Standardized Terminology and Nomenclature for Pancreaticobiliary Cytopathology from the Papanicolaou Society of Cytopathology

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Standardization of Nomenclature

• Unifies reporting of disease categories
• Reduces interobserver variability
• Improves intraobserver reproducibility
• Better aligns patient management options with interpretations
• Improves patient care
PSC Pancreaticobiliary Guidelines

- Committee I - Indications for EUS Guided FNA and Bile Duct Brushings with Pre-procedure Requirements [Douglas Adler, MD - Chair – Endoscopist – University of Utah]
- Committee II – Techniques for Cytologic Sampling of Pancreatic and Bile Duct Lesions [Bill Brugge, MD – Chair - Gastroenterologist – MGH, Harvard Medical School]
- Committee III – Nomenclature and Diagnostic Criteria [Martha Pitman, MD - Chair – Cytopathologist – MGH, Harvard Medical School]
- Committee IV – Utilization of Ancillary Studies in Pancreas FNA and Bile Duct Brushings [Lester Layfield, MD – Chair - Cytopathologist – University of Missouri]
- Committee V – Post-Brushing and FNA Testing Follow-Up and Treatment Options [Dan Kurtycz, MD – Chair – Cytopathologist – University of Wisconsin]

PSC Guidelines for PB Cytology
Published April 2014

- Clinical evaluation, imaging studies, indications for cytologic study, and preprocedural requirements for duct brushing studies and pancreatic FNA. Diagn Cytopathol. 2014;42(4):325-332.
- REVIEW
  – Cancer Cytopathol 2014; 122 (6): 399-411
Pancreatic Pathology: WHO 2010 (abbreviated)

- Epithelial Tumors
  - Benign
    - Acinar cell cystadenoma
    - Serous cystadenoma
  - Pre-Malignant Lesions
    - PanIN
    - IPMN: LGD, IGD, HGD
    - MCN: LGD, IGD, HGD
  - Malignant
    - Ductal adenocarcinoma and variants
    - Acinar cell carcinoma/cystadenocarcinoma/mixed carcinomas
    - IPMN with invasive carcinoma
    - MCN with invasive carcinoma
    - Pancreatoblastoma
    - Solid-pseudopapillary neoplasm
- Neuroendocrine Neoplasms
  - Pancreatic neuroendocrine microadenoma
  - Neuroendocrine Tumor
  - Neuroendocrine Carcinoma

- Negative
- Atypical
- Suspicious
- Positive
### Management of PB Lesions

<table>
<thead>
<tr>
<th>Type</th>
<th>Management</th>
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<td>Malignant</td>
<td>Surgery</td>
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<td>– Low-intermediate grade</td>
<td>Observation with Repeat sampling</td>
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<td>– High-grade/carcinoma</td>
<td>Surgery</td>
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<td>Neoplasm</td>
<td>Surgery or observation</td>
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### General Tiered System in Cytology

- Nondiagnostic
- Negative
- Atypical
- Suspicious
- Positive
Proposed Terminology

I. Nondiagnostic
II. Negative
III. Atypical
IV. Neoplastic
   - Benign
   - Other
V. Suspicious
VI. Positive/Malignant

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 cysts
    - Lymphoepithelial cyst
    - Peripancreatic cysts
Cytology Interpretation of Pancreaticobiliary Lesions

• 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, KRAS, GNAS

Work-up of solid and cystic lesions of pancreas differs.
Two basic questions for Cyst analysis

1. Is the cyst mucinous or non-mucinous?
2. Is the cyst low-grade or high-grade?
Cytological Preparations

No-ROSE

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

CEA and Amylase: Key Points

- Elevated CEA ($\geq 192$ ng/ml) supports a mucinous cyst
  - Does not distinguish IPMN from MCN
  - Level does not correlate with malignancy
  - Rare FP: PCT, GI duplication cyst, LEC

- Amylase levels
  - Elevated in the 1000’s for most PCT
  - Low amylase level tends to exclude a PCT
  - Level does not distinguish IPMN from MCN
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

### Fresh Pancreatic Cyst Fluid

- **<0.5 cc**
- **≥0.5 cc**

#### Molecular

- 1. CEA
- 2. Amylase
- 3. Banking
- Cytospin(s)

