Advances in Pancreatic Cytology

Martha B. Pitman, MD
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Disclosure of Relevant Financial Relationships

Medtronic, Inc. Consultant
Boston Scientific, Consultant
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
BORDERLINE RESECTABLE<sup>d,e</sup> NO METASTASES, PLANNED NEOADJUVANT THERAPY

WORKUP

- Biopsy, EUS-FNA preferred<sup>1</sup>
- Consider staging laparoscopy<sup>2</sup>
- Placement of self-expanding metal stent (preferably a short metal stent) if biliary ductal obstruction is present

Planned neoadjuvant therapy<sup>1</sup>

NEOADJUVANT THERAPY

- Neoadjuvant therapy<sup>3</sup>

Biopsy, EUS-FNA preferred<sup>1</sup>

Cancer not confirmed

Repeat biopsy

Cancer not confirmed (exclude autoimmune pancreatitis [AIP])

Surgical resection<sup>f</sup>

Consider staging laparoscopy if not previously performed

Abdominal (pancreas protocol), pelvic, and chest imaging

Biopsy positive

No jaundice

Disease progression precluding surgery<sup>4</sup>

See Locally Advanced Unresectable (PANC-7) or Metastatic Disease (PANC-9)

Unresectable at surgery<sup>k</sup>

Jaundice

Self-expanding metal stent or biliary bypass ± gastrojejunostomy (category 2B for prophylactic gastrojejunostomy) ± celiac plexus neurolysis (category 2B)

Surgical resection<sup>f</sup>

No jaundice

See Locally Advanced Unresectable (PANC-7) or Metastatic Disease (PANC-9)

See Planned Resection (PANC-5)

There is limited evidence to recommend specific neoadjuvant regimens off-study, and practices vary with regard to the use of chemotherapy and chemoradiation. Acceptable regimens include FOLFIRINOX or gemcitabine + albumin-bound paclitaxel. Subsequent chemoradiation is sometimes included. Most NCCN Member Institutions prefer neoadjuvant therapy at a high-volume center. Performing surgery with a high likelihood of a positive margin is not recommended.

<sup>d</sup>See Principles of Diagnosis, Imaging, and Staging (PANC-A).

<sup>e</sup>See Criteria Defining Resectability Status (PANC-B).

<sup>f</sup>See Principles of Surgical Technique (PANC-C) and Pathologic Analysis: Specimen Orientation, Histologic Sections, and Reporting (PANC-D).

<sup>k</sup>See Principles of Diagnosis, Imaging and Staging #8 (PANC-A).

<sup>1</sup>There is limited evidence to recommend specific neoadjuvant regimens off-study, and practices vary with regard to the use of chemotherapy and chemoradiation. Acceptable regimens include FOLFIRINOX or gemcitabine + albumin-bound paclitaxel. Subsequent chemoradiation is sometimes included. Most NCCN Member Institutions prefer neoadjuvant therapy at a high-volume center. Performing surgery with a high likelihood of a positive margin is not recommended.
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
- Training of interpreter
- Experience of interpreter
- Team approach to diagnosis
Variation of cytopathologists' use of the indeterminate diagnostic categories “atypical” and “suspicious for malignancy” in the cytologic diagnosis of solid pancreatic lesions on endoscopic ultrasound-guided fine-needle aspirates

Renu K. Virk M.D., Roberto Gamez M.D., M.P.H., Swati Mehrotra M.D., Mohammed Atieh D.O., Güliz A. Barkan M.D., Eva M. Wojcik M.D., Stefan E. Pambuccian M.D.

