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Fine-Needle Aspiration Biopsy of Pancreatic Lesions: WHO Reporting System and Diagnostic Strategy

Guoping Cai, MD

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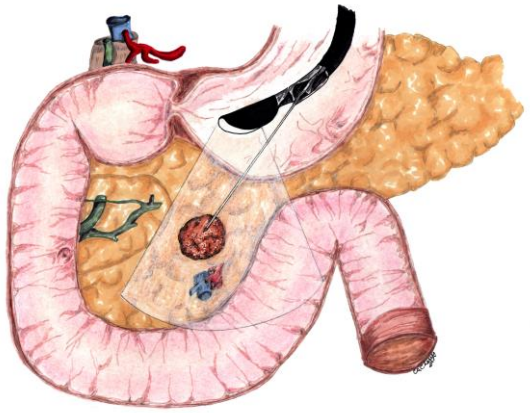
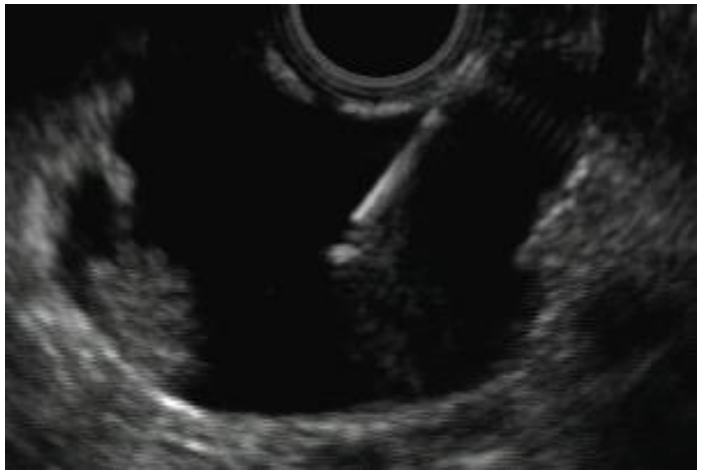
Preoperative Assessment

- Presumptive diagnosis (based on clinical and imaging findings)
- Intraoperative consultation
  - Intraoperative frozen section diagnosis
  - Intraoperative needle aspiration/biopsy
- Preoperative diagnosis
  - Transabdominal FNA or biopsy under CT guidance
  - Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA)
  - Endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB)

Fine-Needle Aspiration Biopsy

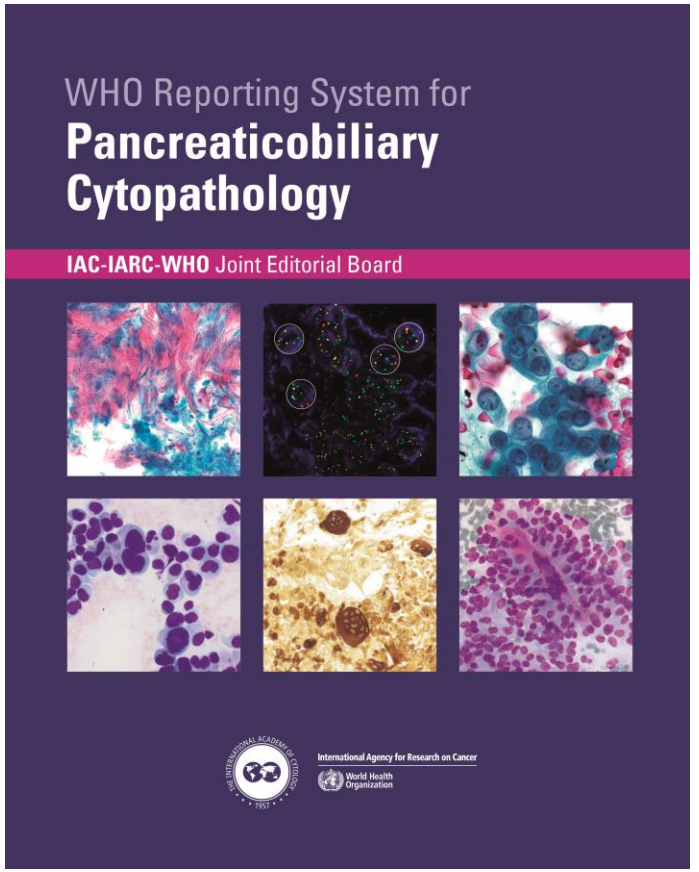
- Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is the choice for diagnostic workup of pancreatic lesions.
  - High sensitivity: 80-85%
  - High specificity: 95-99%
  - Rare complications: < 0.5%
    - Bleeding
    - Pancreatitis





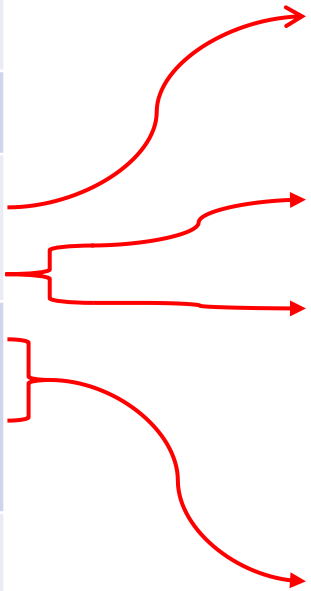
WHO Reporting System

I. Insufficient/inadequate/nondiagnostic
II. Benign/negative for malignancy (non-neoplastic and neoplastic processes)
III. Atypical
IV. Pancreaticobiliary neoplasm, low-risk/grade (PaN-low)
V. Pancreaticobiliary neoplasm, high-risk/grade (PaN-high)
VI. Suspicious for malignancy
VII. Malignant



