CYTOLOGY OF EUS-GUIDED FNA OF THE PANCREAS AND THE UPPER GI TRACT

Barbara A. Centeno, M.D.
Vice-Chair, Clinical Services
Assistant Chief of Pathology
Director of Cytopathology
Department of Anatomic Pathology/Moffitt Cancer Center
Professor
Endoscopic Ultrasound Guided FNA

Courtesy of Dr. William Brugge, Massachusetts General Hospital
Pre-Analytic Work-up

• **Clinical history**
  – Age
    • Pancreatoblastoma in infants
    • Solid-pseudopapillary tumor in younger age group
    • Mucinous cystic neoplasm in middle-aged women
  – Gender
    • Solid pseudo-papillary tumor and mucinous cystic neoplasm in females
  – Previous history of carcinoma
  – Risk factors for ductal adenocarcinoma
  – Familial syndromes
    • Lead to PDAC in young patients (23 yo)
Algorithmic approach of pancreatic masses based on imaging features

Imaging Appearance

Solid

Benign/Non-Neoplastic
- Acute pancreatitis
- Chronic pancreatitis
- Autoimmune pancreatitis
- Ectopic spleen

Neoplastic
- Ductal adenocarcinoma
- Neuroendocrine tumors
- Acinar cell carcinoma
- Pancreatoblastoma
- Lymphoma, plasmacytoma
- Metastasis

Mixed Solid and Cystic

Benign/Non-Neoplastic
- Groove pancreatitis

Neoplastic
- Solid-pseudopapillary neoplasm
- Neuroendocrine tumors
- Ductal adenocarcinoma
- Acinar cell carcinoma
- Metastases and secondary tumors

Cystic

Benign/Non-Neoplastic
- Mature teratoma
- Epidermoid cyst in ectopic spleen
- Lymphoepithelial cyst
- Pseudocyst
- Squamoid cyst of pancreatic ducts

Neoplastic
- Intraductal papillary mucinous neoplasms
- Mucinous cystic neoplasms
- Cystic neuroendocrine tumors
- Serous cystic neoplasm
- Acinar cell cystadenocarcinoma
Pre-Analytic Work-up cont.

• Radiological findings
  – Some lesions may present with characteristic findings
    • *ie*: starburst pattern in serous cystadenoma
    • sausage shaped pancreas in autoimmune pancreatitis
    • Evidence of invasion beyond the pancreas and vascular invasion are features of adenocarcinoma
Pre-Analytic Work-Up cont.

• What kind of technique was used to guide the aspiration
  – Transabdominal approaches more likely to have hepatocytes or mesothelial cells
  – Endoscopic ultrasound will have significant gastrointestinal epithelium
    • Location determines whether it is duodenal or gastric contaminant

• Correlate clinical and imaging findings with cytological and ancillary findings
Esophagus
Squamous Epithelium
Gastric Epithelium
Gastric Epithelium

- Large flat sheets
- Cytoplasm of foveolar cells is columnar, with mucin
- Nuclei round and evenly spaced, uniform in size
Gastric Epithelium

- Sometimes show small grooves, inclusions, and small nucleoli
- Associated with mucin
Gastric Epithelium
Stripped Nuclei

Degenerative grooves

Mucin and stripped nuclei

Inclusions
Gastric Parietal Cells and Chief Cells

Pitfall: May be mistaken for acinar cells or hepatocytes
Gastric Foveolar Epithelium

Cup shaped mucin
Duodenum

- Typically large sheets
- Columnar enterocytes with a microvillus brush border with interspersed goblet cells
Duodenal Epithelium

- Nuclei round and uniform
- Background mucin is typically thin, associated with groups, may have debris
Enterocytes have a microvillous brush border
Primary Neoplasms Of The Pancreas

