, since aspirates of the pancreatic head and uncinate process will traverse the duodenum and aspirates of the neck, body and tail will traverse the stomach. This will determine what type of contaminant one is trying to identify. In general, duodenal epithelium is easier to recognize because of the goblet cells and enterocytes.

Gastric and duodenal contents are usually watery and thin in contrast to the mucin from MCN/IPMN, which is viscous. The mucin of gastrointestinal epithelium appears thinner and wispiest on smears, and is associated with the gastrointestinal epithelium. The mucin of neoplasms contains histiocytes and oncotic cells. Mucin from gastrointestinal epithelium may contain degenerated cells. Mucin from gastrointestinal epithelium may contain degenerated cells.

Key to the diagnosis of a neoplasm is evaluation of the cytological features. Gastric and duodenal epithelium will retain a two-dimensional architecture in which the nuclei are evenly spaced within the sheets. Epithelium from MCN/IPMN will show crowding and nuclear overlap, which may be subtle. The nuclei will be irregularly distributed.

Both gastric and duodenal epithelium may yield single cells. Single columnar cells with abundant, columnar cytoplasm containing mucin that extends to the nucleus, metaplastic mucinous cells, are derived from a neoplasm. The mucin in foveolar cells typically does not fill the entire cytoplasm. Single cells with a brush border are enterocytes and are derived from the duodenum. Goblet cells are seen in IPMN and MCN, but these will be less uniform, and unevenly distributed, in contrast to the goblet cells in the duodenum.

The nuclei from gastrointestinal epithelium will be round and uniform. Neoplastic nuclei are subtly atypical, with nuclear membrane irregularities. Pseudo-inclusions and grooves are typical of neoplastic epithelium, although gastric epithelium may also exhibit grooves and inclusions as a result of degeneration. Gastric epithelium frequently produces stripped nuclei with atypia and grooves in a background of mucin, this pattern should be recognized as part of the spectrum of gastric epithelium, and not over interpreted as being from gastric epithelium.

Even using these criteria, it will be difficult to differentiate gastrointestinal epithelium, particularly gastric epithelium, from neoplastic epithelium. Fortunately, this distinction is not as crucial anymore, since what is critical is recognizing cytological features of high grade dysplasia. Furthermore, ancillary studies assist in diagnosing in neoplastic mucinous cysts.

Extra pancreatic or Retroperitoneal Cysts

Peripancreatic and/or retroperitoneal cysts and cystic neoplasms that appear to be pancreatic in origin on radiological studies, comprise approximately 21% of lesions that are aspirated percutaneously. These lesions may be misdiagnosed if the possibility of an extrapancreatic cyst is not considered. A few examples of these lesions include hydroureter, adrenal cyst, mesothelial cyst, mesenteric lymphangioma, gastric primary tumor, retroperitoneal serous cystadenoma, retroperitoneal leiomyoma, malignant lymphoma in a retroperitoneal lymph node, gastrointestinal stromal tumor, and gastric leiomyosarcoma. Some of these may actually be intrapancreatic, such as schwannoma with cystic degeneration.

Aspirates with oncocytic cells

In the differential diagnosis IOPN and oncocytic are neuroendocrine tumors with oncocytic cytoplasm, acinar cell carcinoma and carcinoma with hepatoid differentiation. Immunohistochemistry will sort out these various entities.

Scant Cellularity of Pancreatic Cysts
Aspirates of pancreatic cystic lesions are frequently scantly cellular. The smears have to be carefully evaluated for the presence of characteristic neoplastic cells. In cyst aspirates, the threshold for adequacy needs to be lower. This is true for diagnosing serous cystadenoma, MCN/IPMN and cystic pancreatic neuroendocrine tumor.

**Ancillary studies**

Ancillary studies are critical for the evaluation of cystic lesions, since cytology alone often lacks adequate sensitivity for the diagnosis of pancreatic cysts due to the difficulty in obtaining sufficiently cellular samples. Analysis of pancreatic cyst fluid for mucin, pancreatic enzymes, and tumor markers improves the sensitivity and specificity of fine needle aspiration for the diagnosis of pancreatic cystic lesions, particularly to rule in or out IPMN or MCN. Cytology is currently the best and only diagnostic modality for many other entities, including pancreatic neuroendocrine tumor, SPPT, and LECP and other lesions occurring in the retroperitoneum and peripancreatic area.

