Pancreatobiliary Cytology

WHO System

Pancreas Cytology Diagnostic Categories: *(based on pancreatic FNA, usually EUS-guided)*

Diagnostic Category	Risk of Malignancy	Clinical management
Insufficient/ Inadequate/ Non-diagnostic	5-25%	Repeat biopsy if concerned
Benign/ Negative for Malignancy	0-15%	Correlate clinically
Atypical	30-40%	Repeat biopsy if concerned
Pancreatobiliary Neoplasm, Low-risk/grade (PaN-low)	5-20%	Correlate clinically
Pancreatobiliary Neoplasm, High-risk/grade (PaN-high)	60-95%	Resection if surgical candidate; possible conservative management
Suspicious for malignancy	80-100%	If surgical candidate, treat as positive. Repeat biopsy before chemoradiation.
Malignant	99-100%	Per clinical stage

Biliary Cytology Diagnostic Categories: *(usually based on bile duct brushing)*

Diagnostic Category	Risk of Malignancy	Clinical management
Insufficient/ Inadequate/ Non-diagnostic	28-69%	Repeat ERCP
Benign/ Negative for Malignancy	26-55%	Correlate clinically
Atypical	25-77%	Repeat ERCP
Suspicious for malignancy	74-100%	If surgical candidate, treat as positive if other factors support malignancy. Repeat biopsy before chemoradiation
Malignant	96-100%	Per clinical stage

Modified from: WHO Reporting System for Pancreatobiliary Cytopathology

Brief Commentary

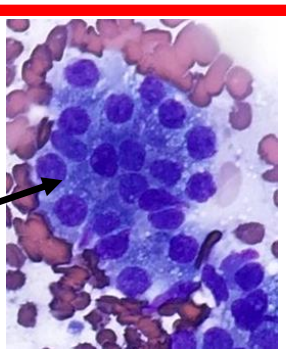
Pancreatobiliary adenocarcinomas have a horrible prognosis, and the surgery is big (if possible), so you want to be *absolutely sure* when you call something "Malignant"—show cases liberally, use strict criteria, and look in the EMR to make sure the diagnosis fits.

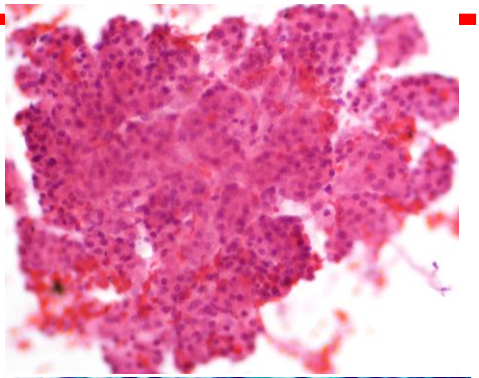
Many of the non-adenocarcinoma diagnoses (e.g., NET, SPN, etc..) require IHC for a definitive diagnosis, so at the time of ROSE, make sure to get a good cell block/core if you're considering one of these DXs.

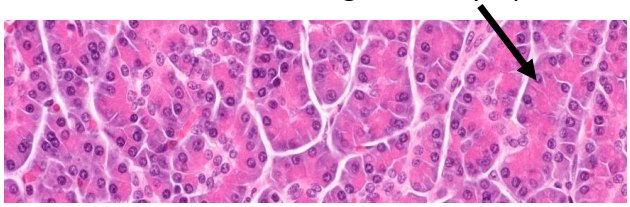
Normal Tissue

Pancreatic acinar cells

Very **cellular** specimens
Cohesive grape-like clusters (few single cells)
Round, eccentric nuclei with fine chromatin.
Small nucleoli. Abundant granular cytoplasm.

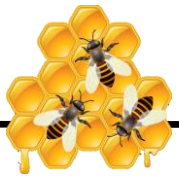


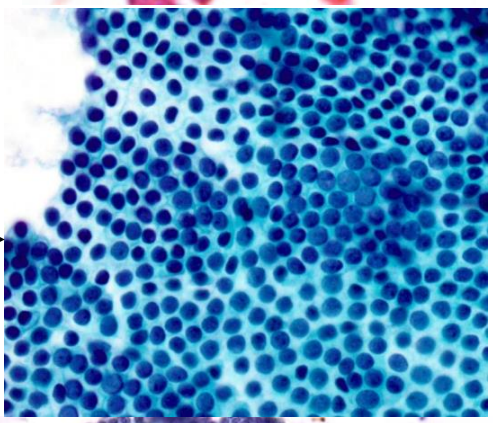




Pancreatobiliary ductal cells

Flat, cohesive sheets with even spacing
→ Think "**Honeycomb**"
Round/oval nuclei with fine chromatin and inconspicuous cytoplasm.
Non-mucinous epithelium.



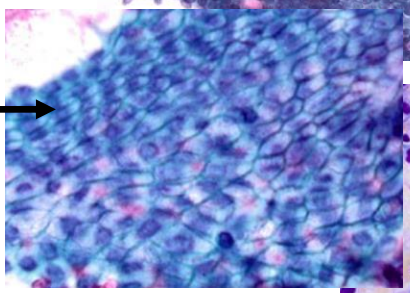


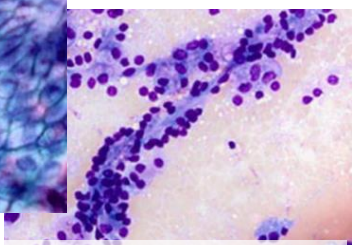
Duodenal epithelium

Flat, monolayer cohesive sheet.
Mostly non-mucinous glandular cells with a brush border.
Interspersed goblet cells (like a stary night)
Lymphocyte "sesame seeds" within epithelium.
Sometimes whole villi!