- Ductal phenotype (ductal neoplasms)
  - Ductal Adenocarcinoma and variants
  - Cystic neoplasms
    - Serous cystic neoplasms (centroacinar cells)
    - Mucinous cystic neoplasms
  - Intraductal papillary mucinous neoplasms
- Acinar cell phenotype
  - Acinar cell carcinomas
  - Acinar cell cystadenoma
  - Acinar cell cystadenocarcinoma
- Islet cell phenotype
  - Pancreatic neuroendocrine tumors
- Unknown histogenesis
  - Pancreatoblastoma
  - Solid pseudo-papillary neoplasms
Solid Tumors Morphology

Ductal Pattern
Ductal adenocarcinoma

Solid Cellular Pattern
Neuroendocrine Tumor
Acinar cell carcinoma
Solid pseudopapillary tumor
Pancreatoblastoma
Ductal Pattern = Ductal Adenocarcinoma

Ductal Pattern with Desmoplasia

Ductal Pattern
Low power view
Solid, Cellular Pattern

Proliferation of neoplastic cells without stromal desmoplasia

NonDuctal Neoplasms
Pancreatic Neuroendocrine Tumor (PanNET)
Acinar Cell Carcinoma (ACC)
Solid Pseudopapillary Tumor (SPPN)
Pancreatoblastoma (PB)
Standardized Terminology and Nomenclature for Pancreatobiliary Cytology: The Papanicolaou Society of Cytopathology Guidelines

Martha B. Pitman, M.D., 1* Barbara A. Centeno, M.D., 2 Syed Z. Ali, M.D., 3 Muriel Genevay, M.D., 4 Ed Stelow, M.D., 5 Mari Mino-Kenudson, M.D., 1 Carlos Fernandez-del Castillo, M.D., 6 C. Max Schmidt, M.D., 7 William Brugge, M.D., 8 Lester Layfield, M.D., 9

1Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts
2H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida
3Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland
4Department of Pathology, Hospital de Pathologie Clinique, Geneva, Switzerland
5Department of Pathology, University of Virginia Medical Center, Charlottesville, Virginia
6Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts
7Department of Surgery, Indiana University Medical Center, Indianapolis
8Department of Medicine, Division of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts
9Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, Missouri
*Correspondence to: Martha Bishop Pitman, M.D., Department of Pathology, Massachusetts General Hospital, 35 Fruit St., Boston, MA 02114, USA. E-mail: mpitman@partners.org
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Management of Pancreatic Masses

• Benign
  – Medical management, minimal intervention or observation

• Neoplastic
  – Surgery or observation

• Premalignant
  – Observation
    • LGD, MGD
  – Resection
    • HGD/Carcinoma

• Malignant
  – Surgery, neoadjuvant therapy, adjuvant therapy
Issues with Cytology Reporting of Pancreatobiliary Specimens

- Nonstandardized reporting lead to confusion for the clinicians treating the patient with a pancreatic mass or lesion
- Lack of epithelial cells used as criteria for nondiagnostic
  - IPMN and MCN not uniformly handled, thick mucin still indicative of underlying neoplasm, even without neoplastic cells
  - Pseudocysts will lack epithelial cells
  - Serous cystadenoma may lack neoplastic cells
  - Cases signed out as c/w cyst contents
- What cytological criteria should be used to interpret a lesion as IPMN or MCN?
  - Atypical mucinous epithelium classified as atypical, rather than neoplastic, or as suspicious for carcinoma
Issues with Cytology Reporting of Pancreatobiliary Specimens cont.

• Was high grade dysplasia in IPMN atypical, suspicious or malignant?
• Serous cystadenoma classified as negative or atypical
• Were neuroendocrine tumor and solid pseudopapillary neoplasms suspicious, positive, or other?
Purpose of Standardization

- Unify reporting of disease categories among pathologists
- Reduce and improve inter and intraobserver variability
- Provide clinically relevant information for patient management
- To reflect the current understanding of the biology of disease entities
Categories

• I. Nondiagnostic
• II. Negative (for malignancy)
• III. Atypical
• IV. Neoplastic: benign or other
• V. Suspicious (for malignancy)
• VI. Positive (for malignancy) or malignant

