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

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EUS-guided FNAB

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

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 cysts
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

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**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

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**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**
Next-Generation Needles

- Need for FFPE tissue
  - Solid-cellular neoplasms
  - Mesenchymal tumors
  - Confirmation of metastatic tumors
  - Search for actionable genetic mutations in PDAC
  - Research on tumor stroma pushes need for stroma from biopsy
  - Neoadjuvant therapy means FNA may be the only tumor tissue ever
Impact on Cytology Specimens

- Decrease
  - # passes
  - Complications
  - ND sample
  - ROSE
  - FNA cytology
  - Cytology specimens
  - Cytology lab RVUs & FTEs
  - Cost overall in the healthcare system

- Increase
  - Tissue for diagnosis
  - Tissue for ancillary tests
  - Preservation of tumor for future clinical trials
  - Documentation of tumor post neoadjuvant therapy
  - Cost per procedure/patient
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

Ductal Cells
Duodenal Contamination
Gastric Contamination

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 Diagnosis of Solid Pancreatic Masses

- **Solid**
  - Chronic pancreatitis
  - Ductal adenocarcinoma
  - Metastasis
  - Pancreatic neuroendocrine tumor
  - Acinar cell carcinoma
  - 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 panc head with irregular borders; atrophy elsewhere

- Cigarette smoking
- Long term DM
- Family history
  - 3 1st degree relatives: 32x
  - 2 1st degree relatives: 6x
  - 1 1st 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
- Colloid Carcinoma
- Signet Ring Cell Carcinoma
- Foamy Gland Carcinoma

Well-Differentiated PDAC

Image courtesy of Dr. Volkan Adsay
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
- Drunken Honeycomb
- Anisonucleosis
- Cytoplasmic mucin
- Parachromatin clearing

- Parachromatin clearing
- Nuclear crowding
- 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

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

Mass Forming Lesion

Type 1 vs Type 2 AIP

<table>
<thead>
<tr>
<th>Type 1 AIP</th>
<th>Type 2 AIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Elderly, 7th decade</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Presentation</td>
<td>Jaundice (75%)</td>
</tr>
<tr>
<td>Systemic Disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Elevated serum IgG4</td>
<td>80%</td>
</tr>
<tr>
<td>IBD</td>
<td>No association</td>
</tr>
<tr>
<td>Histology</td>
<td>Periductal inflammation and one of the following:</td>
</tr>
<tr>
<td></td>
<td>Storiform fibrosis</td>
</tr>
<tr>
<td></td>
<td>Obliterative phlebitis</td>
</tr>
<tr>
<td>Long-term outcomes</td>
<td>Frequent relapses</td>
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</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
- Cellblock and core biopsy samples are small leading to “sampling error”
Epithelial Atypia in AIP

Ancillary Tests: Benign vs Malignant Ductal Epithelium

(AIP Pancreas Fascicle 2007, Chapter 17, Table 17-4)

<table>
<thead>
<tr>
<th>Immunohistochemical Staining in the Diagnosis of Ductal Adenocarcinoma</th>
<th>Benign Glands (chronic pancreatitis, benign bile duct lesions)</th>
<th>Ductal Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA (monoclonal)*</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>RFX</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>CA 125</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>B72.3</td>
<td>0</td>
<td>92</td>
</tr>
<tr>
<td>CA 125</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Smad4 (dpc4)**</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

*abnormal expression is cytoplasmic reactivity
**abnormal expression is reactivity 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): K2A
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)
Differential Diagnosis
Foamy Gland Adenocarcinoma

FGA
CK19+
CA19-9+
SMA-4
RCC
EMA+
PAX8+
Anti-RCC+/-

Differential Diagnosis
Lipid Rich Neuroendocrine Tumor

PanNET
Synapto+
Chromo +
CK7+
RCC
EMA+
PAX8+
Anti-RCC+/-
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
- Oncocytic

IHC: PanNET
- Chromogranin+
- Synaptophysin+
- Trypsin-
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>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 2</td>
<td>AND &lt;3%</td>
</tr>
<tr>
<td>2</td>
<td>2-20</td>
<td>OR 5-20%</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 20</td>
<td>OR &gt;20%</td>
</tr>
</tbody>
</table>


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Conclusions:
Loss of DAXX and ATRX is associated with CIN in pNETs and shorter survival times of patients. These results support the hypothesis that DAXX- and ATRX-negative tumors are a more aggressive subtype of pNET, and could lead to identification of strategies to target CIN in pancreatic tumors.

CIN= chromosomal instability
Poorly-Differentiated Neuroendocrine Carcinoma

Small cell type

Large cell type

Demonstrate p53 and pRB1 mutations

Secondarily Cystic Solid Neoplasms:
Cystic PanNET

Acinar Cell Carcinoma

Clinical
- Adults>>> children
- Mean age ~ 59 years
- Males>females ~ 4:1
- Lipase hypersecretion syndrome= polyarthralgia + multi-focal 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

Benign Malignant

IHC: ACCa

Trypsin

Endocrine markers
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
- Myxoid stroma
- Clinging cells and single cells
- Euchromatin
- Oval, indented, grooved nuclei
- Perinuclear vacuoles/globules
IHC: SPN

Splenule/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

Mahta Agarwal, BSc, PhD, FRCPath, Virginia Murray, Cytotech, Shpane Cakici, Cytotech, and Asela Nasser, MB, BS

ABSTRACT
The Papanicolaou Society of Cytopathology has recently proposed a standardized terminology and nomenclature guidelines for pancreatic cytology. However, the risk of malignancy associated with the new guidelines has been scarcely studied. In this study, a series of pancreatic cytology cases obtained by endoscopic ultrasound-guided fine-needle aspiration from 294 Chinese patients were retrospectively re-categorized according to the new terminology. The re-categorization was done by a cytopathologist with significant experience in pancreatic cytology. The correlation of the old and new terminology was analyzed by comparing the two systems. The results showed that the new terminology system significantly improved the accuracy of diagnosis for pancreatic tumors. The new terminology system was found to be more consistent and easier to use compared to the old system. Overall, the new terminology system was found to be more effective in improving the accuracy of diagnosis for pancreatic tumors.