PSC vs WHO Reporting Systems

PSC Reporting System	WHO Reporting System
1. Nondiagnostic	1. Nondiagnostic
2. Negative for malignancy	2. Benign/negative for malignancy Non-neoplastic Neoplastic
3. Atypical	3. Atypical
4A. Neoplastic: Benign	4. Pancreaticobiliary neoplasm, low-grade
4B. Neoplastic: IPMN/MCN Neuroendocrine tumor Solid pseudopapillary tumor	5. Pancreaticobiliary neoplasm, high-grade
5. Suspicious for malignancy	6. Suspicious for malignancy
6. Malignant	7. Malignant



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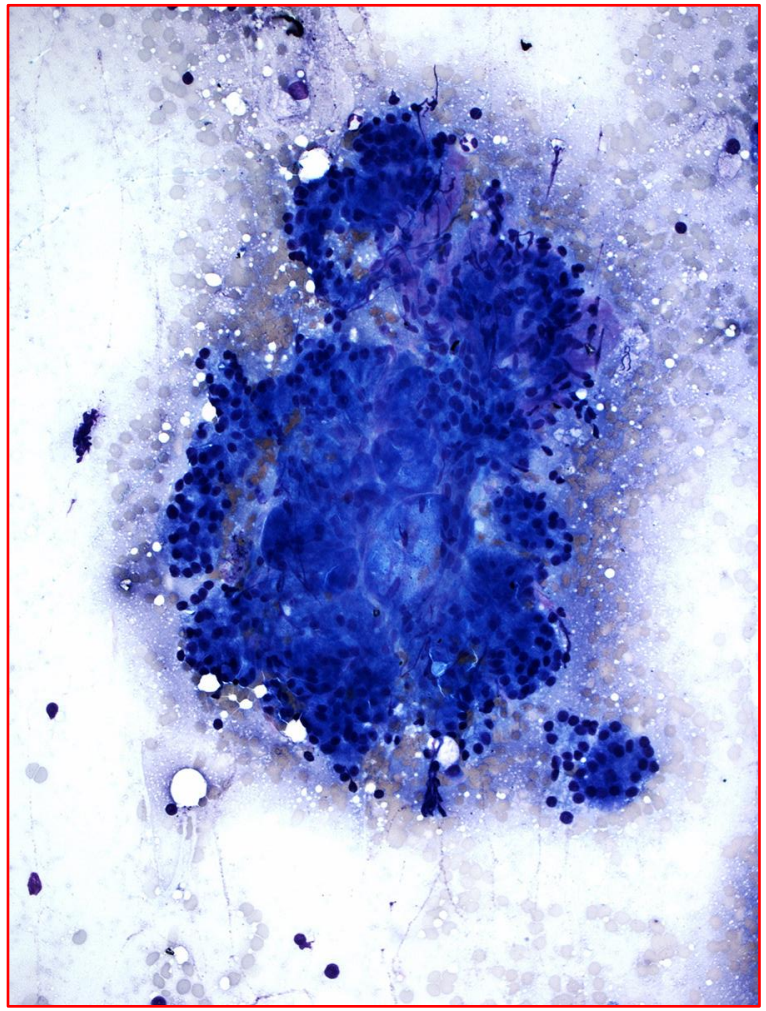
Risk of Malignancy & Management

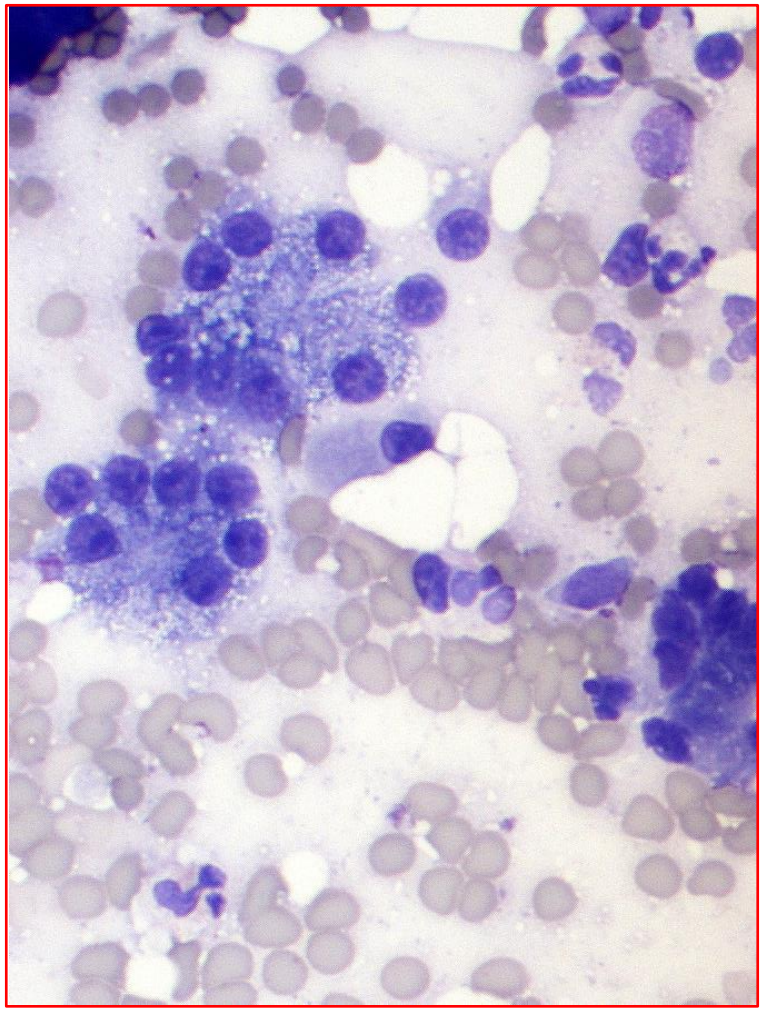
Diagnostic category	Estimated ROM[a]	Clinical management options[b]
Insufficient/inadequate/non-diagnostic	5–25%	Repeat FNAB
Benign / negative for malignancy	0–15%	Correlate clinically
Atypical	30–40%	Repeat FNAB
Pancreaticobiliary neoplasm, low-risk/grade (PaN-low)	5–20%	Correlate clinically
Pancreaticobiliary neoplasm, high-risk/grade (PaN-high)	60–95%	Surgical resection in surgically fit patients; conservative management optional
Suspicious for malignancy	80–100%	If patient to be surgically managed, treat as positive; if patient requires preoperative therapy, repeat FNAB
Malignant	99–100%	Per clinical stage