Gastric epithelium

Foveolar cells with visible mucin cup.
Small sheets, strips, single cells, and pits with oxyntic and chief cells.





Stomach Oxyntic Glands

Mesothelium

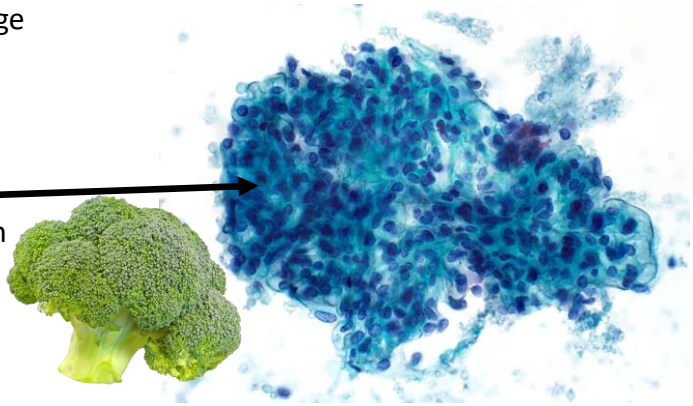
Like elsewhere: 2D sheets.
Round to oval nuclei, moderate cytoplasm.
"Windows" between cells (due to microvilli).

Hepatocytes

Polygonal cells with abundant granular cytoplasm, large round nuclei, and prominent nucleoli.
May see bile or other pigment.

Kidney

Intact **Glomeruli**: looks like vascular broccoli.
Renal **tubular** cells, such as proximal tubular cells with abundant oncocytic cytoplasm.



Insufficient/Inadequate/Non-diagnostic

A specimen that for quantitative or qualitative reasons does not explain the targeted lesion.
No specific cellularity requirement. Any atypia precludes this Dx.

Benign Lesions

Chronic Pancreatitis

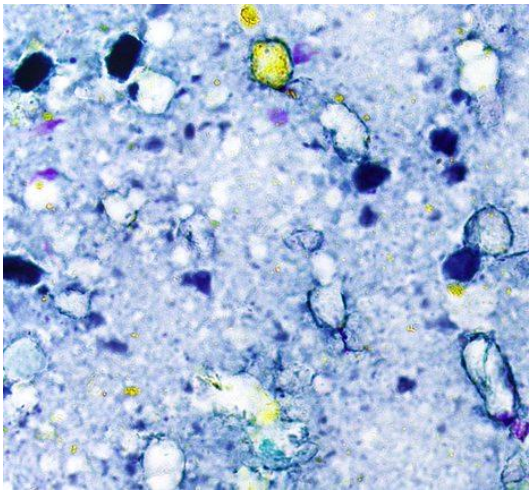
Often diagnosed clinically. Common causes: EtOH, Obstruction, Genetics.

Atrophy of acinar cells→ Usually **hypocellular** and bloody.
Fibrous tissue. Chronic inflammation. Fat necrosis, Calcifications, debris.
Scattered islet cells (can mimic NET).

Acute Pancreatitis—rare to FNA (usually clinical Dx). Acute inflammation, necrotic debris, fat necrosis, histiocytes, calcifications. Reactive atypia common.

Paraduodenal ("Groove") Pancreatitis—inflammation of peripancreatic soft tissue at minor ampulla. Can mimic malignancy. Mixed inflammation, fibrotic/myofibroblastic stroma, hyperplastic Brunner's glands, and debris.

Pseudocyst

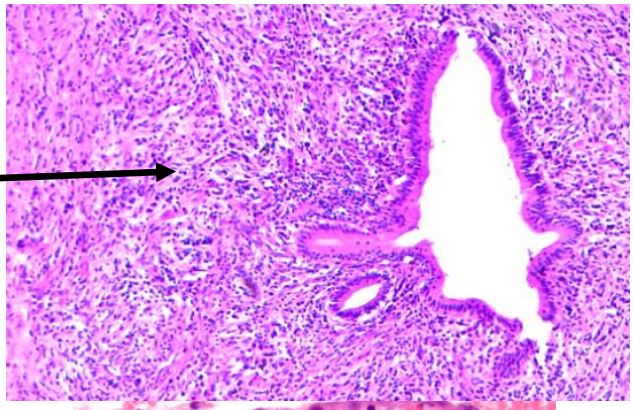


Very common pancreas mass, but often diagnosed clinically.
Fluid-filled spaces (*without* epithelial lining) resulting from pancreas **autolysis**. Often after pancreatitis and/or trauma.

Dirty, proteinaceous, necrotic background.
Inflammation: mostly lymphocytes and **histiocytes**
Debris, including: calcifications, cholesterol crystals, haematoidin.
No significant epithelium

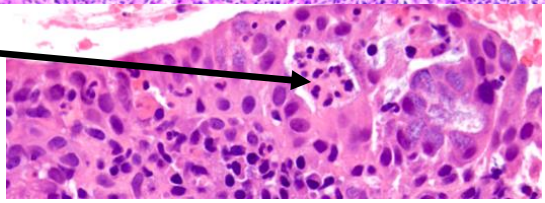
Cyst fluid contains **increased Amylase** (very high)

Autoimmune Pancreatitis



Diffuse or focal fibroinflammatory processes.
Can mimic cancer clinically (mass, jaundice).