**Mucin stains**

Mucicarmine stains and Alcian blue stain aid in identifying background material as mucinous when its nature is questionable. However, mucin from gastrointestinal contaminants may lead to false positive interpretation. The presence of abundant, thick background mucin or cells containing mucin will lead in the direction of a MCN or IPMN and excludes serous cystadenoma and pseudocyst. If the sample is obviously mucinous, mucin stains are not indicated.

**Cyst fluid viscosity**

The original studies published on this topic utilized viscosity measurements as a means of distinguishing between nonmucinous and mucinous lesions. A recent study emphasized again the importance of assessing the viscosity of the fluid.

**Tumor markers**

A large number of tumor markers have been evaluated and reported on in the literature for the evaluation of pancreatic cysts, but currently, CEA levels are the most useful. An elevated CEA level correlates with the presence of a cystic mucinous neoplasm. Serous cystadenomas and pseudocysts will have low levels of CEA. A cut off level of 192 was established in a prospective, multi-institutional series as being indicative of the presence of a mucinous type neoplasm, either MCN or IPMN. However, it is recommended that each laboratory establish its own threshold. CEA levels, however, do not correlate with the grade of dysplasia.

Benign entities, such as enteric duplication cysts, mesothelial inclusion cysts, and lymphoepithelial cyst of the pancreas may also produce elevated CEA levels. Therefore, CEA levels should not be used as the sole criterion for the diagnosis of a mucinous cystic neoplasm.

**Enzymes.**

Elevated amylase levels are associated with a pseudocyst. Elevated amylase levels may also be encountered in IPMN. Typically, serous cystadenomas and MCN will not have elevated amylase levels. LECP may have elevated amylase levels.

**Mutations**

Kris mutations are identified in both MCN and IPMN. GNAS mutations are identified in IPMN. Neither of these mutations identifies high grade dysplasia. Cytology still remains the gold standard as some entities, such as IOPN, may have neither.

**ROLE OF PANCREATIC CYST FLUID CYTOLOGY IN THE PRE-OPERATIVE WORK-UP**

**MCN/IPMN**

Aspiration of pancreatic cysts is performed to differentiate benign processes or indolent neoplasms from pre-invasive precursor lesions, MCN and IPMN. As shown so far, a combination of cyst fluid analysis for cytology, viscosity, tumor markers, enzymes and mutational analyses may identify MCN or IPMN. If the sample is sufficiently cellular, the cytology may be diagnostic of
nonneoplastic entities, such as LECP, and nonmucinous neoplasms such as SPPT or serous cystadenoma.

Many times, the cyst fluid studies are not available when the pathologist is reviewing the cyst fluid cytology, or the material was insufficient for ancillary testing, but yet, the slides show background mucin and mucinous cells that are not clearly neoplastic, and possibly contaminant. This scenario is most problematic for IPMN. What is the role of cytology in this situation?

The most recent international consensus guidelines for the management of IPMN recommend resection for all main duct IPMN. Side branch IPMN >= 3 cm need to be resected if associated with high risk stigmata. Cytology suspicious or positive for malignancy is considered a high risk stigmata\(^\text{35}\). What is meant by positive cytology may be open to interpretation, however, what is helpful is to identify significant atypia that may indicate the presence of high grade dysplasia in the cyst. At our institution, we assess the amount of dysplasia in the cells and report on whether there is high grade dysplasia or not, or at least moderate dysplasia when sufficient cells are present.

Features that represent moderate dysplasia include enlarged nuclei with a cigar shaped appearance in groups with nuclear overlapping or stratification. High grade dysplasia is characterized by groups forming small papillary tufts with nuclear membrane irregularity and high N/C within the cells. Other features that relate to high grade dysplasia include single dysplastic cells, signet ring cells, or groups with three dimensionality and crowding and anisonucleosis.

These criteria and management guidelines take some heat of the pathologist, particularly when trying to distinguish gastric foveolar epithelium from IPMN with low grade dysplasia, which in the side branches, has a foveolar phenotype. Making this distinction does not matter, and a report with a descriptive diagnosis and a differential diagnosis of gastric epithelium vs. IPMN with low grade dysplasia should be sufficient. The key is to identify neoplastic cells with significant atypia indicating the probable presence of high grade dysplasia in the cyst.