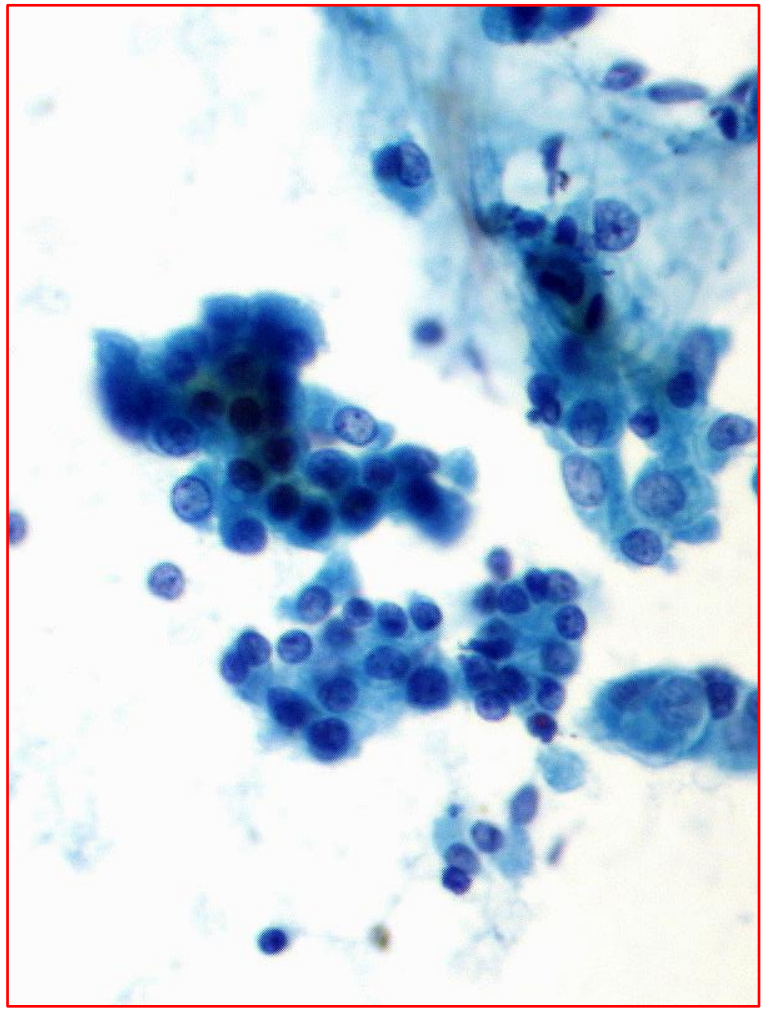
Non-diagnostic

- A specimen categorized as non-diagnostic is one that for qualitative and/or quantitative reasons does not permit a diagnosis of the targeted lesion.
  - Obscuring/preparation artifacts
  - Gastrointestinal epithelium only
  - Acellular aspirate of a solid mass
  - Acellular aspirate of a cystic mass (without thick mucin, cyst fluid analysis or molecular testing)
  - Normal elements only in the setting of a clearly defined solid or cystic mass

