EUS-FNA of Solid and Cystic Lesions: Part 1: Solid Masses

Martha Bishop Pitman, M.D.
Director, Cytopathology
Massachusetts General Hospital
Professor of Pathology
Harvard Medical School
Boston, MA

EUS-guided FNAB

- Linear e-scope
- Color Doppler
- Aspiration needle
- Transgastric or transduodenal aspiration
- Cytology specimen

Transgastric: body and tail
Transduodenal: head

Differential Diagnosis

- Solid
  - Chronic pancreatitis
  - Ductal adenocarcinoma
  - Acinar cell carcinoma
  - Pancreatic endocrine neoplasm
  - Solid pseudopapillary tumor
  - Pancreatoblastoma
  - Metastasis
- Cystic
  - Pseudocyst
  - Serous cyst
  - Mucinous cyst (MCN and IPMN)
  - Cystic degeneration of typically solid tumors
    - PEN
    - SPN
    - Other
  - Other more rare cysts
    - Simple cyst
    - Lymphoepithelial cyst
    - Peripancreatic cyst
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

Quality FNA

Quality specimen
- High cellularity
- Cells representative of the lesion

Quality preparations

Quality interpretation
- Training of interpreter
- Experience of interpreter
- Team approach to diagnosis

EUS-FNAB

- Smaller transducers
- Sharper needles
- Disposable needles
- Needle ridges
- Sharp stylet
- Beveled needle
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

Newer Needles

Acquire- Boston Scientific
Cytohistology

Autoimmune pancreatitis  Poorly-differentiated PDAC

Normal Pancreas  (bivalved pancreatic head)

Courtesy of 4th Series AFIP Fascicle on Tumors of the Pancreas

Normal Pancreas  

Acini and intercalated cells
Normal Pancreas

Ducts and Islets

Normal Pancreas

Acinar Cells
Normal Pancreas

Ductal Cells
Differential Diagnosis of Solid Pancreatic Masses

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

Images: AFIP Pancreas fascicle 2007

Differential Diagnostic Approach to Evaluating the Slide

- Solid
  - Glandular smear pattern versus solid cellular smear pattern
    - Glandular smear pattern
      - Malignant or not
    - Solid cellular smear pattern
      - Endocrine or not
- Cyst
  - Extracellular mucin
    - Yes: thick or thin
    - No: CEA elevated? KRAS positive?
  - Epithelial cells
    - Yes: mucinous?
      - Yes: low grade or atypical or malignant
  - Multimodal parameters: gender, symptoms, location, imaging
Clinical and Radiological Features of PDAC

- 60-80 y.o. M>F
- Radiating epigastric pain with wt. loss
- Jaundice
- Migratory thrombophlebitis
- Sudden onset DM
- Double duct sign on CT
- Hypodense mass in pan head with irregular borders; atrophy elsewhere

- Cigarette smoking
- Long term DM
- Family history
  - 3° degree relatives: 32x
  - 2° degree relatives: 6x
  - 1° degree relatives: 2.3x
- Germline mutations
  - PJS ([STK11/LKB1]): 132x
  - FAMMM ([p16/CDKN2A]:
  - FANC
  - BRCA2
  - Familial CP ([PRSS1/SPINK1])
High Grade Adenocarcinoma

- Marked nuclear atypia
- Hyperchromasia
- Pleomorphism
- Overlapping
- Prominent nucleoli
- Single atypical cells
- Mitoses
- Coagulative Necrosis
Variants of PDAC:
Adenosquamous Carcinoma

Variants of PDAC:
Undifferentiated Carcinoma with Osteoclast-type Giant Cells

Variants of PDAC
- Colloid Carcinoma
- Undifferentiated Carcinoma
- Signet Ring Cell Carcinoma
- Foamy Gland Carcinoma
Limitations of Cytology compared to Histology

- Lack of architecture
  - Cannot see distribution of ductal structures
  - Cannot see abnormal localization
  - Cannot see if ducts are adjacent to medium sized vessels, wrapping around nerves or isolated in fat
  - Cannot see contours or angulation of ducts
  - Cannot see luminal contents
  - Cannot see stromal reaction