* only for laboratory systems where the information system requires it.
NONDIAGNOSTIC

Category I
Nondiagnostic

• **Definition:** A non-diagnostic cytology specimen is one that provides no useful information or diagnostic information about the sampled mass.
  – Discordant imaging and cytology findings
  – Cyst fluids that yield insufficient material for ancillary studies
• Any cellular atypia precludes a non-diagnostic category
• Caveats:
  – Nondiagnostic does not equal unsatisfactory
  – Unsatisfactory indicates that a specimen cannot be evaluated,
  – Acellular cyst fluid without other specific features is still representative of the cyst aspirated
Nondiagnostic

Cytological Criteria

- Preparation or obscuring artifact
- Gastrointestinal contaminant
- Benign acinar and ductal epithelium derived from a solid or cystic mass lesion
- Acellular aspirates of a pancreatic mass or pancreatic brushing
- Acellular aspirate of a cyst without evidence of mucinous etiology
  - Lack of thick, background mucin and/or oncotic cells
  - Lack of elevated CEA
  - Lack of KRAS or GNAS mutations
Incidental Cysts in the Pancreas

- Incidental cysts of the pancreas are common
- More likely to be neoplastic rather than benign
  - 30% of all incidental masses were IPMN in one series
  - 55% were MCN or IPMN (preinvasive precursors), only 4% were pseudocyst
- Pathologist is reviewing the cyst fluid to assess for the presence of pre-invasive precursors lesions
  - Pseudocyst diagnosis of exclusion
Cyst Contents
NEGATIVE

Category II
Negative

- **Definition:** Adequate cellular and/or extracellular material to evaluate and or define a nonneoplastic lesion identified on imaging
- Includes the presence of normal pancreatic parenchyma in the appropriate clinical setting
  - Vague fullness of the pancreas
  - No distinct pancreatic mass
Differential Diagnosis

• Benign pancreas
• Acute Pancreatitis
• Chronic Pancreatitis
• Autoimmune pancreatitis
• Splenule/Ectopic spleen/Accessory spleen
• Pseudocyst
• Lymphoepithelial cyst
Atypical

• *Definition:* Cells with cytoplasmic, nuclear, or architectural features not consistent with normal or reactive cellular components of the pancreas or bile ducts, and insufficient features to classify them as a neoplasm or suspicious for a high grade malignancy
  – The findings do not explain the lesion identified on imaging
  – Follow-up evaluation is warranted
Atypical Cytological Criteria

• Cytological specimen contains cellular or extracellular tissue that displays morphological features that cannot comfortably be classified as reactive or benign, but which are also insufficient to classify as definitively neoplastic or as suspicious for malignancy.

• Examples:
  – Biliary brush specimens with mucinous epithelium and other atypical findings
  – Atypical mucinous epithelium in a pancreatic aspirate
    • PanIN
    • Gastric contaminant
    • IPMN
  – Cellular component is suggestive of a PanNET or SPN but the sample is of insufficient quantity or quality for definitive diagnosis
NEOPLASTIC

Category IV
Neoplastic: Benign

• *Definition*: The cytological specimen is sufficiently cellular and representative, with or without the context of clinical, imaging and ancillary studies to be diagnostic of a benign neoplasm.

• Neoplasms included in this category:
  – Serous cystadenoma
  – Schwannoma
  – Cystic teratoma
Neoplastic: Other

• **Definition:** Defines a neoplasm that is either premalignant, or a low grade malignant neoplasm.