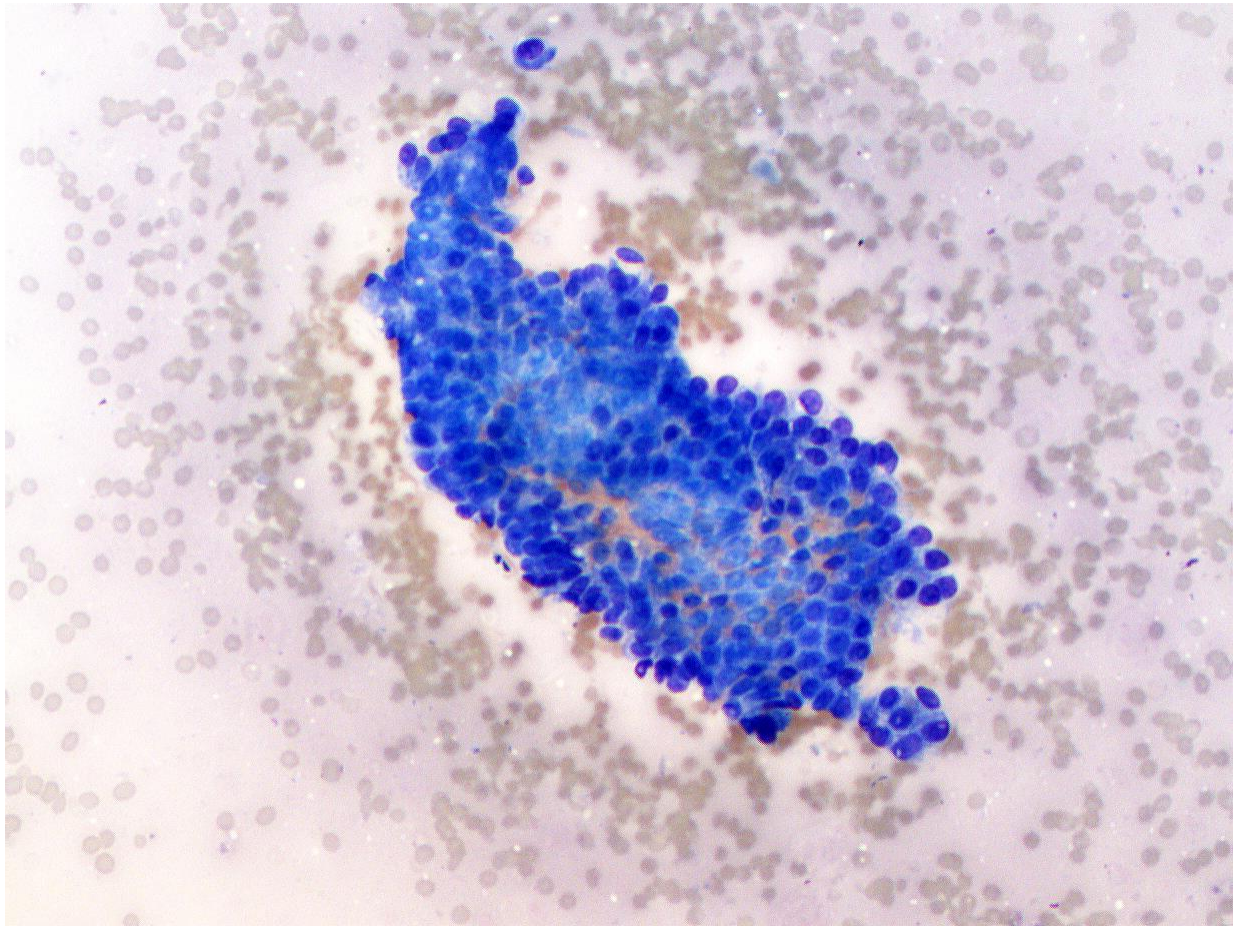
Pancreatic Acinar Cells

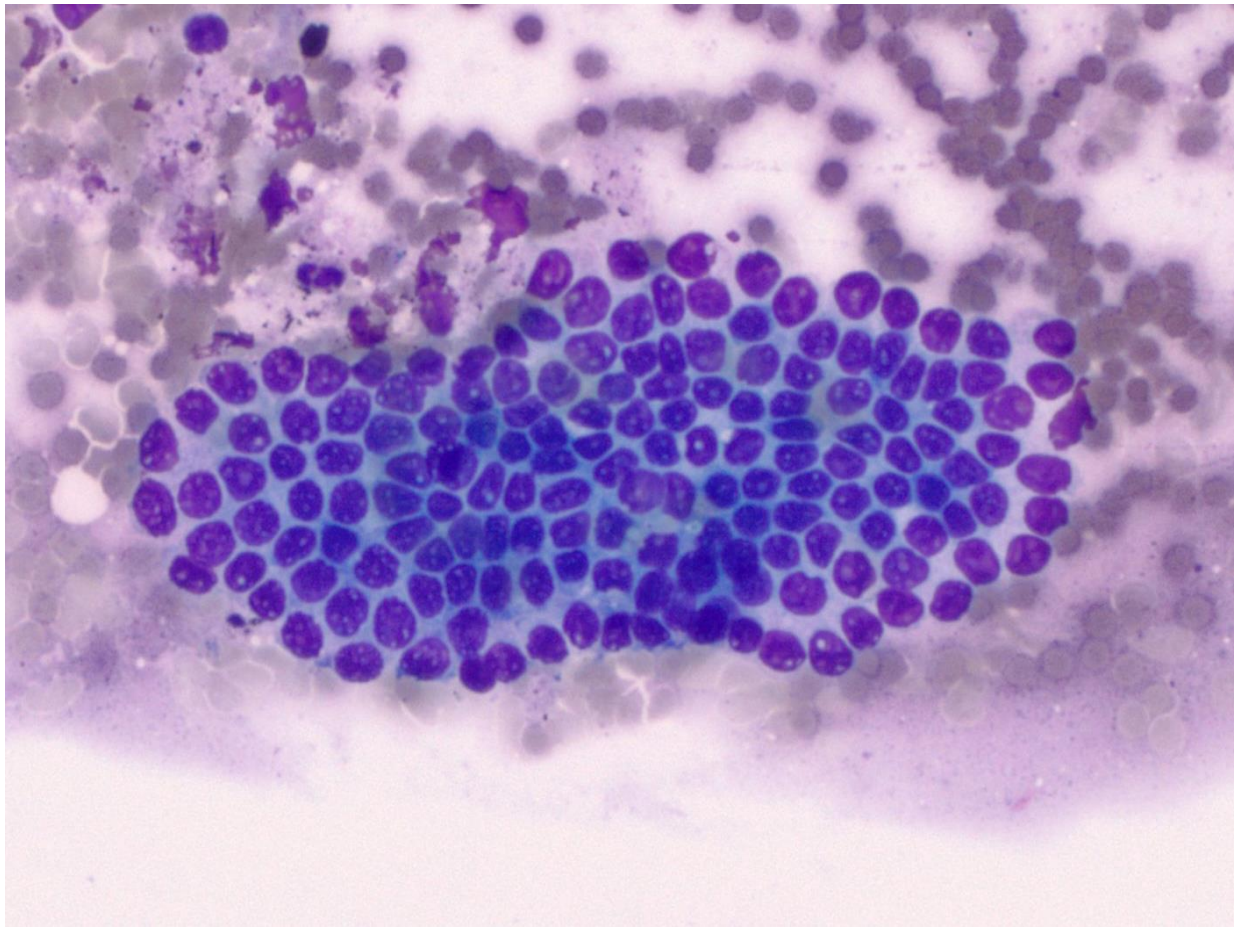






Pancreatic Ductal Cells





Atypical

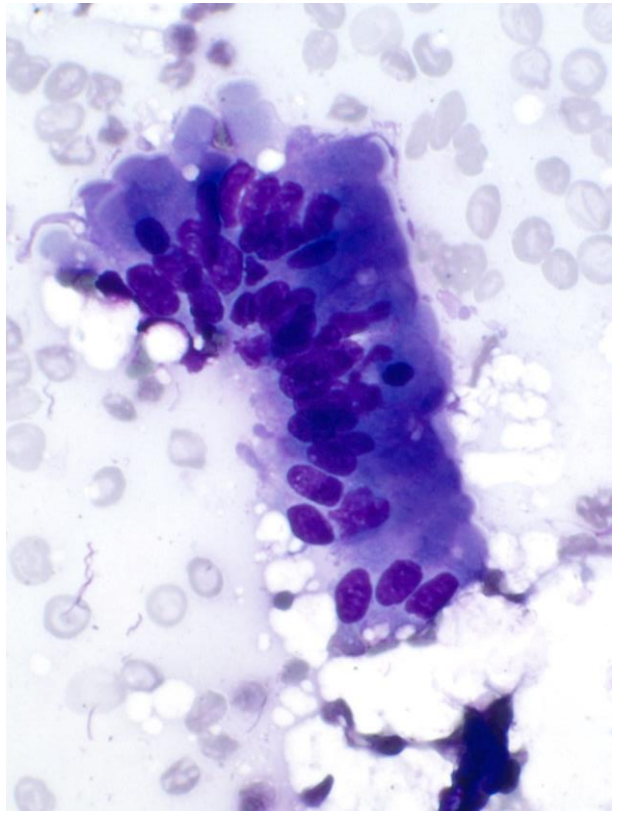
- An atypical specimen demonstrates features predominantly seen in benign lesions and minimal features that cannot exclude the possibility of a malignant lesion, but with insufficient features either in number or quality to diagnose a process or lesion as:
  - benign
  - pancreaticobiliary neoplasm, low-grade (PaN-low)
  - pancreaticobiliary neoplasm, high-grade (PaN-high)
  - suspicious for malignancy
  - malignant

Atypical

- Possible causes:
  - Low cellularity
  - Lack of rapid on-site evaluation, limited sampling
  - Poor specimen preparation or preservation artifacts
  - Lack of sufficient material for ancillary tests
  - High threshold for a malignant diagnosis
- Not a waste basket !!!

Atypical

- An atypical diagnosis should include:
  - Multidisciplinary discussion
  - Consensus review
  - Expert consultation
  - Use of ancillary tests
  - Repeat sampling with rapid on-site evaluation
- Ancillary tests:
  - Cyst fluid analysis
  - Molecular testing



Suspicious for Malignancy

- A specimen categorized as “suspicious for malignancy” demonstrates some cytopathological features suggestive of malignancy, but with insufficient features either in number or quality to make an unequivocal diagnosis of malignancy.

ATYPICAL	SUSPICIOUS
Favor benign, cannot exclude malignancy	Favor malignant, cannot exclude reactive