Type 1: IgG4-related pancreatitis
3 key findings: **1) Increased plasma cells** (IgG4 positive: on biopsy >40 cells/mm^2, on resection >200 cells/mm^2),
2) Storiform fibrosis, 3)Phlebitis
More common form of autoimmune pancreatitis.
Frequently also increased serum IgG4 (~70%)

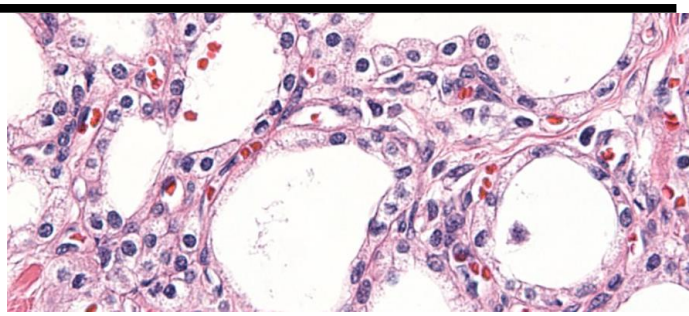


Type 2: Granulocytic epithelial lesions (PMNs in ducts and acini)

Easier to see on cell block. Dx requires clinical correlation.

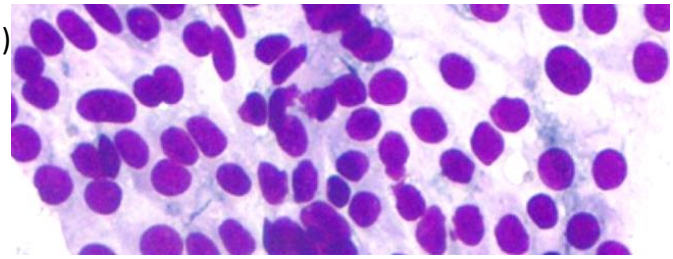
Both **treated with steroids** (*not* surgery!!)

Serous Cystadenoma



Benign non-mucinous epithelial neoplasm
Variably sized cysts lined by glycogen-rich cuboidal cells
Small, bland, round nuclei. Clear cytoplasm.

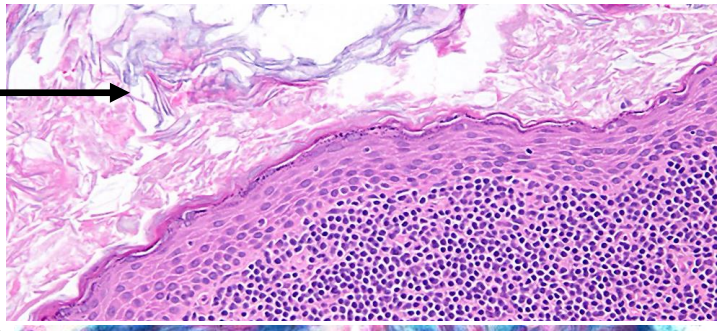
Smears are often paucicellular.
No necrosis, mucin, or atypia.
Cell blocks can be very helpful for staining!



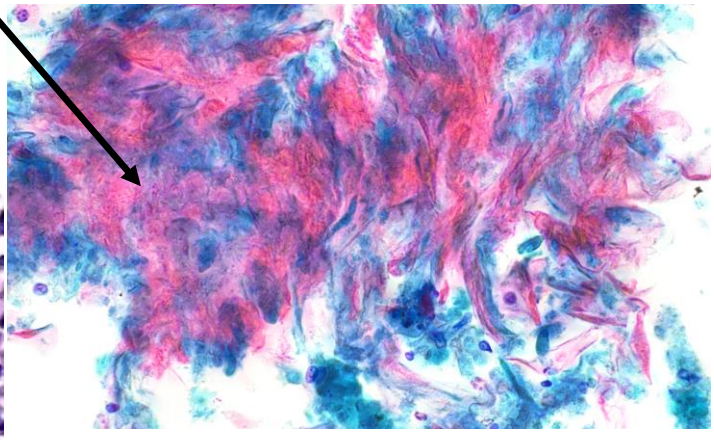
Stains: **Glycogen**→ PAS + → digested by diastase (PAS -)
(+) Inhibin, Glut1, CAIX, AE1/AE3

Often older women. Many are incidental findings.
Tumors have VHL mutations → Associated with VHL
Malignant transformation is exceedingly rare.

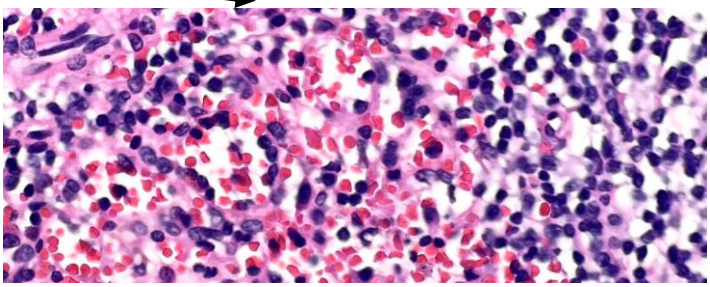
Other Lesions



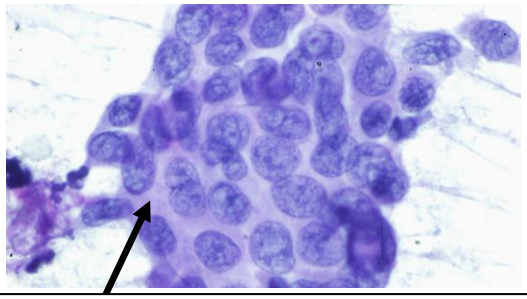
Lymphoepithelial Cyst
Cyst lined by squamous epithelium overlying benign lymphoid tissue with germinal centers. Filled with keratinaceous debris.
(similar to keratinaceous cysts in head and neck)



Splenule (Accessory Spleen)
Extra/ectopic spleen tissue
Heterogeneous lymphocytes and vascular structures
No tingible body macrophages.
CD8 can be used to highlight sinusoidal endothelial cells.



Atypical



Indeterminate category.
Cells with a spectrum of cellular atypia and/or architectural atypia that cannot be confidently classified as benign or malignant.