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
Drunken Honeycomb
Anisonucleosis
Parachromatin clearing

Parachromatin clearing
Cytoplasmic mucin
Irregular nuclear membranes
Visible cytoplasmic mucin
Drunken honeycomb
Exaggerated vacuolated cytoplasm

Well-differentiated Adenocarcinoma
Cell block preparation of needle rinsings

Chronic Pancreatitis
- mostly ductal cells
- scantily cellular
- some islet cells
- monolayered sheets
- cohesive, few single cells
- maintained polarity
- minimal nuclear overlap
- mild anisonucleosis
- smooth nuclear membranes
- rare/normal mitoses
- no coagulative necrosis
Chronic Pancreatitis

Key Cytologic Features:
- Fragments of acinar tissue with acini splayed apart by fibrosis
- Stromal fragments
- Inflammatory cells (lymphocytes, plasma cells, macrophages and siderophages)
- Background debris and calcification
- Ductal epithelium with only mild cytologic atypia
- No definite features of neoplasia

Autoimmune Pancreatitis

Autoimmune Pancreatitis: Mass Forming Lesion
Type 1 vs Type 2 AIP

<table>
<thead>
<tr>
<th></th>
<th>Type 1 AIP</th>
<th>Type 2 AIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Elderly, 7th decade</td>
<td>Middle age, 5th decade</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>M/F – 1:1</td>
</tr>
<tr>
<td>Presentation</td>
<td>Jaundice (75%)</td>
<td>Pain</td>
</tr>
<tr>
<td></td>
<td>Acute Pancreatitis (15%)</td>
<td></td>
</tr>
<tr>
<td>Systemic Disease</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Elevated serum IgG4</td>
<td>80%</td>
<td>Uncommon</td>
</tr>
<tr>
<td>IBD</td>
<td>No association</td>
<td>Present in 16-30% of cases</td>
</tr>
<tr>
<td>Histology</td>
<td>Periductal inflammation and</td>
<td>Periductal inflammation and</td>
</tr>
<tr>
<td></td>
<td>one of the following:</td>
<td>one of the following:</td>
</tr>
<tr>
<td></td>
<td>• Storiform fibrosis</td>
<td>• Ductal/lobular abscess</td>
</tr>
<tr>
<td></td>
<td>• Obliterative phlebitis</td>
<td>• Ductal ulceration with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>neutrophils</td>
</tr>
<tr>
<td>Long-term outcomes</td>
<td>Frequent relapses</td>
<td>No relapses</td>
</tr>
</tbody>
</table>

Autoimmune Pancreatitis


AIP: Core Biopsy

Type 1                      Type 2
IgG4 Staining

- IgG4 positive plasma cells relative to generic IgG positive plasma cells
- No defined criteria
- Heterogeneity of plasma cell distribution in the tissue
- Cell block and core biopsy samples are small leading to "sampling error"

Epithelial Atypia in AIP

Ancillary Tests:
Benign vs Malignant Ductal Epithelium

<table>
<thead>
<tr>
<th></th>
<th>Benign Glands (chronic pancreatitis, benign bile duct lesion)</th>
<th>Ductal Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA *</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>B72.3</td>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td>CA 125</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>bClust</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>Bcl (Bcl2)**</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>Meso**</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

*Abnormal expression is cytoplasmic reactivity
**Abnormal expression is in >20% of nuclei
***Abnormal expression is loss of cytoplasmic and nuclear activity
The Immunohistochemical Expression Pattern of SMAD4, p53, and CDX2 is Helpful in Diagnosing Pancreatic Ductal Adenocarcinoma in Endoscopic Ultrasound-Guided Fine Needle Aspirations

Jian Shen M.D., Ph.D., Edmund S. Cibas M.D., and Xiaohua Qian M.D., Ph.D.
Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts
Modern Path 2007; 20(Suppl 2): 82A

SMAD4 Loss

Present in ~50% of PDAC; supports a malignant interpretation

Metastases

- Rare compared to primary malignancy
- Any tumor can metastasize to the pancreas
- Renal cell carcinoma common metastasis that mimics primary PDAC
  - Solitary mass
  - Decades after nephrectomy (avg. 10 yrs)