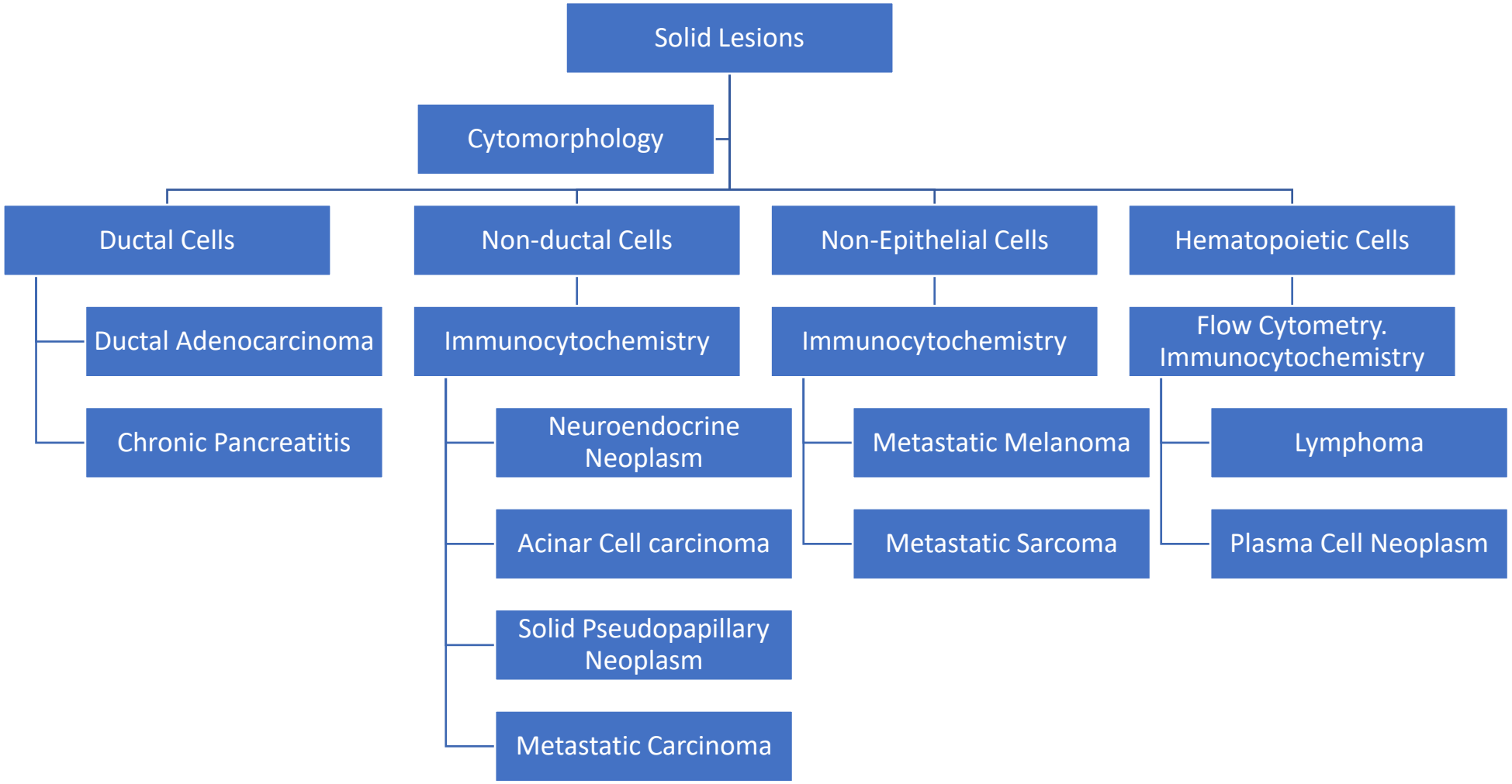
Suspicious for Malignancy

- Well-differentiated adenocarcinoma: when all cytopathological criteria are not present and/or a limited number of lesional cells are identified.
- Neoplastic mucinous cysts: a cyst demonstrates high-grade epithelial atypia in a background of necrosis and high-risk imaging features, findings that raise concern for invasive carcinoma.
- Inadequate material for confirmatory ancillary studies for the characterization of malignancies such as neuroendocrine tumor or acinar cell carcinoma.
- Insufficient tissue for the confirmation of a possible metastasis, lymphoma, or poorly differentiated neuroendocrine carcinoma.

Common Pancreatic Lesions

- Solid/neoplastic
  - Adenocarcinoma
  - Neuroendocrine neoplasm
  - Acinar cell neoplasm
  - Pancreatoblastoma
  - Solid pseudopapillary neoplasm
  - Secondary tumors
- Solid/nonneoplastic
  - Chronic pancreatitis
  - Autoimmune pancreatitis
  - Accessary spleen/splenule
- Cystic/neoplastic
  - Serous cystadenoma
  - Intraductal papillary mucinous neoplasm
  - Mucinous cystic neoplasm
  - malignant tumors undergoing cystic changes
- Cystic nonneoplastic
  - Pseudocyst
  - Lymphoepithelial cyst
  - Duplication cyst