Often either scant sampling or only mild atypia.

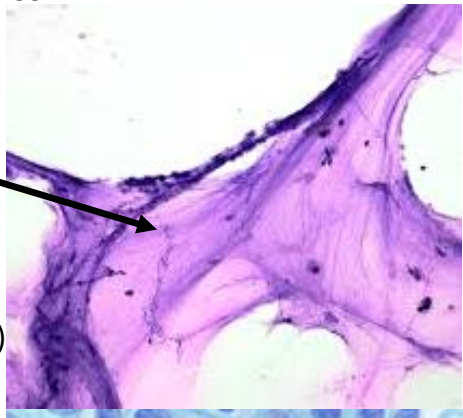
Prompts clinical reconsideration and repeat sampling if clinical concern persists

For example: Although very jumbled (architecturally complex), these cells are around all the same size and round, so if there weren't many of them, I'd likely stop at atypical.

Mucinous/Papillary Neoplasms

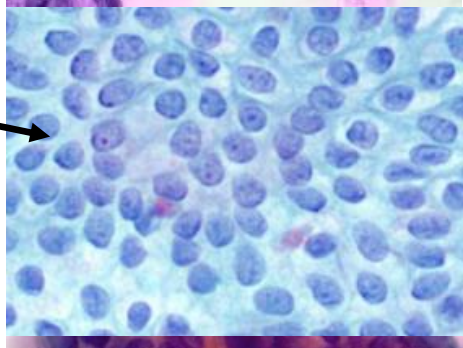
Further classified as "Pancreatobiliary neoplasm," "*Low-risk/grade*" or "*High-risk/grade*" based on atypia.

On imaging/grossly usually **cystic**. Can be seen in pancreas and/or biliary tree.

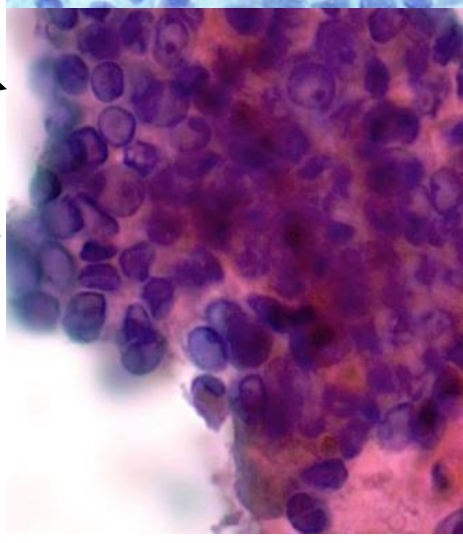


Main DDX is GI tract contamination.

Findings that favor a *neoplasm*:
1)"**Neoplastic mucin**"—thick, copious, tenacious colloid-like mucin
(clinically, they describe this as a "string sign" as it's stringy grossly)
vs. thin, wispy mucin, which could be a GI tract contaminant.
2) **Atypia** (cytologic or architectural)
3) **Papillary architecture**
4) **Cyst fluid analysis** (elevated CEA and/or specific mutations like KRAS)



Low-grade/risk: (~5-20% risk of malignancy)
Neoplastic cells look *almost* like gastric foveolar epithelium
Polarized nuclei, evenly spaced, mildly crowded
Moderate to **abundant cytoplasmic mucin**
Round to ovoid nuclei, smooth contours, and even chromatin



High-grade/risk: (~60-95% risk of malignancy)
Higher N:C ratio cells (often less mucin)
Crowded, **disorganized**
Abnormal chromatin (hyper or hypochromatic)
Small cells (compared to a 12 µm duodenal enterocyte)
Frequent background **necrosis**.
Some genetic alterations (e.g., TP53 mutations)

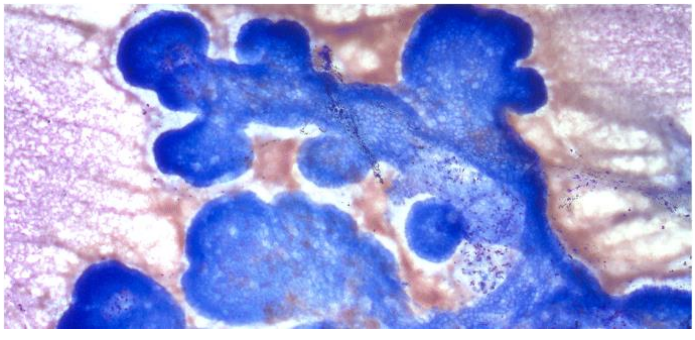
Grade based on worst component.
When in doubt grading, categorize as low grade but raise the possibility of a higher-grade lesion in a comment.

Note: Often it is challenging to definitively distinguish between these specific neoplasms on cytology specimens. It's fine to just call it a "Pancreaticobiliary Neoplasm" and grade the atypia!

Nonetheless, I've included more info below in case you get an awesome sample and feel like you can (or are studying ;-)

Intraductal Papillary Mucinous Neoplasm (IPMN)

Mucin-producing epithelial neoplasm of the pancreatic ducts (main or branch) with papillary architecture.



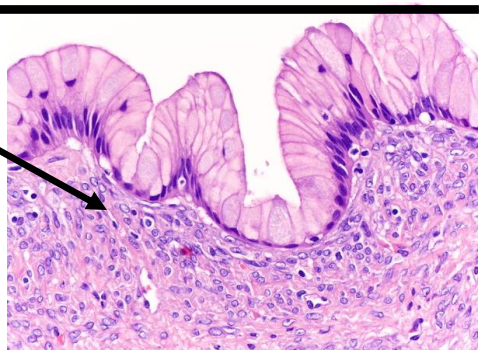
Visible on imaging or grossly
(vs PanIN, which is only seen microscopically)

Can see big papillary architecture.