#### Cytology

+/- mucin stains
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. Dagililar, MD, William R. Brugge, MD, and Mari Mino-Kenudson, MD
Cancer Cytopathology epub ahead of print Aug. 2013

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 Glandular Epithelium

Martha Bishop Pitman M.D., et al. (Cancer Cytopath 2010)
Components of Cytology Report

- Adequacy Statement
- Diagnostic Category
- Subcategory and/or Comments

Proposed Terminology

I. Nondiagnostic
II. Negative
III. Atypical
IV. Neoplastic
    - Benign
    - Other
V. Suspicious
VI. Positive/Malignant
Category I

Non-diagnostic

Knowing which structures are passed through helps recognize contaminants.
Nondiagnostic

**Definition:**
- A non-diagnostic cytology specimen is one that provides no diagnostic or useful information about the lesion sampled. Any cellular atypia precludes a non-diagnostic report.

**Cytological Criteria:**
- Preparation artifact precludes evaluation of the cellular component
- Obscuring artifact precludes evaluation of the cellular component
- Gastrointestinal epithelium only
- Normal pancreatic tissue elements in the setting of a clearly defined solid or cystic mass by imaging
- Acellular aspirates of a solid mass or pancreatobiliary brushing
- Acellular aspirate of a cyst without evidence of a mucinous etiology such as thick colloid-like mucin, elevated CEA or KRAS mutation (See Section IV)

Duodenal Contamination
Gastric Contamination

Example Report

- Evaluation limited by preparation artifact
- Nondiagnostic
- Tissue entrapped in blood clot and fibrin precluding cytological evaluation.
NOT non-diagnostic

Example Report

- Satisfactory for Evaluation
- Nondiagnostic
- Gastrointestinal contamination only.
Example Report

• Satisfactory for Evaluation
• Nondiagnostic
• Normal acinar and ductal epithelium. The biopsy does not explain the well-defined pancreatic mass seen on imaging.

Category II

Negative (for malignancy)
Negative (for malignancy)

- Indicates the absence of malignancy
- Can be further qualified by stating the type of benign lesion present
- When not further qualified, negative is not synonymous with a specific benign lesion

Negative (for malignancy)

- False negative rate for a solid mass lesion is approximately 15% and for a cystic lesion may be as high as 60%.
- False negative rate for bile duct brushings is higher than for EUS-FNA.
- Malignancy risk for Negative EUS-FNA is 23%.
- Malignancy risk for Negative brushing sample is 25%.

Subcategories for Negative

- Benign pancreas
- Chronic Pancreatitis
- Autoimmune Pancreatitis
- Pseudocyst
- Lymphoepithelial Cyst
- Splenule/Accessory Spleen

Benign Pancreatic Tissue

Correlation with imaging: no visible mass
Example Report

• Satisfactory for Evaluation
• Negative for Malignancy
• Benign acinar and ductal epithelium. See note.

• Note: The biopsy may be representative in the setting of only vague fullness on imaging. Clinical correlation required.

Pancreatitis

• Chronic Pancreatitis
  – Active (acute): background inflammation, fat necrosis, calcific debris

• Autoimmune Pancreatitis
  – cellular stromal fragments embedded with lymphocytes and plasma cells
  – Few ductal cells present
Lymphoepithelial Cyst

- Anucleate squames and abundant keratinous debris
- Mature superficial squamous cells
- Lymphocytes are usually present but amount is variable and may be quite scant
- +/- Cholesterol clefts

Example Report

- Evaluation limited by poor cellular preservation
- Negative (for malignancy)
- Lymphoepithelial cyst.
Pseudocyst

- Inflammation, histiocytes
- Granular, junky background
- No cyst lining epithelium
- Yellow pigment, crystals
- High amylase (1000’s)
- Low CEA
- No mutations

Example Report

- Satisfactory for Evaluation
- Negative (for malignancy)
- Nonmucinous cyst fluid consistent with pseudocyst (See note).