Results

The six cytopathologists diagnosed 10% of cases as indeterminate; 82 (7.4%) as “atypical” and 29 (2.6%) as “suspicious”. The individual cytopathologists’ indeterminate diagnosis rates varied twofold (6.67-12.80%) and did not correlate with their experience, total or annual volume of EUS-FNAs. Of the 56/99 (56.57%) cases with follow-up, the underlying rate of malignancy was 47% (33/71; for “atypical” and 87.5% (21/24); for “suspicious”). The underlying rates of malignancy were 33-67% for “atypical” and 80-100% for “suspicious” diagnoses made by individual cytopathologists. The rate of indeterminate diagnoses decreased from 11.55 to 7.88% after the implementation of departmental consensus review.
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

- Smaller transducers
- Sharper needles
- Disposable needles

- Needle ridges
- Sharp stylet
- Beveled needle
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

Images: AFIP Pancreas fascicle 2007
High Grade Adenocarcinoma

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

Image courtesy of Dr. Volkan Adsay
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
Well-differentiated Adenocarcinoma
Well-differentiated Adenocarcinoma
Well-differentiated Adenocarcinoma
ProCore: Well-differentiated Adenocarcinoma
Core Biopsy

Shark Core: Well-differentiated Adenocarcinoma
Well-differentiated Adenocarcinoma: Cellbock
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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Intraductal Papillary Mucinous Neoplasm

Intra-ductal

Branch duct IPMN

Combined disease
• 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></th>
<th>Whipple</th>
<th>Middle pancreatectomy</th>
<th>Distal pancreatectomy</th>
<th>Other</th>
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<tbody>
<tr>
<td>Frequency, (%)</td>
<td>368, (43.2%)</td>
<td>63, (7.4%)</td>
<td>373, (43.8%)</td>
<td>47, (5.5%)</td>
</tr>
<tr>
<td>Complications (%)</td>
<td>40%</td>
<td>49.2%</td>
<td>36.4%</td>
<td>32.4%</td>
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<tr>
<td>Pancreatic fistula</td>
<td>12.5%</td>
<td>35.5%</td>
<td>18.2%</td>
<td>8.8%</td>
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<tr>
<td>Delayed gastric emptying</td>
<td>6.5%</td>
<td>0%</td>
<td>0.3%</td>
<td>0%</td>
</tr>
<tr>
<td>Other major complication</td>
<td>12.9%</td>
<td>12.7%</td>
<td>12.6%</td>
<td>11.8%</td>
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<tr>
<td>Median length of stay, days</td>
<td>8 days</td>
<td>6 days</td>
<td>6 days</td>
<td>8 days</td>
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<tr>
<td>Operative mortality, n</td>
<td>2</td>
<td>0</td>
<td>1</td>
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## Outcomes

<table>
<thead>
<tr>
<th></th>
<th>MCN</th>
<th>MD IPMN</th>
<th>BD IPMN</th>
<th>SCA</th>
<th>CNET</th>
<th>SPN</th>
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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>
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<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>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3-year survival (%)</td>
<td>94.0%</td>
<td>83.0%</td>
<td>88.0%</td>
<td>97.0%</td>
<td>98.0%</td>
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<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>
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</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 walls
  – Main duct 5-9mm
  – Non-enhancing mural nodule
  – Abrupt change in MPD size with distal pancreatic atrophy

• EUS-FNA ➔ Susp/Pos cytology ➔ Surgery
Challenges in Cyst Characterization by CT: Morphologic Overlap

MCN  Pseudocyst  IPMN

Cohen-Scali F et al. Radiology 2003
Khurana B et al. AJR 2003
Kim S et al. AJR 2006
Nonspecific EUS Imaging

- Broad differential diagnosis:
  - Mucinous
    - BD-IPMN
    - MCN
  - Nonmucinous
    - Macrocystic SCA
    - Lymphangioma
  - Benign
  - Malignant (≥HGD)
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
No-ROSE

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

Molecular analysis

≥0.3 cc PCF after vortex

Residual

Pancreatic Cyst Fluid (PCF)

Centrifuge

Amnioned casing

Rapidly rotating rotor

Cell button: Cytospin

Cytology

CEA

≥0.3 cc PCF

Amylase

Supernatant
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**
  - 3p25, 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

Martin Jones, MBBS1*, Zongli Zheng, MD, PhD1*, Jessica Wang, MD1, Emily Albanese1, Abdurrahman Kadayifci, MD2, Dora Dias-Santagata, PhD2, Long Le, MD2, William R. Brugge, MD2, Carlos Fernandez-del Castillo, MD3, Mari Mino-Kenudson1, MD, A. John Iafrate, MD, PhD1*, and Martha Pitman, MD1* States | *Co-first authors | Co-senior authors
Gastrointestinal Endoscopy (in press)