Mucinous Cystic Neoplasm (MCN)

Mucin-producing epithelial cystic neoplasm with <u>ovarian-type stroma.</u>

Since ovarian stroma is necessary for this diagnosis, a good cell block/biopsy is often necessary for a definitive Dx.



Pancreatic Intraepithelial Neoplasia (PanIN)

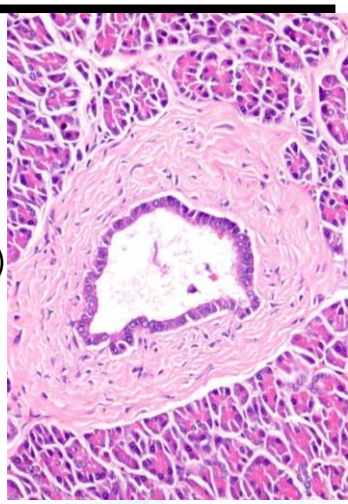
Microscopic, non-invasive epithelial neoplasm growing within pre-existing pancreatic ducts. Very common. If low-grade → very low risk of progression.

As it's microscopic, it can't be targeted and this is therefore an <u>incidental finding</u>.

<u>Low cellularity</u> of atypical cells in a <u>background of normal</u> pancreatic tissue (acini) So, make sure you see a decent quantity of epithelium before diagnosing a mucinous neoplasm or adenocarcinoma to protect against overcalling PanIN.

On cytology, likely often categorized as "Atypical"

Can also see in biliary tract: Biliary Intraepithelial Neoplasia (BilIN)



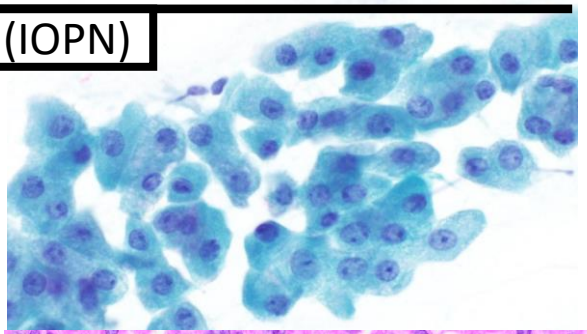
Intraductal Oncocytic Papillary Neoplasm (IOPN)

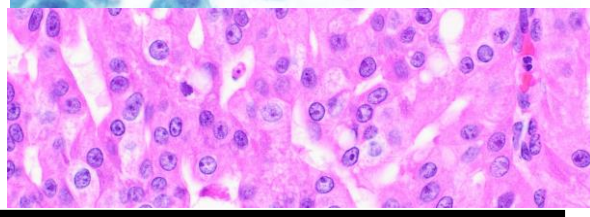
Epithelial neoplasm with complex papillae lined by oncocytic epithelium that is mucin depleted.

Hypercellular smears. ***<u>No</u>*** significant mucin.
Polygonal, oncocytic cells with granular cytoplasm

Genetic rearrangements of PRKACA/B

Although cytologically/architecturally high-grade, only 1/3 of cases are invasive and even those are relatively indolent.



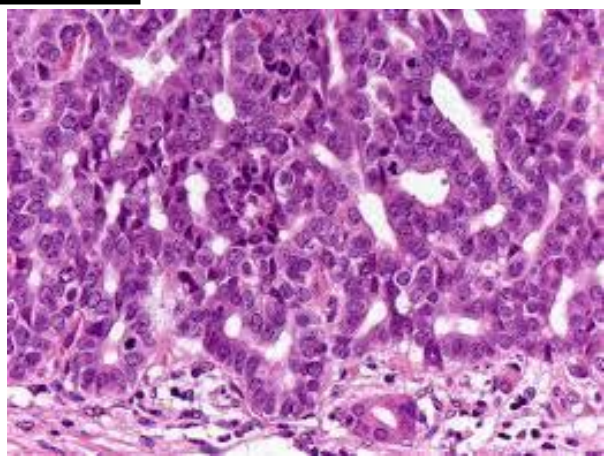


Intraductal Tubulopapillary Neoplasm (ITPN)

Intraductal epithelial neoplasm with ductal differentiation, high-grade dysplasia, lacking overt mucin production.

Back-to-back tubules (best seen on cell block)
Cuboidal tumor cells with no mucin
High-grade atypia
Hypercellular smears with NO significant mucin

Frequently associated with invasive cancer, but still more indolent.



Suspicious for Malignancy

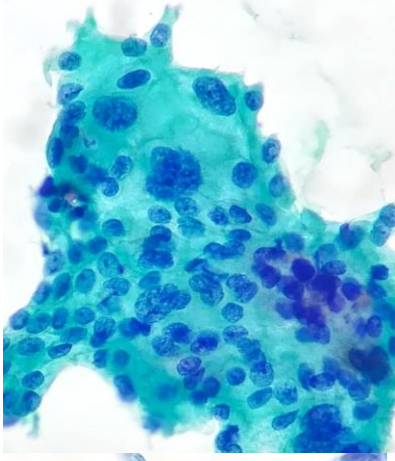
Used when a specimen has some features of malignancy, but insufficient quantity/quality of findings for a definitive diagnosis.

Often used in the setting of limited/focal atypia, well-differentiated adenocarcinoma, or unusual clinical scenario to avoid false positive.