**SUMMARY**

Evaluation of pancreatic cysts needs to begin with knowledge of the clinical and imaging findings. Cytology is the only test that will yield a diagnosis for many lesions including cystic neuroendocrine tumor, SPPT and LECP. Cytology will also on most occasions identify a cystic lesion as being mucinous, either an MCN or IPMN. Grossly viscous fluid, abundant background mucin with histiocytes and oncotic cells are indicative of the presence of a mucinous cyst. Since aspirates of cystic neoplasm may be scanty cellular, the smears need to be carefully evaluated for other cytological features. Even a few cells will be diagnostic for a neoplasm.

Cyst fluid viscosity, CEA and amylase levels and mutational analysis of KRAS and GNAS will help sort out those cases in which the cytological findings are inconclusive, either due to an absence of diagnostic epithelium or gastric or duodenal contamination. Cystic mucinous neoplasms yield fluids with a high viscosity. Elevated CEA levels are indicative of the presence of a neoplastic mucinous cyst and exclude the presence of pseudocyst, serous cystadenoma and gastrointestinal contaminants. A cyst with a high amylase and low levels of CEA is a pseudocyst and finally serous cystadenoma should have low amylase and CEA levels. KRAS and GNAS mutations occur in cystic mucinous neoplasms but not in nonmucinous neoplasms.

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Outline

- Cytology of normal ductal cells and contaminants
- Cytology of inflammatory and benign cystic lesions and cystic neoplasms
- Pitfalls and challenges
- Ancillary studies
- Role of cytology in the management of IPMN
Cystic And Intraductal Lesions

- Inflammatory
  - Pseudocysts/Retention Cysts
- Congenital/Hereditary/Undetermined
  - simple or solitary true cysts, polycystic diseases
  - Lymphoepithelial cysts
- Infectious
  - Abscess
- Neoplastic
  - Inherently cystic
    - serous cystadenoma and mucinous cystic neoplasms
  - Neoplasms with cystic degeneration
    - Solid pseudopapillary neoplasm
    - Cystic pancreatic neuroendocrine tumor
  - Intraductal neoplasms
Integrative Approach To The Work Up Of Pancreatic Masses

• Clinical history
  – Age
    • Solid-pseudopapillary tumor in younger age group, mucinous cystic neoplasm in middle aged females
  – Gender
    • Solid pseudo-papillary tumor and mucinous cystic neoplasm in females
  – Previous history of carcinoma

• Radiological findings (this is critical*)
  – Cystic vs. solid vs. solid and cystic: if cystic, is there a connection to the ductal system?
  – Multiple cysts or single cyst
  – Location of the cyst
  – Certain Imaging techniques more likely to have contaminants
    • Location determines whether it is duodenal or gastric contaminant

• Cytological findings

• Ancillary studies

• Correlate all findings
Ductal Cells
Gastric epithelium at low power
Gastric Epithelium

- Sometimes show small grooves, inclusions and small nucleoli
- Associated with mucin
Gastric Epithelium

- Large flat sheets
- Cytoplasm of foveolar cells is columnar, with mucin
- Nuclei round and evenly spaced, uniform in size
- Opening to pits appear as acinar structures
Cup shaped mucin
Gastric Epithelium
stripped nuclei

Inclusions

Degenerative grooves

Mucin and stripped nuclei
Gastric Parietal Cells and Chief Cells

Pitfall: May be mistaken for acinar cells or hepatocytes
Duodenum

- Enterocytes with a microvillus brush border with interspersed goblet cells
- Background mucin is typically thin, associated with groups, may have debris
Duodenal Epithelium

- Lamina propria
Enterocytes have a microvillous brush border
Degenerated duodenal cells
Incidental Cysts in the Pancreas

• Incidental cysts of the pancreas are common

• More likely to be neoplastic rather than benign
  – 30% of all incidental masses were IPMN in one series
  – 55% were MCN or IPMN (preinvasive precursors), only 4% were pseudocyst