1Massachusetts General Hospital, Department of Pathology, Boston, MA, United States | 2Massachusetts General Hospital, Department of Medicine, Boston, MA, United States | 3Massachusetts General Hospital, Department of Surgery, Boston, MA, United States

- 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 with accuracy
Diagnostic Morphology of Carcinoma

Already invasive- prognosis decreases ~50%
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. 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
Benign/Low Grade Glandular Epithelium

High-Grade Atypical Epithelial Cells in Pancreatic Mucinous Cysts are a More Accurate Predictor of Malignancy than “Positive” Cytology
Martha Bishop Pitman M.D, et.al. (Cancer Cytopath 2010)
Cytohistology: CB

IPMN-HGA

IPMN-LGA
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
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
Neoplastic: Other

**Patient:** Jane Doe
**Medical Record Number:** 123 45 6789
**Date of Birth:** January 1, 1959

**Clinical History:** 57 year old female with an incidentally discovered pancreatic cyst on CT scan for chest pain.

**Procedure:** Endoscopic ultrasound-guided fine needle aspiration biopsy with Muray ™ mini-forceps biopsy.

**Specimens Submitted:** A. pancreas head cyst fluid  B. Mini-forceps biopsy

**Imaging Features:**
- CT scan shows a 2.5 cm unilocular cyst with thin wall and no mural nodule.
- EUS shows a 3.0 cm unilocular cyst with no septations and a mural nodule.

**Ancillary Studies:**
- Biochemical Testing: CEA - 548 ng/ml; Amylase - 35,000 U/L. See the Chemistry Lab Results for details.
- Genetic Analysis using anchored multiplex next-generation sequencing: KRAS and GNAS mutant; SMAD4 deletion. See Center for Integrated Diagnostics Report for details.

**Cytomorphological Findings:** The cyst fluid contains a background of extracellular mucin and necrosis as well as single small (<12 micron) cells with a high nuclear to cytoplasmic ratio, irregular chromatin and focally irregular nuclear membranes. Cytoplasmic vacuoles are also focally present. The mini-forceps biopsy shows a small fragment of tissue with predominantly low-grade dysplasia, but a detached fragment of markedly atypical epithelial cells consistent with the high-grade atypia noted in the cyst fluid. No ovarian-type subepithelial type stroma is identified.

**Integrated Cytopathology Diagnosis:** Neoplastic: Other Intraductal papillary mucinous neoplasm with high-risk features including a mural nodule on EUS and high-grade atypia on cytology, findings warranting surgical resection. See note.

**Note:** A mucinous etiology of the cyst is supported by the elevated CEA, the extracellular mucin and the mucinous epithelial cells on cytology, as well as the genetic analysis showing KRAS and GNAS mutations. The high-risk for malignancy features include the mural nodule in the cyst noted on EUS coupled with the high-grade epithelial atypia noted on cytology in the cyst fluid and forceps biopsy. High-risk is also supported by the SMAD4 deletion noted on mutational analysis. These findings support a diagnosis of at least high-grade dysplasia; invasive carcinoma cannot be excluded. Surgical resection is warranted for complete histological evaluation and potential cure.

**Similar Patients at The State General Hospital Medical Center:**
Data from patients with similar imaging and cytological findings have a >92% chance of having at least high-grade dysplasia found in the cyst on histological evaluation. The morbidity from surgery is ~10% and risk of death from surgery ~1%.

**Management Recommendations:** According to a multidisciplinary group of experts in pancreatology, patients with worrisome to high-risk features for malignancy and who are good surgical candidates should undergo surgical resection of the cyst with the goal of cure (Tanaka M, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. Pancreatology. 2012 May-Jun;12(3):383-97.)
Important Information Regarding CME/SAMs

The Online CME/Evaluations/SAMs claim process will only be available on the USCAP website until September 30, 2017.

No claims can be processed after that date!

After September 30, 2017 you will NOT be able to obtain any CME or SAMs credits for attending this meeting.
THANK YOU