- Note: The cyst is inflammatory with only GI epithelium and no recognizable cyst lining epithelium. Yellow pigment is present. The cyst fluid CEA is 2 ng/ml and amylase 56,469 U/L, results that support the diagnosis [or correlation with cyst fluid analysis recommended].
Splenule/accessory spleen

- Lymphoid tissue
- Histiocytes
- Blood vessels
- Fibrin aggregates with embedded lymphocytes and plasma cells

Example Report: Negative vs. ND

- Satisfactory for Evaluation
- Negative for Malignancy
- Mucinous cyst debris of uncertain etiology. No high-grade epithelial atypia identified. Correlation with imaging and ancillary studies required.
Category III

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 a lesion identified on imaging studies.
  – Follow-up evaluation is warranted.
Heterogeneous category which includes samples with reactive change, low cellularity, (pre)malignant lesions with nonspecific cytology and cases reflecting cytopathologist’s diagnostic caution.

Premalignant Lesions of Biliary Tract and Pancreas

- PanIN (I to III)
- BilIN (I to III)
- IPMN (various degrees of dysplasia)
- Cystic Mucinous Neoplasm (various degrees of dysplasia)
In general, the concept of dysplasia is most often applied to bile/pancreatic duct brushings and FNAs of cystic lesions.

Atypia including Dysplasia

- PanIN unlikely to be recognized in a cytology specimen as such and more likely to be over-diagnosed
  - Value of correlating cytological findings with imaging
  - PanIN is a subclinical lesion
- Concept of dysplasia best developed in histologic material.
  - BillIN is poorly defined cytologically

Low grade dysplasia is assigned to the atypical category.

Categorization of Dysplasia

• Bile duct brushings
  • Low-grade dysplasia – Atypical category
  • High-grade dysplasia – Suspicious category

• Mucinous Cysts (IPMN or MCN)
  • All grades of dysplasia - Neoplastic: Other category
Risk of malignancy of Atypical Category

- EUS-FNA: 79%
- Duct Brushings: 62%


Atypical

- Mild-moderate cellular atypia, NOS
- Mucinous/ductal epithelium with mild-moderate nuclear atypia (from a solid lesion or not clearly from a mucinous cyst)
Example Report

- Evaluation limited by scant cellularity
- Atypical
- Atypical bile duct epithelium with mucinous metaplasia and mild nuclear atypia.

Specimens are interpreted as Atypical when:

- A specimen is suggestive of a neuroendocrine tumor or solid-pseudopapillary neoplasm but tissue is of insufficient quality and quantity to make a specific diagnosis
Example Report

• Satisfactory for Evaluation
• Atypical
• Atypical epithelial proliferation suggestive but not diagnostic of a neuroendocrine tumor.
• No tissue for ancillary studies is available.

Category IV

Neoplastic:
Benign
Other
Neoplastic: Benign

- **Definition:**
  - This interpretation category connotes the presence of a cytological specimen sufficiently cellular and representative, with or without the context of clinical, imaging, and ancillary studies, to be diagnostic of a benign neoplasm.

Serous cystadenoma

- Sparse cellularity
- Clean or bloody background
- Flat sheets and loose clusters
- Bland cuboidal cells
- Clear, finely vacuolated or granular cytoplasm with indistinct borders
- Associated hemosiderin-laden macrophages
- Low CEA; low amylase
- No *KRAS* mutation
Example Report

- Evaluation limited by scant cellularity
- Neoplastic: Benign
- Scant nonmucinous cuboidal epithelium in a nonmucinous cyst fluid with low CEA (4ng/ml) and amylase (12 U/L) consistent with the serous cystadenoma.

Neoplastic: Other

- **Definition:**
  - This interpretation category defines a neoplasm that is either premalignant such as IPMN or MCN with low, intermediate or high grade dysplasia, or a solid-cellular neoplasm such as well-differentiated PanNET or SPN.
Neoplastic: Other

- Pancreatic neuroendocrine tumor
- Solid-pseudopapillary neoplasm
- Mucinous cyst (IPMN or MCN), not otherwise specified (NOS), e.g. only thick, colloid-like mucin or elevated CEA or positive KRAS, if known
- Mucinous cyst (IPMN or MCN) with low-grade atypia/dysplasia
- Mucinous cyst (IPMN or MCN) with high-grade atypia/dysplasia
- GIST