• Neoplasms included in this category:
  – Neoplasm that is preinvasive cancer
    • IPMN or MCN with LGD, MGD, HGD
    • IOPN
  – Solid cellular neoplasm
    • Pancreatic neuroendocrine tumor
    • Solid pseudopapillary neoplasm
  – Extra-adrenal paraganglioma
  – Gastrointestinal Stromal tumor
Rationale

• Established to provide a category for neoplasms that were either not clearly benign, such as serous cystadenoma, nor clearly aggressive, and high grade in their behavior, such as ductal adenocarcinoma.
• Standardize cytological nomenclature and terminology to correlate with WHO 2010 classification and terminology.
  – The words tumor and neoplasm connote a neoplasm, but not a malignancy
• Patients with neoplasms in this category may have the option of being managed conservatively
  – PanNET may be observed
  – IPMN with low risk features may be observed.
• The categories of atypical and suspicious connote an indeterminate interpretation.
• Does not define these a benign or malignant
Mucinous Cysts

- Nonneoplastic and neoplastic mucinous cysts
  - Nonneoplastic mucinous cyst (mucinous duct lesion)
  - Foregut cysts
  - Nonneoplastic cysts lack mutations
- Cytological and cyst fluid features may overlap
  - Mucin only
  - Elevated CEA
  - Lack KRAS and GNAS
- Mucinous cyst, not otherwise specified
  - Characteristic mucin with oncotic cells, no epithelium for evaluation
- Mucinous cyst with low grade epithelial atypia (low grade and moderate grade dysplasia) (IPMN or MCN)
- Mucinous cyst with high grade epithelial atypia (high grade dysplasia and adenocarcinoma)
Criteria for Mucinous Cyst

• Fluid viscous, clear or white
• Cytology shows mucinous background as described, +/- neoplastic epithelium
• Ancillary studies
  – CEA elevated
  – Mutational analyses
    • KRAS mutated in IPMN and MCN
    • GNAS mutations in IPMN
    • RNF43 mutations in IPMN and MCN
Neoplastic: Other

• Imaging: Large, multiloculated cyst in the tail of the pancreas with evident connect to the pancreatic ductal system

• Report:
  – Adequacy: Satisfactory
  – Interpretation: Thick background mucin, histiocytes, and oncotic cells, consistent with IPMN
  – Category: Neoplastic: Other (other)
  – Comment: No neoplastic epithelium is present for evaluation of dysplasia. The cytological findings correlated with the imaging findings support the above interpretation.
Mucinous cyst, NOS

Background
Management of IPMN
2012 Consensus Guidelines

• Consensus conference of the International Association of Pancreatology has proposed guidelines for the management of patients with pancreatic cysts suspected to be IPMNs

• These guidelines use a combination of clinical history, gender, imaging characteristics, cytology and cyst fluid analysis to determine therapeutic and surveillance strategies.

• Resection without tissue confirmation:
  – Main duct IPMN > 10 mm
  – Obstructive jaundice a/w cyst in HOP
  – Enhancing solid component
Management of IPMN
2012 Consensus Guidelines

• Worrisome features
  – Patient evaluated by EUS
  – These include:
    • Cyst >= 3 cm
    • Thickened, enhancing cyst walls
    • Main duct 5-9 mm
    • Nonenhancing mural nodule
    • Abrupt change in caliber of pancreatic duct with distal pancreatic atrophy

• EUS worrisome features
  – Definite mural nodule
  – Main duct features suspicious for involvement
  – Cytology: suspicious or positive
    • Strongly consider surgery if > 3 cm
    • Consider surgery if 2-3 cm
What is suspicious or positive cytology?

- Cells that indicate the presence of high grade dysplasia or invasive carcinoma
  - Role of cytology: Screening for high risk cytological features
- Terms:
  - High grade dysplasia present or absent
  - High grade epithelial atypia: incorporates high grade dysplasia and invasive carcinoma
    - absence of high-grade atypia with the absence of high risk imaging features of dilated MPD and mural nodule gives a NPV of > 90%
Low-Grade Dysplasia

- Abundant columnar mucin containing cytoplasm
- Basally located nuclei
- Columnar cytoplasm with mucin
- Basally located nuclei
Moderate dysplasia

Pseudostratification
High-Grade Dysplasia

Papillary tufts, nuclei extend to luminal border

Mitoses
Cytological Criteria of High-Grade Epithelial Atypia in the Cyst Fluid of Pancreatic Intraductal Papillary Mucinous Neoplasms