Diagnostic Approach for Solid Lesions



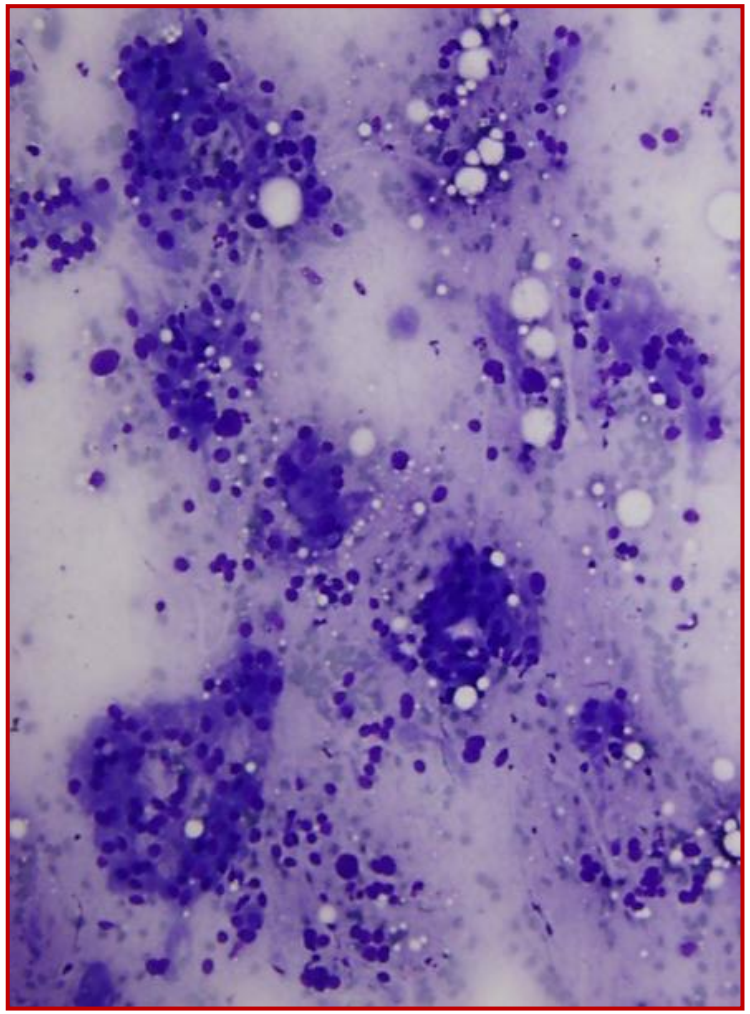
Ductal Adenocarcinoma

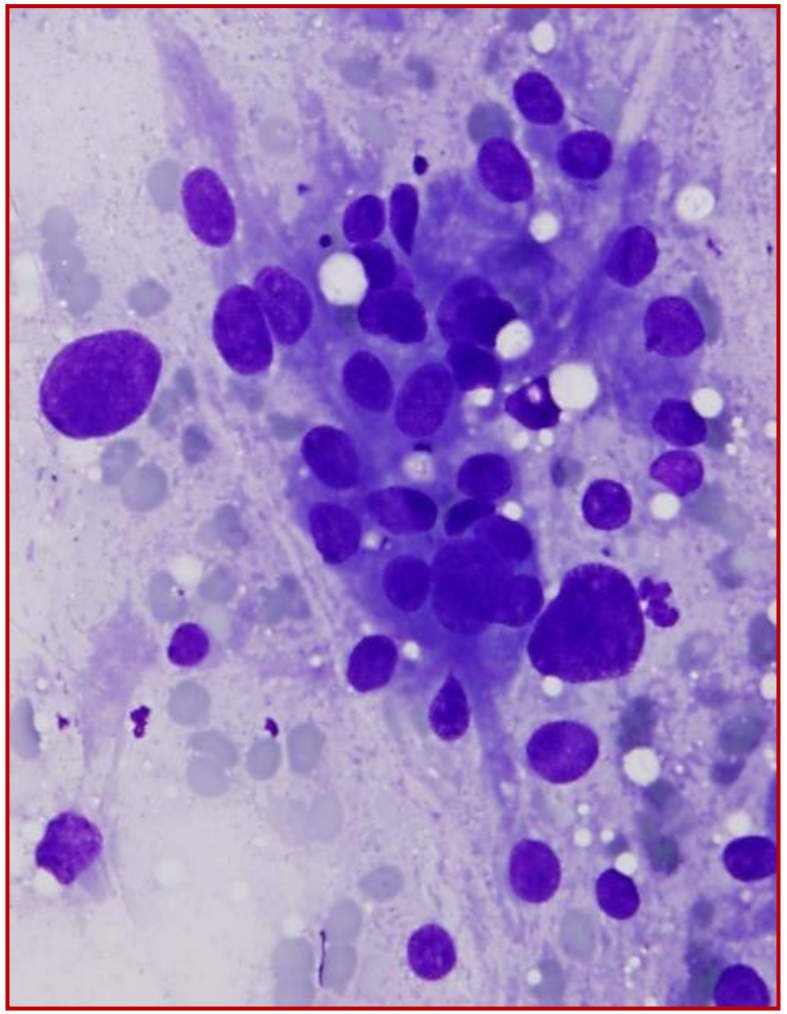
- The diagnosis of pancreatic ductal adenocarcinoma is usually straightforward when sufficient cellular material is present.
- In cases that are indeterminate, ancillary studies may help to provide a diagnosis of malignancy.
- Ancillary studies are essential in patients with:
  - a history of previous malignancy and possible metastases to the pancreaticobiliary tract
  - undifferentiated malignancies
  - neoplasms with cytopathological features of a non-ductal neoplasm.

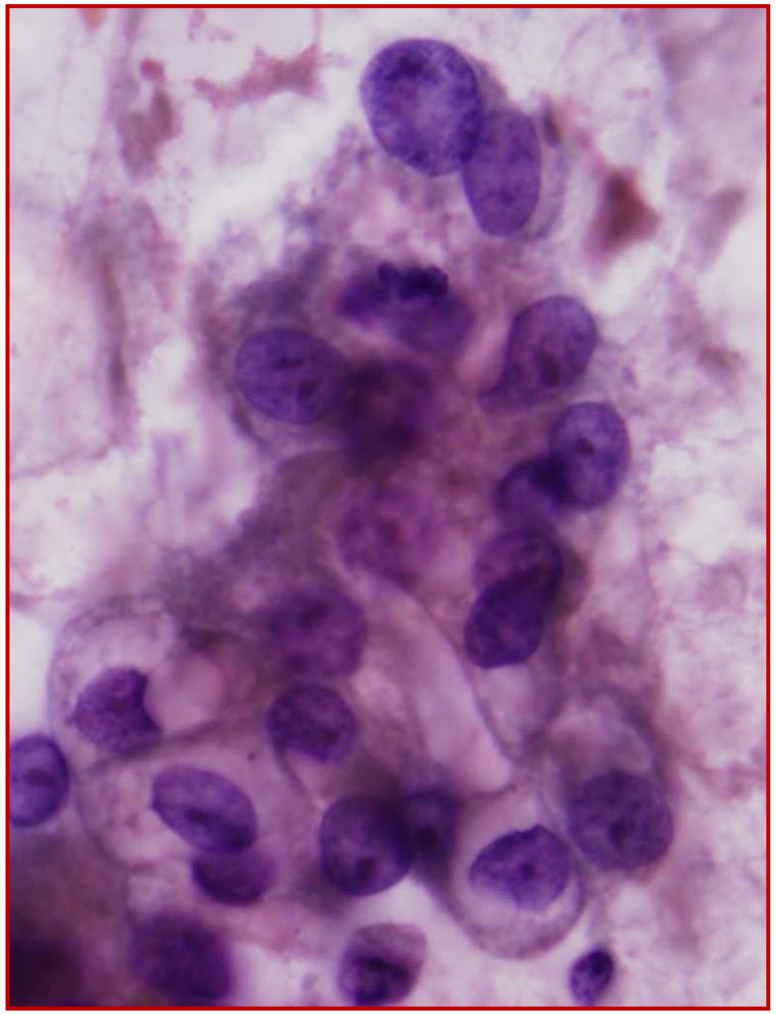
Ductal Adenocarcinoma

- Moderate to high cellularity with predominantly ductal cells
- Disorganized clusters with nuclear crowding or overlapping
- Isolated/single atypical cells
- Nuclear atypia:
  - Anisonucleosis, nuclear enlargement, irregular nuclear contours, coarse or clearing chromatin, mitotic figures
- Background: clean, inflammatory, mucinous or necrotic