Can also use when there are underlying inflammatory/reactive changes, which can cause atypia.
For example, when a biliary stent is present, I consider downgrading my diagnosis
(e.g., Malignant→ Suspicious)

Malignant

Pancreatic Ductal Adenocarcinoma *(PDAC)*



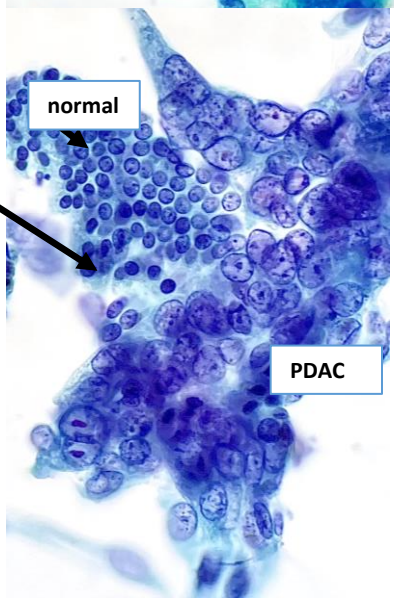
Invasive pancreatic epithelial neoplasm with glandular and ductal differentiation.
Often locally advanced or metastatic at diagnosis→ *unresectable*

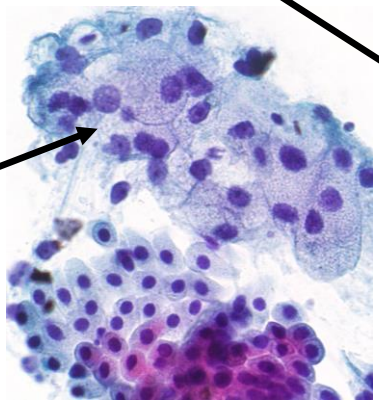
Unless you have a good core that allows you to see infiltration/invasion, correlation with clinical findings is essential. PDAC usually forms an **irregular, solid mass**. If the lesion is purely cystic, consider high-grade dysplasia in a cystic neoplasm (see prior section). If well-circumscribed, consider non-adenocarcinoma Dx's below.

On Bx: Disorganized, irregular, infiltrating glands with desmoplastic stroma

The Criteria I use:

1) **Pleomorphic nuclei** (anisonucleosis) with at least **4:1** variation in cell size
2) **Architectural disarray** ("drunken honeycomb")
3) **Irregular nuclear contours**
4) *Isolated* malignant cells





Possible additional features
Cellular smears with crowded tissue fragments
Mucinous cytoplasm (sometimes foamy)
Hyper or hypochromatic nuclei
± Nucleoli, Necrosis

(Because I require 4:1 size variation, I often find Pap stained smears the most helpful/reliable as there is less "air dry" artifact.)

Specific subtypes to recognize:
Adenosquamous carcinoma—both glandular and squamous components (≥30% squamous). Aggressive.
Colloid carcinoma—>80% of the epithelium is suspended in extracellular mucin pools.
Undifferentiated carcinoma, anaplastic subtype—discohesive, single large pleomorphic tumor cells with abundant cytoplasm. Rhabdoid features. Occasional CK+ giant cells, Loss of E-cadherin.
Undifferentiated carcinoma, Sarcomatoid type—Pleomorphic spindle cells with high N:C ratios.
Undifferentiated carcinoma with Osteoclast-like giant cells—benign osteoclast-like giant cells admixed with variable carcinoma component.

(NOTE: On a biopsy you can't be definitive about one of these subtypes, but you could suggest it)

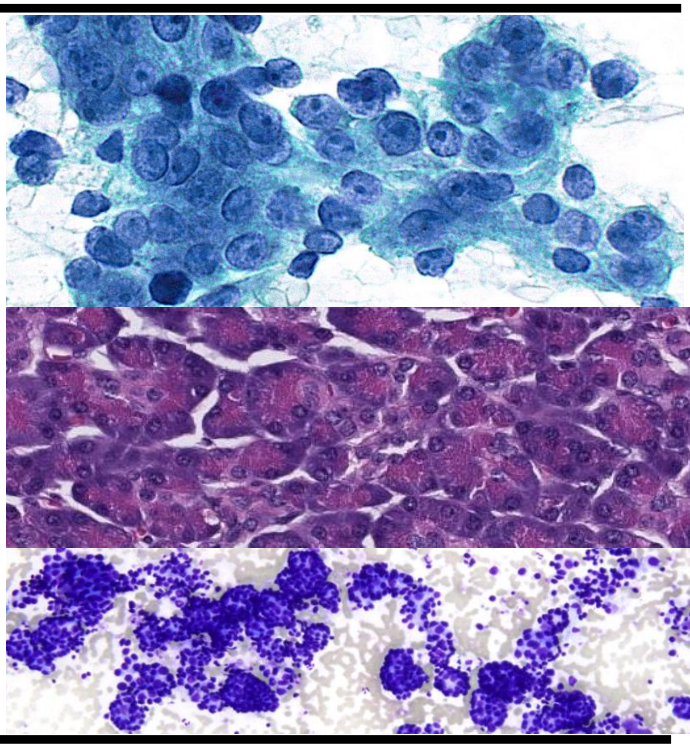
Cholangiocarcinoma

Primary adenocarcinoma arising from the bile duct.

Use the same criteria as for PDAC (above). Essentially, a similar disease as PDAC (adenocarcinoma of ductal epithelium, just in the biliary tree instead of the pancreas).

Particularly if there are stents/stones, have a high threshold for malignancy as these can cause considerable reactive/reparative changes.

Pancreatic Acinar Cell Carcinoma



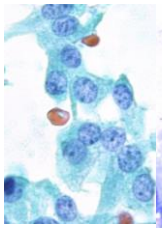
Malignant pancreatic epithelial neoplasm with acinar cell differentiation.