• Pathologist is reviewing the cyst fluid to assess for the presence of pre-invasive precursors lesions
  – Pseudocyst diagnosis of exclusion
Cyst Contents
Pseudocysts
Gross: Abundant turbid, brown material
Lymphoepithelial Cyst Of The Pancreas

- Occurs most often in men
- Cyst lined by squamous epithelium with adjacent lymphocytic infiltrate
- Elevated CEA in fluid
Lymphoepithelial Cyst Of The Pancreas
Serous Cystadenoma
Classic CT and gross appearance
Serous Cystadenoma
Typical histopathology and cytopathology
• Gross: Scant fluid
• No mucin*
Pre-Invasive Precursors

IPMT

MCN
Typical cytology of IPMT or MCN

- Gross: viscid, clear to white fluid, may have visible strands of mucus

Mucin
Neoplastic epithelium
Background Mucin

- Thick, diffuse, colloid like film
- Pink on PAP
Psammomatous calcifications a feature of IPMN

Ferning

Cell block showing thick, feathery type material
Cytopathology report: Thick background mucin, histiocytes, and oncotic cells, consistent with IPMN or neoplastic mucinous cyst
Comment: No neoplastic epithelium is present for evaluation of dysplasia
Intraductal Papillary Oncocytic Neoplasm
Intraductal Oncocytic Papillary Neoplasm
Cystic Pancreatic Neuroendocrine Tumor
Pitfalls

• Mucinous epithelium
  – GI contaminant vs. Mucinous neoplasia

• Extrapancreatic cysts
  – May be confused with mucinous neoplasia

• Aspirates with Oncocytic cells

• Scant cellularity
GI Contaminant vs. IPMN/MCN

- Thin, watery mucin in GI contaminant
  - Will lack oncotic cells, histiocytes
  - Thick, tenacious mucin typical of IPMN or MCN

- GI contaminant more cellular with groups showing more even architecture, nuclei generally show the same chromatin pattern
  - Duodenum will have goblet cells evenly distributed within epithelium
  - Gastric epithelium may have degenerative grooves and inclusions, but will be associated with other cells that look gastric
  - Contaminant should lack definitive features of neoplasia, such as nuclear enlargement, architectural abnormalities in the groups

- Ancillary studies such as CEA beneficial
- If it is still not clear whether it is contaminant, particularly gastric vs. IPMN foveolar type (low grade dysplasia), may not matter
  - Patient with side branch IPMN less than 3 cm will be followed
Gastric Foveolar epithelium

IPMN with foveolar lining
Gastrointestinal Background vs. Neoplastic Background

Duodenal

Neoplastic
Neoplastic Mucinous Epithelium vs. Gastric Epithelium

IPMN: irregular spacing  Gastric: Regular spacing
Neoplastic Mucinous Epithelium VS. Gastric

IPMN: Papillary

Gastric: Flat sheet
Extrapancreatic cysts

• 21% of lesions that were aspirated percutaneously
  – Peripancreatic and/or retroperitoneal cysts and cystic neoplasms
    • retroperitoneal serous cystadenoma
  – Nonneoplastic
    • Hydroureter, adrenal cyst, mesothelial cyst (elevated CEA)
  – Mesenchymal neoplasms
    • lymphangiomas, smooth muscle tumors, gastrointestinal stromal tumor
Extrapancreatic cysts cont.

- May occur with EUS-guided FNA
  - Tumors secondarily involving pancreas (GIST)
  - Gastric tumors invading pancreas
- These lesions may be misdiagnosed if the possibility of an extrapancreatic cyst is not considered
  - Cyst fluid studies may be misleading
Oncocytic Cells

- Oncocytic neoplasms
  - MUC1, MUC 6

- PanNET with oncocytic cytoplasm
  - Neuroendocrine markers

- Hepatoid adenocarcinoma
  - HepPar, AFP

- Acinar cell carcinomas
  - Trypsin
Scantly Cellular Samples

- Scantly cellular specimens
  - Serous cystic neoplasm
  - High grade cells in IPMN
  - Cystic pancreatic neuroendocrine tumor

- Assess carefully for neoplastic cells

- Lower quantitative threshold for diagnosing neoplasia
Ancillary Studies in Pancreatic Cyst Fluid

