Approach to the Diagnosis of Pancreatic Cysts

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Outline

• Background
  - Foundational clinical and pathological features of pancreatic cysts
• Purpose of Pre-Operative Evaluation of Pancreatic Cysts
  - Two questions to answer from FNA
• Pancreatic Cyst Fluid Triage
• Cytology and Ancillary Testing
• Reporting using the PSC PB Terminology System

Pancreatic Cysts

• Primary Differential Diagnosis
  - Pseudocyst
  - Serous cyst
  - Mucinous cyst
  - (MCN and IPMN)
  - Cystic degeneration of typically solid tumors
  - PanNET
  - SPN

Modified from 1 2 3
Cytology Interpretation

- Multimodal Approach
  - Clinical Information
    - Patient age and gender
    - Symptoms
    - Past medical history
  - Radiological Information
    - Location of mass in the pancreas (and thus organ traversed for EUS)
    - Cyst characteristics
      - Size, contours, invasion
      - Cyst structure: uni- or multilocular; thick/thin wall, Ca++, nodule/mass in the wall
      - Gross cyst contents: thick, viscous, thin, water, clear, brown
  - Ancillary tests: CEA, amylase, IHC, molecular analysis

Pancreatic Pseudocyst

Clinical
- Non-neoplastic cyst
  - Associated with pancreatitis, trauma, surgery

Radiology
- Unilocular, non-septated
- Thick walled
- No mural nodule

Histology
- Cyst lining of histiocytes and inflammatory cells; no epithelial lining, hence, pseudocyst

Serous Cystadenoma

- Clinical
  - Benign, slow growing neoplasm
  - women>>men, mean age 7th decade
  - Associated with VHL, with deletion of 3p25 in many cases
  - Often asymptomatic, but can hemorrhage and cause pain
- Radiology
  - Circumscribed, multi-lobulated
    - Microcystic with fibrous septae, central scar, calcifications in ~30-40%; can be oligocystic
- Histology
  - "glycogen-rich" cuboidal cyst lining
  - PAS+/dPAS-
Serous Cystadenoma

- Oligocystic Variants
- Vascular Septae that bleed

Hemosiderin-laden macrophages = surrogate marker in cyst fluid

- Clinical
  - Neoplasm with malignant potential
  - F:M = 20:1
  - Most are low-grade benign
  - Prognosis excellent for non-invasive completely resected tumors
  - Resection recommended despite grade

- Radiology
  - Body and tail (90%)
  - Do not communicate with the pancreatic ductal system
  - Thick walled (Ca++ in 20%)
  - Thin or thick septa

Mucinous Cystic Neoplasm

(pro-malignant neoplasm with malignant potential)

- Not connected to the pancreatic ducts
- Lined by mucinous, generally non-papillary epithelium
- Subepithelial “ovarian-like stroma” required for histological diagnosis
  - Do not see stroma on FNA of cyst contents
Intraductal Papillary Mucinous Neoplasm
(pre-malignant neoplasm with malignant potential)

- Variously papillary mucinous epithelium of variable cell type and heterogenous atypia
- No association with ovarian-like stroma under the epithelium

Epithelial Types of IPMN

- Gastric: low-grade
- Intestinal: intermediate-grade
- Pancreaticobiliary: high-grade

Invasive tubular carcinoma
Invasive colloid carcinoma
Invasive tubular carcinoma
Secondarily Cystic Solid Neoplasms: SPN

- Clinical
  - 89% in young women, mean age ~28 years
  - 1/3 in head, 1/3 in body and 1/3 in tail
- Radiology
  - Shows large solid and cystic neoplasm
- Histology
  - Pseudopapillary architecture from necrosis
  - Myxoid stroma around vessels
  - Bland polygonal cells with round to coffee-bean nuclei, perinuclear vacuoles and cytoplasmic hyaline globules
  - Beta-catenin mutation is almost all

Secondarily Cystic Solid Neoplasms: PanNET

- Clinical
  - ~10% of PanNETs
- Radiology
  - Round well-circumscribed cyst
  - Thick wall
  - Anywhere in the gland
- Histology
  - Typical well-differentiated NET
  - Synaptophysin and chromogranin +
  - Low amylase and CEA in fluid
  - No mutations in typical NGS panel

Two basic questions for Cyst analysis

1. Is the cyst mucinous or non-mucinous?
2. Is the cyst low-grade/low-risk or high-grade-high-risk?
Mucinous

- High-risk
  - Mucinous IPMN with LGD
  - Mucinous IPMN with HGD
  - Cystic PanNET
  - Cystic Acinar Cell carcinoma
  - SPN

- Non-mucinous
  - Non-neoplastic Mucinous cyst
  - GI duplication cyst
  - Pancreatic Cyst with LGD
  - Pancreatic Cyst with IGD
  - IPMN/MCN with HGD

Surgery

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Patient Demographics
- Age, gender, medical history (e.g., symptoms, pancreatitis)

Imaging Features
- Location, size, shape, septations, cyst wall thickness, mural nodule