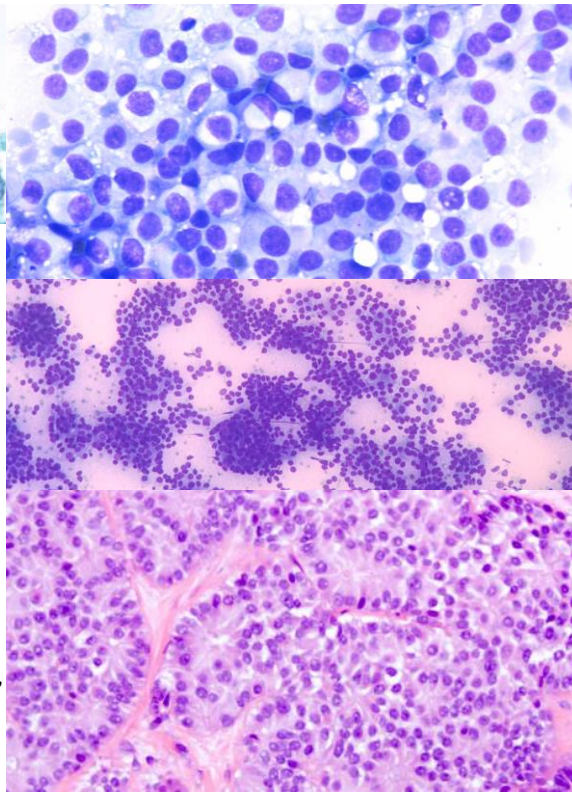
Classically older men.
Lipase hypersecretion (rare) → subcutaneous fat necrosis, fever

Hypercellular smears. 3D fragments and single cells.
Lobulated/**acinar architecture.**
Large nuclei with prominent nucleoli.
Eccentric **granular cytoplasm** (full of zymogens)
Granular background (ruptured cell zymogens)

IHC: **(+)Trypsin, Chymotrypsin, and BCL10**
(-) Neuroendocrine markers (vs PNET); only patchy nuclear β catenin (vs diffuse nuclear in SPN).
Ki67 10-50%

Neuroendocrine Tumor





Well-differentiated tumors with neuroendocrine differentiation. (aka PanNET)

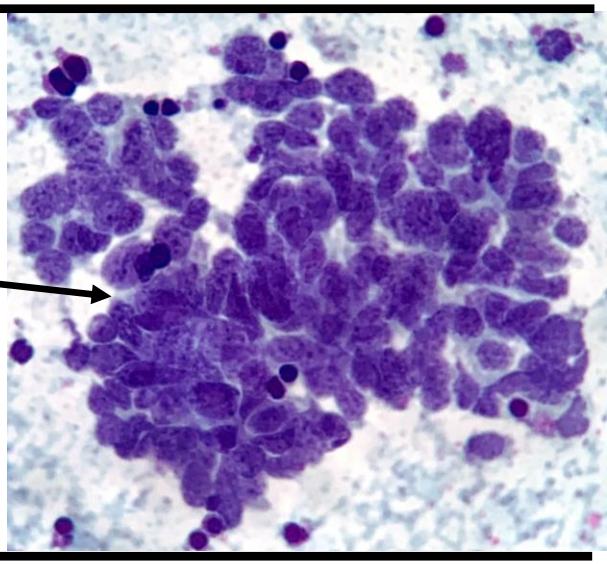
Indolent, malignant, slow-growing tumors. >5mm.
(<5mm = microtumor/microadenoma)
Can be functional (hormone-secreting, often discovered earlier due to symptoms) or non-functional.

Loosely cohesive cells.
Epithelioid to plasmacytoid.
Round nuclei with stippled "salt and pepper" chromatin.
Dense, granular cytoplasm.
Cellular smears. No necrosis.

Bx: Nested and/or trabecular architecture
IHC: **(+)Synaptophysin, Chromogranin**

Can attempt to *provisionally* grade on Bx/FNA
G1= <3% Ki67 (<2 mits/mm^2), G2= 3-20% Ki67 (2-20mits/mm^2), G3=>20% Ki67 (>20 mits/mm^2).
(True grading requires examination of the whole tumor and counting in a hotspot)

Neuroendocrine Carcinoma



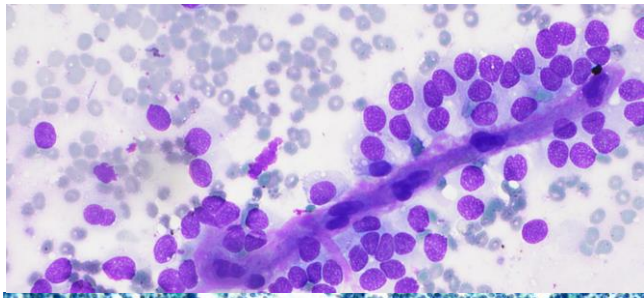
High-grade malignant neoplasm with neuroendocrine differentiation (aka PanNEC) *(similar cells to lung NEC!)*

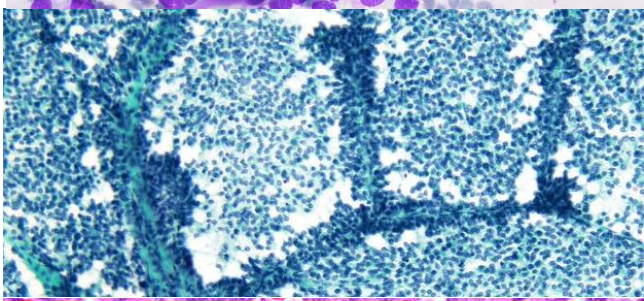
Small cell type:
Angulated, pleomorphic nuclei with molding.
Hyperchromatic, coarsely stippled chromatin
Minimal cytoplasm (high N:C ratio)
Abundant mitoses, necrosis, and apoptotic bodies

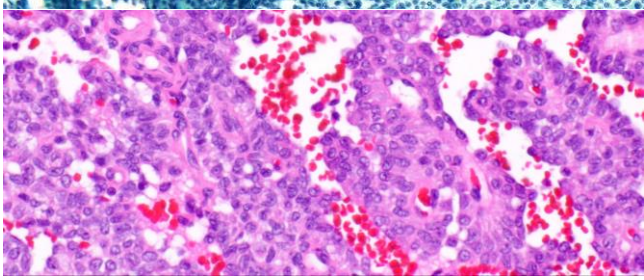
Large cell type:
Large nuclei with prominent nucleoli
Abundant cytoplasm. Anisonucleosis. Necrosis.