CDX2

SMAD4

p53

Metastases

- Rare compared to primary malignancy
- Any tumor can metastasize to the pancreas
- Renal cell carcinoma common metastasis that mimics primary PDAC
  - Solitary mass
  - Decades after nephrectomy (avg. 10 yrs)
Differential Diagnosis
Foamy Gland Adenocarcinoma

- CK19+  CA19-9+
- SMAD4 -  EMA+
- PAX8+  Anti-RCC-
Differential Diagnosis
Lipid Rich Neuroendocrine Tumor

PanNET  Synaptoph+  Chromo+  CK7+
RCC    EMA+  PAX8+  Anti-RCC+/-

Solid Cellular Neoplasms

PanNET  ACC  PanNET  SPN
PanNET  ACC  PanNET  SPN
Pancreatic Neuroendocrine Tumor
[PanNet, aka PEN, PET]

- **Clinical**
  - Any age; 40-50 y.o.
  - M=F
  - MEN, VHL syndromes
  - Hormone effects in functional
    PanNet: insulin, glucagon for
    example

- **Radiological**
  - Pancreatic tail>>head/body
  - Round, well-circumscribed
  - Sometimes cystic, CA++
  - Octreotide scan+

- **Histology**
  - Cellular monomorphic
    population of polygonal cells with
    various organoid patterns with
    scan stroma (occasionally
    hylanized or amyloid stroma)

---

**Classic Morphology: PanNET**

- Single cells mostly
- Plasmacytoid
- Coarse, stippled chromatin
- +/- nucleoli

---

**Variant Morphology: PanNET**

- Lipid laden
- Prominent nucleoli
- Osteocytic

---
IHC: PanNET

Grading of NETs

- Best performed on resected specimen
  - Requires evaluation of “hottest” area
- If resectable, grading on FNA material not necessary, wastes resources
- If unresectable, grading helps to separate G1/G2 from G3 and direct treatment
  - Usually apparent on HE

Grading GEP NETs

( WHO, ENETS )

<table>
<thead>
<tr>
<th>GRADE</th>
<th>MITOSES</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 2</td>
<td>&lt; 3%</td>
</tr>
<tr>
<td>2</td>
<td>2-20</td>
<td>3-20%</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 20</td>
<td>&gt; 20%</td>
</tr>
</tbody>
</table>

Poorly-Differentiated Neuroendocrine Carcinoma

Small cell type

Large cell type

Secondarily Cystic Solid Neoplasms:
Cystic PanNET
Acinar Cell Carcinoma

- **Clinical**
  - Adults vs. children
  - Mean age ~ 59 years
  - Males > females ~ 4:1
  - Lipase hypersecretion syndrome (polyarthralgia and multifocal subcutaneous fat necrosis)
- **Radiological**
  - Solid, circumscribed
  - Large and bulky
  - Rarely cystic

---

**Image: AFIP Pancreas fascicle 2007**

---

**Classic Morphology: Acinar**

- Cohesive groups and single cells
- Many stripped naked nuclei
- Granular cytoplasm (and background)
- +/- nucleoli

---

**Benign versus Malignant Acinar Cell Population**

---
IHC: ACCa

Classic Morphology: PBL

- Clinical
  - Most common malignant pancreatic neoplasm of children
  - 2/3’s occur in children and 1/3 in adults
  - Half in Asians
  - Often identical to acinar cell carcinoma on FNA
  - Diagnosis depends on identifying squamoid corpuscle on smears or cell block

Solid-Pseudopapillary Neoplasm

- Clinical
  - Rare but may represent up to 6% of all pancreatic neoplasms and 24% of resected cysts
  - 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
Classic Morphology: SPN

- Papillary branching
- Malignant stroma
- Clinging cells and single cells
- Euchromatin
- Oval, indented, grooved nuclei
- Perinuclear vacuoles/globules

IHC: SPN

- Beta-catenin
- CD10
- CD56

Spenule/accessory spleen

- Lymphoid tissue
- Histiocytes
- Blood vessels
- CD8+ cells indicating splenic endothelial cells
Standardized Terminology and Nomenclature for Pancreaticobiliary Cytopathology from the Papanicolaou Society of Cytopathology

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