Gross feature of cyst fluid
- Thick, white, sticky, mucous, thin, brown, yellow

Cytology

Ancillary Testing
- Biochemical
- CEAs, Amylase
- Molecular
- Cytology - Cytospin, Cellblock
- Direct smears - If fluid thick enough

Tissue Triage - Cysts

No-ROSE
- Fresh undiluted cyst fluid
- CEAs, Amylase
- Molecular
- Cytology
  - Cytospin
  - Cellblock
- Direct smears
  - If fluid thick enough
Ancillary Tests for Mucinous Neoplasia

- CEA > 192 ng/ml
  - Mucinous cyst (rare FP = LEC)
- Genetic Testing
  - VHL
  - KRAS mutation
  - GNAS mutation
  - RNF43 mutation
  - P53 mutation
  - P16 deletion
  - SMAD4 deletion
- Early mutations of mucinous neoplasia with no correlation with grade
- Late mutations of mucinous neoplasia indicating high-risk for malignancy

CFA cut-off levels lab and study dependent


- CEA > 800 ng/ml
  - Neoplastic mucinous cysts
- CEA < 5 ng/ml
  - Serous cystadenoma
  - Pseudocyst
- Amylase < 250 U/L
  - Not a pseudocyst
Two basic questions for Cyst analysis

1) Is the cyst mucinous or non-mucinous?
   1) Gross examination
   2) CEA >192 ng/mL
   3) KRAS/GNAS/RNF43
   4) Cytology

2) Is the cyst low-grade or high-grade?

Acellular thick, colloid-like mucin is NOT non-diagnostic!
Pancreatic Pseudocyst cytology

- Cyst debris with blood, proteinaceous material and yellow hematoidin-like pigment (grossly brown and thin fluid)
- Variable inflammation
- NO cyst lining epithelium (beware of contamination, mucin and epithelium)
- CEA low; amylase usually in the 1000's; no mutations

Serous Cystadenoma

- Cuboidal non-mucinous epithelial cells
- Hemosiderin-laden macrophages in a clean or bloody, non-pseudocyst like background
- Grossly bloody or thin and clear
- CEA and amylase low
- NO KRAS/GNAS
- 3p deletions (3p25, VHL)

Cytohistology: CB SCA PAS/D
Two basic questions for Cyst analysis

1) Is the cyst mucinous or non-mucinous?
2) Is the cyst low-grade or high-grade?
   1) Cytology!!
   2) Late molecular mutations

Ideal World- Recognize HGD

Diagnostic Morphology of Carcinoma

Already invasive; prognosis decreases ~50%
Atypical Epithelial Cells

Intraductal Papillary Mucinous Neoplasm of the Pancreas: Cytologic Analysis and Correlation with Histologic Grade

PJ Michaels, EF Brachtel, BC Bounds, WR Brugge, and MB Pitman

(Cancer Cytopathol 2006; 108:174-179.)

Low grade dysplasia  Moderate dysplasia  HGD/Carcinoma

Morphological Overlap with AEC

Histologically Confirmed LGD/IGD
Cytological Criteria of High-Grade Epithelial Atypia in the Cyst Fluid of Pancreatic Intraductal Papillary Mucinous Neoplasms

Martha B. Pitman, MD, Barbara A. Centeno, MD, Ebubekir S. Dagliilar, MD, William R. Brugge, MD, and Mari Mino-Kenudson, MD

Cancer Cytopathology 2014;122(1):40-47.

HGA is most accurately identified in mucinous cyst fluids by:

1. an increased N/C ratio,
2. an abnormal chromatin pattern
3. background necrosis

Grading Epithelial Atypia in EUS-FNA of Intraductal Papillary Mucinous Neoplasms: An international interobserver concordance study

Martha B Pitman MD1, Barbara A Centeno MD2, Muriel Genevay MD3, Ricardo Fonseca, MD4 and Mari Mino-Kenudson MD1.

Cancer Cytopathology 2013;121(12):729-736.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Four Reviewers</th>
<th>Randolph's Kappa</th>
<th>Cohen's Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2, 3-4</td>
<td>54%</td>
<td>0.45</td>
<td>0.74</td>
</tr>
<tr>
<td>0-1, 2-4</td>
<td>52%</td>
<td>0.44</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* Two most experienced reviewers

0= GI contamination; 1=LGD; 2= IGD; 3= HGD and 4= PDAC

Table 3. Kappa Coefficient for Two-Tiered Cytological Grading of Branch-Duct IPMN Cyst Fluids
I. Nondiagnostic

II. Negative: Normal pancreatic tissue, splenule, LEC, pancreaticitis (AIP), indeterminate bile duct lesions

IV. Neoplastic
- Benign: SCA, NET microadenoma
- Other: IPMN, MCN, PanNET, SPN

V. Suspicious: Suggestive but not diagnostic of PDAC, Acinar Cell Ca., PanNEC

VI. Positive/Malignant: PDAC, Acinar Cell Ca., PanNEC

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**Reporting Pancreatic Cytology**