Ki67 > 20% and/or mitoses >20/mm^2

Solid Pseudopapillary Neoplasm







Low-grade malignant pancreatic tumor composed of poorly cohesive epithelial cells forming solid and pseudopapillary structures that lack a specific line of differentiation. *(aka SPN)*

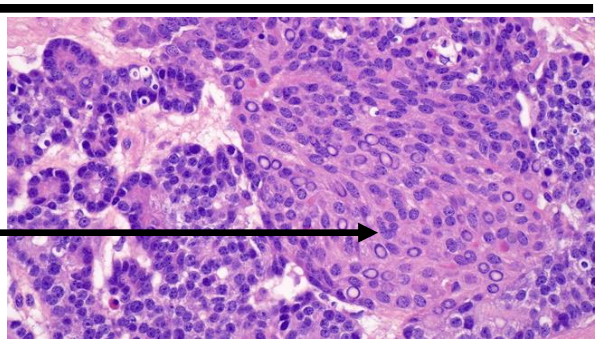
Classically **young women** in the body/tail.

Neoplastic cells cling to branching papillary fonds with central vessels.
Monomorphic bland polygonal/plasmacytoid cells
Round to convoluted nuclei with some **grooves**
Indistinct cytoplasmic borders.
Often vacuolated cytoplasm.
Hyaline globules and myxoid stroma

IHC: **(+) nuclear β-catenin** (diffuse, strong), CD99 (perinuclear dot-like), PR, CD10;
(-) E-cadherin; Ki67 usually low (~3%)

Warning: may express some neuroendocrine markers, so do a panel (see next page)

Pancreatoblastoma



Rare malignant epithelial neoplasm with trilineage (i.e., acinar, neuroendocrine, or ductal) differentiation with squamoid nests.

Acinar growth predominates. Squamoid nests are distinguishing feature. Hard to diagnose by cytology!

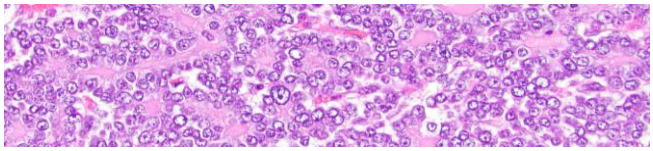
Most common in **children**.

Metastases

Most common source: Renal cell carcinoma.
Other primary sites: lung, GI tract, melanoma, and breast.

IHC Panel for solid, "blue" pancreatic tumors:

Main DDX: SPN, PanNET, Acinar cell carcinoma, Pancreatoblastoma. Also, potentially, ITPN.



Antibody	Pattern	Solid Pseudopapillary Neoplasm	Neuroendocrine Tumor	Acinar Cell Carcinoma	Pancreatoblastoma
AE1/AE3	Cytoplasmic	+ (focal)	+	+	+
AR	Nuclear	+	−/+	−	−
BCL10	Cytoplasmic	−	−	+	+
CD99	Dot-like paranuclear	+	−	−	−
Chymotrypsin	Cytoplasmic	−	−	+	+
Cyclin D1	Nuclear (100%)	−	−	+	+ (squamoid corpuscles)
E-cadherin	Nuclear/cytoplasmic or absent	+	−/+	−	−
LEF1	Nuclear (95%)	+	−	−	−
PR	Nuclear	+	−/+	−/+	+ in stromal components
Synaptophysin	Cytoplasmic	+ (focal)	+	−	−/+
TFE3	Nuclear (75%)	+	−/+	−/+	−
Trypsin	Cytoplasmic	−/+	−/+	+	+

***Possible starting panel**:* Synaptophysin, Chromogranin, Chymotrypsin, β-catenin.

Modified from: WHO Reporting System for Pancreatobiliary Cytopathology

Ancillary Testing

Cyst Fluid Analysis

ROSE not recommended for cyst fluid (low cellularity→ wastes fluid)

Ideally send for:

1) **CEA** → increased in mucinous cysts (cutoff usu. 192 ng/mL)
2) **Amylase** → increased in pseudocysts (<250 U/L makes pseudocyst unlikely)
3) **Molecular profiling**

Mutations and their associations:

KRAS → neoplastic mucinous cyst
GNAS → supports an IPMN, specifically
P53, SMAD4 or CDKN2A [P16] deletion → "high risk"
mTOR pathway (PIK3CA, PTEN, ATK1)→ "high risk"
VHL→ Serous cystadenoma
MEN1→ PanNET
CTTNNB1→ Solid Pseudopapillary Neoplasm
PRKACA/B fusion→ IOPN

In situ Hybridization

FISH can look for aneuploidy, deletions, amplifications, and rearrangements in a small number of cells. Frequently performed on bile duct brushings of strictures→ esp. useful if inflamed!

Many just use the "UroVysion" FISH probes used to look for urothelial carcinoma in urine.
→ has probes for CDKN2A, chromosomes 3, 7 and 17
→ chromosomal rearrangements/aneuploidy and/or CDKN2A deletions support cancer.

Pancreas-specific probe kits also exist and include TP53, EGFR, etc…