Well-differentiated PanNET

- highly cellular aspirate with “solid-cellular” smear pattern
- predominantly isolated cells, bare nuclei
- pseudorosettes and small clusters
- uniform, round/oval nuclei
- eccentric nuclei (“plasmacytoid” appearance)
- finely stippled (“salt and pepper”) chromatin
- moderate to abundant cytoplasm, typically granular but may be vacuolated or oncocytic
Solid-Pseudopapillary Neoplasm

- highly cellular aspirate with solid-cellular smear pattern
- myxoid or hyalinized vascular stalks lined by neoplastic cells
- delicate finely vacuolated cytoplasm with indistinct cell borders
- perinuclear vacuole
- PAS/d positive hyalin globule
- round/oval, bean shaped nuclei
- nuclear grooves
- inconspicuous nucleoli
- foam cells, necrotic debris in the background

Mucinous Cysts [IPMN and MCN]

- Generally hypocellular specimen
- Variable extracellular mucin
- thick, colloid-like mucin with or without cyst lining epithelium is diagnostic of a mucinous cyst
- low-grade dysplasia/atypia: benign appearing mucinous epithelium in sheet and groups, often indistinguishable from gastric epithelium
- high-grade dysplasia/atypia (intermediate to high-grade): atypical epithelial cells in small, tight bud-like clusters or singly with increased N/C ratio, irregular nuclear membranes and variably vacuolated cytoplasm
Example Report

- Satisfactory for Evaluation
- Neoplastic: Other
- Mucinous cyst fluid with low-grade dysplasia (See note).
- Note: Benign-appearing mucinous epithelium is present from this transduodenal FNA in a background of abundant extracellular mucin. [If available, add CEA is elevated at 357 ng/ml supporting the diagnosis].

Example Report

- Evaluation limited by Absent Epithelial Component
- Neoplastic: Other
- Cyst fluid with thick colloid-like extracellular mucin containing cyst debris consistent with a neoplastic mucinous cyst
Category V

Suspicious

• **Definition:**
  – A specimen is suspicious for malignancy when some but not all of the criteria of a specific malignant neoplasm are present, mainly pancreatic adenocarcinoma. The cytological features raise a strong suspicion for malignancy, but the findings are qualitatively and/or quantitatively insufficient for a conclusive diagnosis.
The category “suspicious for malignancy”

• Represents 5 to 12% of cases
• Shows high interobserver variability

If the category “suspicious for malignancy” is used for the basis for operative intervention, confirmatory ancillary testing information or substantial clinical and radiological findings must be present and discussed during a treatment planning conference or similar correlation procedure.
Malignancy Risk of the “suspicious for malignancy” category is:

EUS-FNA: 96%

Brushing specimens: 74%

Suspicious for Malignancy

Ancillary testing may or may not be helpful in evaluating cases assigned to “suspicious for malignancy” category

- KRAS Testing
- Digital Image Analysis
- FISH Testing
**KRAS Mutational Analysis**

- Mutations found in 91% of pancreatic adenocarcinomas
- Mutations also found in ductal hyperplasia, metaplasia, chronic pancreatitis
- May help in separation of autoimmune pancreatitis from carcinoma
- Insufficient specificity for clinical usage

**Digital Image Analysis**

- Aneuploidy and tetraploidy support diagnosis of carcinoma
- Most useful in brushing specimens
- Does not significantly improve diagnostic accuracy above cytology alone
Fluorescence In-Situ Hybridization

- Commercially available kit
- Targets pericentromeric regions of chromosomes 3, 7 and 17 and chromosomal band 9p21
- Can be automated
- Sensitivity 90%, Specificity 94%
- Overall best ancillary test for identifying carcinoma
- PPV 98%, NPV 75%

Example Report

- Satisfactory for Evaluation
- Suspicious (for Malignancy)
- Rare atypical epithelial cells suspicious for adenocarcinoma.
Example Report

- Satisfactory for Evaluation
- Suspicious (for Malignancy)
- Solid cellular neoplasm with features suspicious for acinar cell carcinoma. Tissue for confirmatory ancillary studies is not available.