Mucin Production Established
- Thick, colloid-like extracellular mucin
- Cellular or inflammatory debris within the mucin
- Thin mucin covering the slide confirmed with special stains for mucin (mucicarmine or Alcian blue pH2.5)

Elevated CEA (192 ng/ml is ~ 80% accurate)

**Reporting of Mucinous Cysts**

- Neoplastic Epithelial Cells Identified
  - Low grade mucinous epithelium, e.g. low-grade atypia (low-grade to intermediate-grade dysplasia)
  - High-grade epithelium, e.g. high-grade atypia (at least high-grade dysplasia/carcinoma in-situ, but quality and quantity of atypia is insufficient for diagnosis of adenocarcinoma)

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**Table 3. Absolute Risk and Relative Risk of Malignancy of the Diagnostic Categories in The Pancreaticobiliary System for Reporting Pancreaticobiliary Cytology**

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Absolute Risk of Malignancy (%)</th>
<th>Relative Risk</th>
<th>p-value (Relative to Nondiagnostic Category)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Nondiagnostic</td>
<td>1.3</td>
<td>1.3</td>
<td>0.07</td>
</tr>
<tr>
<td>II. Negative</td>
<td>0.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>III. Atypical</td>
<td>28.9</td>
<td>28.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>IV. Neoplastic, Benign</td>
<td>0.0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>IV. Neoplastic, Other, all grades of atypia</td>
<td>26.3</td>
<td>26.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>IV. Neoplastic, Low Grade atypia</td>
<td>4.3</td>
<td>4.3</td>
<td>0.23</td>
</tr>
<tr>
<td>IV. Neoplastic, High Grade atypia</td>
<td>90.9</td>
<td>90.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>V. Suspicious</td>
<td>100.0</td>
<td>100.0</td>
<td>-</td>
</tr>
<tr>
<td>VI. Positive or Malignant</td>
<td>100.0</td>
<td>100.0</td>
<td>-</td>
</tr>
</tbody>
</table>

* Denotes a statistically significant p-value <0.05
Risk of Malignancy in the Categories of The Papanicolaou Society of Cytopathology System for Reporting Pancreaticobiliary Cytology

Raza S. Hoda, M.D., Elizabeth B. Finer, Ronald N. Arpin III, M.S. SCT(ASCP), Matthew Rosenbaum, M.D., Martha B. Pitman, M.D.

accepted to JASC

Table 4: Performance Characteristics of Pancreatic EUS-FNA Biopsy, Stratified by Category for Present Case

<table>
<thead>
<tr>
<th>Diagnostic Categories</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinar, Nontumorous</td>
<td>99.2</td>
<td>61.7</td>
<td>98.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Acinar, Nontumorous</td>
<td>95.7</td>
<td>80.0</td>
<td>86.2</td>
<td>96.0</td>
</tr>
<tr>
<td>Acinar, Nontumorous</td>
<td>92.2</td>
<td>98.8</td>
<td>95.5</td>
<td>94.5</td>
</tr>
<tr>
<td>Acinar, Nontumorous</td>
<td>78.1</td>
<td>108</td>
<td>87.6</td>
<td></td>
</tr>
<tr>
<td>Acinar, Nontumorous</td>
<td>65.2</td>
<td>108</td>
<td>87.7</td>
<td></td>
</tr>
</tbody>
</table>

Example Case

- 60 year old female with no history of pancreatitis, no weight loss and no jaundice is found to have a 3 cm cyst in the pancreatic body by CT scan performed due to abdominal pain. Slight stranding of the pancreas adjacent to the cyst is noted but otherwise the pancreas and ductal system is normal. No connection to the pancreatic duct is noted. Serum lipase is normal.
Cytology- ThinPrep

- CEA 13,350 ng/ml
- Amylase 46 U/L

Final Diagnosis

SPECIMEN ADEQUACY
Satisfactory for evaluation

INTERPRETATION
NEOPLASTIC: OTHER

DIAGNOSIS:
Mucinous cyst with high-grade epithelial atypia (see note).

Note: A mucinous cyst is established from the elevated CEA (by report 13,350 ng/ml), viscous appearance on gross inspection of the cyst fluid and the presence of mucinous epithelium on cytology. The presence of mucinous epithelial cells in hyperchromatic crowded groups and singly with markedly elevated mucin or extracellular mucin, acinar cell changes and irregular nuclear membranes supports the diagnosis of high-grade epithelial atypia, which is consistent with malignant mucinous epithelial cells. The diagnosis of cancer is based on the presence of abnormal nuclear and cytoplasmic changes, as well as the presence of high-grade epithelial atypia in malignant mucinous epithelial cells. Pancreatic carcinoma is excluded based on the absence of pancreatic, low nuclear grade H-H, and the presence of benign mucinous epithelium. The differential diagnosis includes a mucinous cystic neoplasm (MCN) and branch duct intraductal papillary mucinous neoplasm (IPMN). The presence of a connection to the pancreatic duct, papillary and glandular cell formation and the absence of fibrovascular stroma is inconclusive for IPMN. Surgical resection is advised.