Martha B. Pitman, MD\textsuperscript{1}; Barbara A. Centeno, MD\textsuperscript{2}; Ebubekir S. Daglilar, MD\textsuperscript{3}; William R. Brugge, MD\textsuperscript{3}; and Mari Mino-Kenudson, MD\textsuperscript{1}

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Duodenum: 12 u

LGEA: cells 12u

HGEA: cells <12 u
High-grade epithelial atypia

- Includes high grade dysplasia and invasive carcinoma

- Criteria
  - High N/C
  - Nuclear membrane irregularities
  - Abnormal chromatin
    - Hypo- or hyperchromasia
  - Background necrosis

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SUSPICIOUS

Category V
Suspicious

• Some, but not all of the criteria of a specific malignant neoplasm are present, mainly for pancreatic adenocarcinoma diagnosis. The features are qualitatively or quantitatively insufficient for a conclusive diagnosis.

• Confirmatory ancillary testing or substantial clinical and radiological findings must be present and discussed during a treatment planning conference, or similar correlation conference.
POSITIVE

Category VI
Positive/Malignant

• Unequivocal display of malignant cytological change
• Diagnoses:
  – Adenocarcinoma and its variant
  – Neuroendocrine carcinoma, small and large cell type
  – Pancreatoblastoma
  – Acinar cell carcinoma
  – Lymphoma
  – Metastases
  – Sarcomas in this region, secondarily involving the pancreas
Ancillary Testing
Solid Masses

- Flow cytometry
  - Work-up for probable lymphomas

- Immunohistochemistry
  - Reactive Glands vs. Adenocarcinoma
    - S100P, IMP3, mesothelin, DPC4, VHL
  - Differential diagnosis of solid cellular neoplasms
    - PanNET, SPN, ACC, PB
      » PanNET: CK +, neuroendocrine markers+
      » SPN: β-catenin +, vimentin+, loss of e-cadherin
      » ACC: chymotrypsin, trypsin, and bcl 10+

- Metastases
  - IHC panel depending on the differential diagnosis
Ancillary Testing
Cystic Masses

• Biochemical
  – Viscosity
    • Elevated in MCN/IPMN/IOPN
    • Ostwald viscometer, or finger pull
  – CEA: >192ng/ml
    • False positives from duplication cysts, mesothelial inclusion cysts, LECP, etc
  – Amylase:
    • >1000s for pseudocyst
    • Elevated level does not mean it is a pseudocyst, falsely elevated in IPMN, MCN and other neoplasms
Ancillary Testing
Cystic Masses

- **Molecular (for pancreatic cysts)**
  - **RAS**
    - supports a neoplastic mucinous cyst, does not distinguish between IPMN or MCN
  - **GNAS**
    - Supports IPMN
    - No correlation with grade
  - **RNF43**
    - Mucinous neoplastic etiology
    - Does not distinguish between IPMN and MCN
Ancillary Testing
Cystic Masses

• Molecular (for pancreatic cysts)
  – 3P deletions
    – 3p25, VHL gene, supports serous cystadenoma
    – Other 3p deletions also found in serous cystadenoma
  – \textit{CTNNB1}
    – Mutations support solid pseudopapillary neoplasm
  – \textit{TP53, CDKN2A, SMAD4/DPC4}
    – Loss supports a high risk cyst
    – High grade dysplasia or risk to progression
Pancreatic Cytohistology

Barbara Ann Centeno
Edward B. Stelow
Martha Bishop Pitman
EUS-FNA OF THE UPPER GI TRACT

• Utilized for masses in the submucosal and deep layers of the GI tract
• 80-90% sensitivity

• Layers:
  – Mucosal and muscularis mucosa
    • NETs
  – Submucosal
    • lipoma
EUS-FNA OF THE UPPER GI TRACT

- Muscularis propria
  - GIST, schwannoma, leiomyoma
- Serosa/adventitia
  - Metastatic disease