Category VI

Positive (for malignancy)
Positive/Malignant

- Definition: unequivocal display of malignant cytologic characteristics
- Adenocarcinoma of the pancreaticobiliary ducts, and variants
- Acinar cell carcinoma
- High-grade neuroendocrine carcinoma (small and large cell type)
- Pancreatoblastoma
- Lymphoma
- Metastases

Malignancy risk for malignant category is essentially 100%.
Pancreatic Ductal Carcinoma and carcinomas of biliary and main pancreatic duct (85-90% of all carcinomas)

Cytological Criteria
Well-differentiated PDAC

- Variable cellularity with predominance of one cell type, i.e., ductal cells
- Cohesive small to medium-sized sheets of cells with smooth borders, and rarely single cells
- Three-dimensional fragments
- Moderate nuclear enlargement with high N/C ratios, cellular crowding and overlap
- Loss of nuclear polarity, often pale nuclei with chromatin clearing and/or clumping, nuclear membrane irregularity with clefts and notches
- Lack of prominent nucleoli
- Mild to moderate anisonucleosis (typically more than 4 to 1 in the same gland/duct)
- Rare mitoses
- Uncommon necrosis
Example Report

- Satisfactory for Evaluation
- Positive (for Malignancy)
- Well-differentiated adenocarcinoma (See note).

- Note: An immunohistochemical stain for SMAD4 shows loss of staining in the suspicious cells supporting a malignant interpretation.

Cytological Criteria
Moderately Differentiated Pancreatic Ductal Adenocarcinoma

- Above features with addition of one or more of the following
- Larger cellular sheets/fragments with increased numbers of single cells
- More extensive cellular pleomorphism
- Marked anisonucleosis and occasional prominent nucleoli
- More crowded three-dimensional or syncytial tissue fragments
Cytologic Criteria
Poorly Differentiated Pancreatic Ductal Adenocarcinoma

- Extreme pleomorphism, with almost total lack of glandular differentiation
- Larger loosely cohesive syncytial tissue fragments
- Significant populations of single large malignant cells
- High N/C ratio, nuclei with coarse dark chromatin and often macro nucleoli
- Occasional bizarre nuclei with triangular shapes and/or multinucleated cells
- Mitoses and karyorrhexis
- Prominent necrosis

Example Report

- Satisfactory for Evaluation
- Positive (for Malignancy)
- Adenocarcinoma.
Major Diagnostic Criteria for Cholangiocarcinoma

- Nuclear molding
- Chromatin clumping
- Enlarge nuclei
- Loss of polarity (loss of “honey comb” pattern
- Cell-in-cell arrangements
- Increased N/C ratio
- Greater than 4-fold variation in nuclear size in a single group of cells

Example Report

- Satisfactory for Evaluation
- Positive (for Malignancy)
- Adenocarcinoma consistent with cholangiocarcinoma.
Cytologic Criteria
Acinar Cell Carcinoma

- Usually hypercellular, mostly small to mid-sized cellular fragments and few single cells
- Prominent acinar formation without lobular arrangements (in well-differentiated tumors that can be confused with “rosettes” of a PanNET), rare syncytia
- Uniform population of cells larger than ductal carcinoma, minimal pleomorphism
- Finely granular to denser cytoplasm, granularity is often basophilic
- Mildly increased N/C ratios, single round to oval nucleus which are eccentrically placed, course chromatin, single prominent nucleoli, focal anisonucleosis
- Significant anisonucleosis in high-grade tumors
- Numerous bare “stripped off” nuclei, rare intranuclear inclusions
- Rare necrosis
- Trypsin +

Example Report

- Satisfactory for Evaluation
- Positive (for Malignancy)
- Acinar cell carcinoma (See note).

- Note: An immunohistochemical stain shows the tumor cells to be positive for trypsin supporting the diagnosis.
Lymphoma of the Pancreas

- Most lymphomas involving pancreas are secondary
- Essentially all are non-Hodgkin lymphomas
- 2/3 are large B-cell lymphomas
- Most involve head of pancreas

Cytologic Criteria for Lymphoma

- Cellular smears of non-cohesive cells
- Predominance of large immature lymphoid cells
- Background lymphoglandular bodies
- Cells have scant cytoplasm
- Flow cytometry discloses monoclonal cell population
Metastatic Neoplasms to Pancreas

- Lung (23%)
- Breast (8%)
- Melanoma (5%)
- Kidney (15%)

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