Advances in Pancreatic Cytology
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Disclosure of Relevant Financial Relationships
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Advances in Pancreatic Disease that impact Cytology
• Neoadjuvant therapy for pancreatic cancer
  • Definitive diagnosis required
  • FNA specimen = only tumor available for that patient after treatment in some cases
• Conservative management for most BD-IPMN
  • New PB terminology and integrated cytology reports support this effort
  • Moray™ Micro-Forceps Biopsy

Neoadjuvant Therapy for borderline resectable PDAC
• FOLFIRINOX (5-fluorouracil/leucovorin/irinotecan/oxaliplatin) chemotherapy
• +/- Gemcitabine or other chemotherapy
• +/-radiation therapy

• Potential to change 10-20% of borderline resectable disease to resectable
Post-neoadjuvant therapy with complete response.

EUS-guided FNA

- Technique of choice
- Controversial
- Requires significant experience for quality aspiration and interpretation

Quality FNA

- Quality specimen
- High cellularity
- Cells representative of the lesion
- Quality preparations
- Quality interpretation

Second Opinion:
Expert Consultation

- Recommended for indeterminate diagnoses that would lead to repeat biopsy
- Issues noted in my practice:
  - FNA is diagnostic but pathologist lacks experience and confidence to make a definitively malignant diagnosis
    - Mostly well-differentiated PDAC
  - CB is diagnostic but only on levels and/or with support from IHC
Optimal Preparation of EUS-FNAB of Solid Masses

- Direct Smears (ROSE)
  - Alcohol fixed
  - Air dried
- Cell Block Preparations
  - Rinsings and dedicated pass into RPMI or formalin
  - Enrich material with large bore needle (19g) or pro-core
  - If cellularity appears too scant for cell block, process fluid as cytospin, ThinPrep or SurePath
- Dedicated pass for flow cytometry if lymphoma is suspected or lymphoid dominant lesion noted on rapid interpretation

EUS-FNAB

Newer Needles

Acquire- Boston Scientific

Cytohistology

- Autoimmune pancreatitis
- Poorly-differentiated PDAC

Differential Diagnosis of Solid Pancreatic Masses

- Solid
  - Chronic pancreatitis
  - Ductal adenocarcinoma
  - Metastasis
  - Pancreatic neuroendocrine tumor
  - Acinar cell carcinoma
  - Pancreatoblastoma
  - Solid-pseudopapillary neoplasm
High Grade Adenocarcinoma

- Marked nuclear
  - atypia
  - hyperchromasia
  - pleomorphism
  - overlapping
- Prominent nucleoli
- Single atypical cells
- Mitoses
- Coagulative Necrosis

Criteria for Well-differentiated Adenocarcinoma

- Irregular cellular distribution in a sheet (drunken honeycomb)
- Anisonucleosis 4:1 in a group
- Parachromatin clearing
- Irregular nuclear membranes, often subtle
- Abundant cytoplasm, often visibly mucinous
Well-differentiated Adenocarcinoma

Core Biopsy

Shark Core: Well-differentiated Adenocarcinoma

Well-differentiated Adenocarcinoma: Cellblock

Well-differentiated Adenocarcinoma: p53
Well-differentiated Adenocarcinoma: ki-67

Well-differentiated Adenocarcinoma: SMAD4 nuclear loss

**Advances in Pancreatic Disease that impact Cytology**

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**PRESENTATION TITLE**

**Intraductal Papillary Mucinous Neoplasm**

- Intra-ductal
- Branch duct IPMN
- Combined disease

**IPMN**

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

**Pancreatic Cysts**

- Differential Diagnosis
  - Pseudocyst
  - Lymphoepithelial cyst
  - Serous cyst
  - Mucinous cyst
    - (MCN and IPMN)
  - Cystic degeneration of typically solid tumors
    - PanNET
    - SPN
    - other
  - Other more rare cysts
### Surgical procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Frequency (%)</th>
<th>Complications (%)</th>
<th>Pancreatic fistula</th>
<th>Delayed gastric emptying</th>
<th>Other major complications</th>
<th>Median length of stay, days</th>
<th>Operative mortality, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whipple</td>
<td>368, (43.2%)</td>
<td>40%</td>
<td>12.5%</td>
<td>6.5%</td>
<td>12.5%</td>
<td>5 days</td>
<td>2</td>
</tr>
<tr>
<td>Middle pancreatectomy</td>
<td>63, (7.4%)</td>
<td>49.2%</td>
<td>35.5%</td>
<td>0%</td>
<td>12.7%</td>
<td>6 days</td>
<td>0</td>
</tr>
<tr>
<td>Distal pancreatectomy</td>
<td>373, (43.8%)</td>
<td>36.4%</td>
<td>18.2%</td>
<td>0.3%</td>
<td>12.6%</td>
<td>6 days</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>47, (5.5%)</td>
<td>32.4%</td>
<td>8.8%</td>
<td>0%</td>
<td>11.8%</td>
<td>8 days</td>
<td>1</td>
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</table>

### Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>MCN</th>
<th>MD</th>
<th>BD</th>
<th>SCA</th>
<th>CNET</th>
<th>SPN</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>199</td>
<td>180</td>
<td>146</td>
<td>137</td>
<td>62</td>
<td>29</td>
</tr>
<tr>
<td>Malignant (%)</td>
<td>10.3%</td>
<td>33.7%</td>
<td>13.7%</td>
<td>0.0%</td>
<td>10.7%</td>
<td>0.0%</td>
</tr>
<tr>
<td>3-year survival (%)</td>
<td>94.0%</td>
<td>83.0%</td>
<td>88.0%</td>
<td>90.0%</td>
<td>98.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>5-year survival (%)</td>
<td>90.0%</td>
<td>78.0%</td>
<td>80.0%</td>
<td>90.0%</td>
<td>98.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

### The International Consensus Guidelines 2012 for the Management of IPMN and MCN of the Pancreas


- **High Risk Stigmata** — Surgery if clinically feasible
  - Obstructive jaundice in a patient with a cyst in the pancreatic head
  - Enhancing solid component of the cyst
  - Main pancreatic duct dilatation ≥10mm

- **Worrisome Features** — EUS-FNA
  - Cyst ≥3cm
  - Thickened/enhancing cyst wall
  - Main duct 5-9mm
  - Non-enhancing mural nodule
  - Abrupt change in MPD size with distal pancreatic atrophy

- EUS-FNA — Susp/Pos cytology — Surgery

### Nonspecific EUS Imaging

- **Broad differential diagnosis:**
  - Mucinous
    - BD-IPMN
  - MCN
  - Nonmucinous
    - Macrocystic SCA
    - Lymphangioma
    - Benign
    - Malignant (≥HGD)

### Challenges in Cyst Characterization by CT: Morphologic Overlap

Cohen-Salzi F et al. Radiology 2003
Khurana R et al. AJR 2003
Kim S et al. AJR 2006

### Small Cysts with “Benign” Imaging are not all low-grade

Two basic questions for Cyst analysis

1) Is the cyst mucinous or non-mucinous?
2) Is the cyst low-grade or high-grade?

Value of EUS-FNA

- Obtain fluid and tissue
  - Biochemical testing (CEA, amylase)
    - Mucinous vs. non-mucinous
  - Cytology
    - Mucinous vs. non-mucinous
    - Grade: low-grade/risk vs. High-grade/risk
  - Molecular analysis
    - Gene mutations
      - neoplasia
      - late mutations associated with high-risk

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)
    - Mass characteristics
      - Solid or cystic
      - 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, molecular analysis

Cytological Preparations

No-ROSE

- Cysts
  - Direct smears
    - If fluid thick enough
  - Fresh undiluted cyst fluid
    - CEA; Amylase
    - Molecular
    - Cytology
      - Cytospin
      - Cellblock

Pancreatic Cyst Fluid Triage
Two basic questions for Cyst analysis

1) Is the cyst mucinous or non-mucinous?
   1) Gross examination
   2) CEA (best test)
   3) Cytology

2) Is the cyst low-grade or high-grade?
   1) Cytology!!

Gross Cyst Fluid

Mucinous cyst fluid
Pseudocyst fluid

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

Mucin with LBC processing

CEA cut-off levels: lab and study dependent

Molecular Tests

- KRAS
  - Mutation(s) support a neoplastic mucinous cyst
  - Does not distinguish IPMN and MCN
  - Does not correlate with grade
- GNAS
  - Mutation supports IPMN over MCN
  - Does not correlate with grade
- RNF43
  - Mutation supports a mucinous cyst
  - Does not distinguish IPMN and MCN
- 3p deletions
  - 3p21, VHL gene, supports SCA
  - Other 3p deletions also noted in SCA
- CTNNB1 (beta-catenin) deletion
  - Mutation(s) support SPN
- TP53, CDKN2A loss SMAD4 loss support a HR cyst
Impact of Next-Generation Sequencing on the Clinical Impression of Pancreatic Cysts

- NGS supported the imaging impression in 78% but changed it in 12%
- NGS defined a cyst as mucinous in 48% of cysts with a non-elevated CEA
- KRAS and/or GNAS mutations supported a diagnosis of IPMN in 71% of cases without an elevated CEA
- KRAS mutation reclassified 19% of cysts non-neoplastic by imaging and with low CEA

Two basic questions for Cyst analysis

1) Is the cyst mucinous or non-mucinous?
   1) Gross examination
   2) CEA (best test)
   3) Cytology

2) Is the cyst low-grade or high-grade?
   1) Cytology!!

Ideal World- Recognize HGD

Diagnostic Morphology of Carcinoma

Epithelial Cells with HGA

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. Daglilar, 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

Benign/Low Grade Glandular Epithelium

Cytohistology: CB

Moray™ Micro-forceps biopsy

Ancillary Tests: IPMN/MCN
- IHC insufficiently specific to be diagnostic of grade in premalignant cysts
- SMAD4 loss does tend to support HGA - loss of nuclear staining
I. Nondiagnostic
II. Negative: Normal pancreatic tissue, splenule, LEC, pancreatitis (AIP)
III. Atypical: Suggestive but not diagnostic of NET or SPN; 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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