- **Viscosity**
  - Elevated in MCN/IPMN/IOPN

- **CEA**
  - Elevated in mucinous neoplasms
  - Remains the standard for separating mucinous from nonmucinous cysts
  - False positives for some nonmucinous cysts, such as LCOP

- **Amylase**
  - Elevated in pseudocyst, and intraductal papillary mucinous tumors
  - Low in serous cystadenoma and mucinous cystic neoplasm

- **Mutational analysis**
  - Kras detected in IPMN and MCN
  - GNAS in IPMN
  - These mutations can identify the cyst as a mucinous type
Ductal Carcinoma
Pre-Invasive Precursors
MCN and IPMN (Mucinous)
vs.
Pseudocyst
Benign or Indolent Neoplasms (Nonmucinous)
Criteria for Mucinous Cyst

- Fluid viscous, clear or white
- Cytology shows mucinous background as described, +/- neoplastic epithelium
- Ancillary studies
  - CEA elevated
  - Mutational analyses
    - Kras mutated
    - GNAS mutations in IPMN
Management of IPMN  
2012 Consensus Guidelines

• Consensus conference of the International Association of Pancreatology has proposed guidelines for the management of patients with pancreatic cysts suspected to be IPMNs

• These guidelines use a combination of clinical history, gender, imaging characteristics, cytology and cyst fluid analysis to determine therapeutic and surveillance strategies.

• Resection without tissue confirmation:
  – Main duct IPMN > 10 mm
  – Obstructive jaundice a/w cyst in HOP
  – Enhancing solid component
Management of IPMN
2012 Consensus Guidelines

• Worrisome features
  – Patient evaluated by EUS
  – These include:
    • Cyst >= 3 cm
    • Thickened, enhancing cyst walls
    • Main duct 5-9 mm
    • Nonenhancing mural nodule
    • Abrupt change in caliber of pancreatic duct with distal pancreatic atrophy

• EUS worrisome features
  – Definite mural nodule
  – Main duct features suspicious for involvement
  – Cytology: suspicious or positive
    • Strongly consider surgery if > 3 cm
    • Consider surgery if 2-3 cm
What is suspicious or positive cytology?

• Cells that indicate the presence of high grade dysplasia or invasive carcinoma
  – Role of cytology: Screening for high risk cytological features

• Terms:
  – High grade dysplasia present or absent
  – High grade epithelial atypia: incorporates high grade dysplasia and invasive carcinoma
    • absence of high-grade atypia with the absence of high risk imaging features of dilated MPD and mural nodule gives a NPV of > 90%
Low Grade Dysplasia

Abundant columnar mucin containing cytoplasm

Basally located nuclei

Columnar cytoplasm with mucin

Basally located nuclei
Moderate dysplasia

Pseudostratification
High-Grade Dysplasia

Papillary tufts, nuclei extend to luminal border

Mitoses
High-grade epithelial atypia

• Small cells
• high N/C
• Nuclear membrane irregularities
• Abnormal chromatinin
  – Hypo- or hyperchromasia
• Background necrosis
Is it IPMN or gastric foveolar epithelium?

Mucinous epithelium present: possibly gastric or neoplastic, Negative for high grade dysplasia
Algorithmic Approach for the Cytological Evaluation of Pancreatic Cysts

Is there characteristic background mucin?

YES

Evaluate for neoplastic cells

NO CELLS

Descriptive

CELLS PRESENT

Report if there is high grade dysplasia

NO

Descriptive

YES

Does the aspirate show neoplastic cells?

NO

Work-up as a solid lesion

YES

Descriptive
Summary cont.

• Characteristic mucin is diagnostic, and should be reported as consistent with neoplastic mucinous cyst in the appropriate setting

• CEA and amylase remain standard ancillary studies

• Elevated CEA does not correlate with high grade dysplasia or adenocarcinoma
Summary

• Sensitivity much lower for the detection cystic neoplasms due to scant cellularity
• Correlate with imaging findings: this is key
• Goals
  – Identify preinvasive precursor lesions: IPMN and MCN
    • Common source of incidental cysts
    • Determine if there is high grade dysplasia, sometimes will put moderate dysplasia into high grade category
  – Identify other cystic neoplasms, particularly solid neoplasms with cystic degeneration