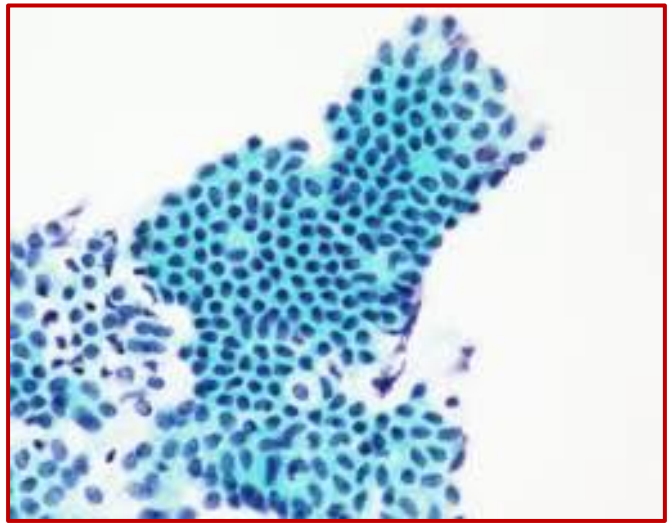
Ductal Adenocarcinoma

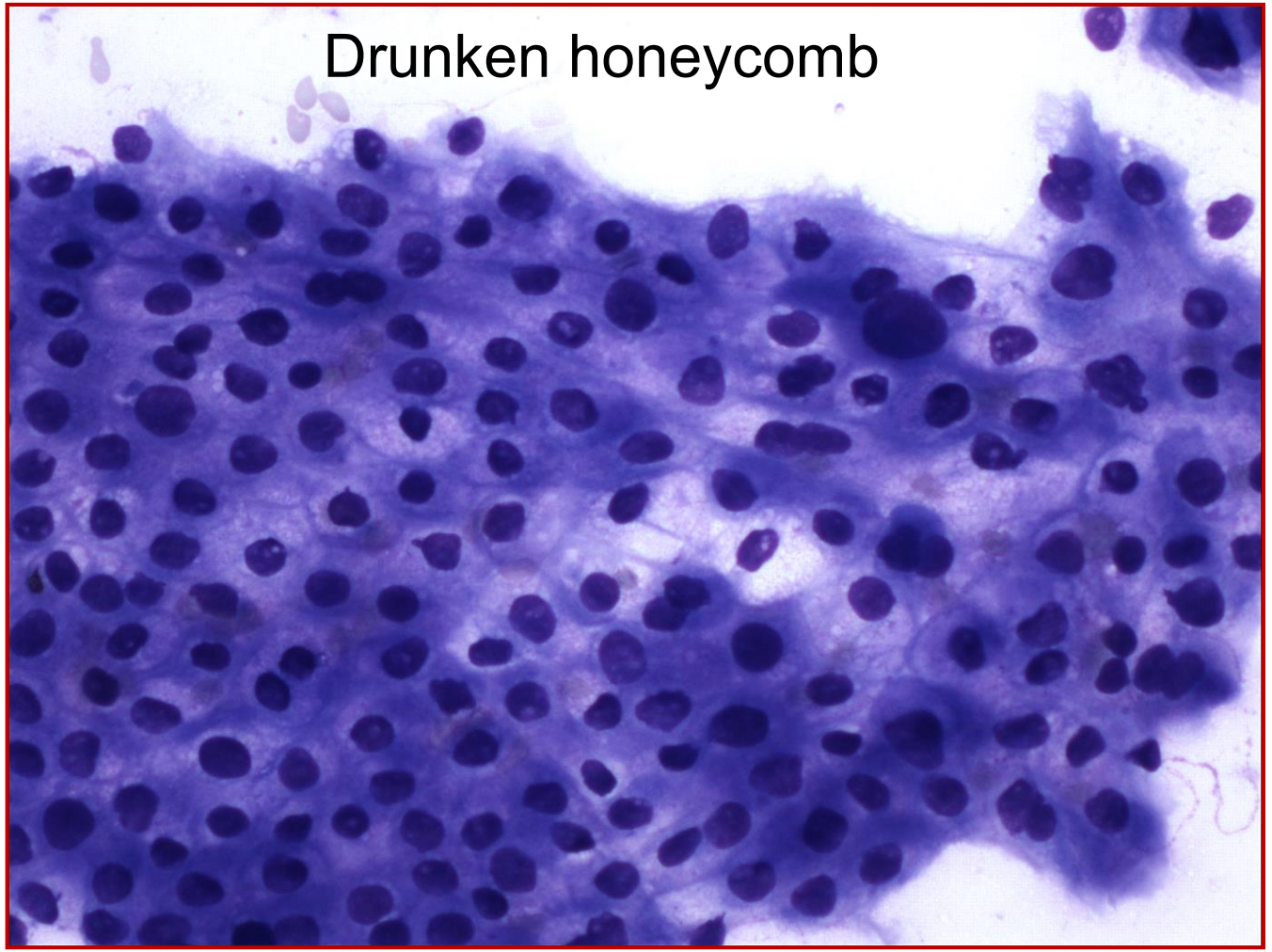






Well-Differentiated Adenocarcinoma

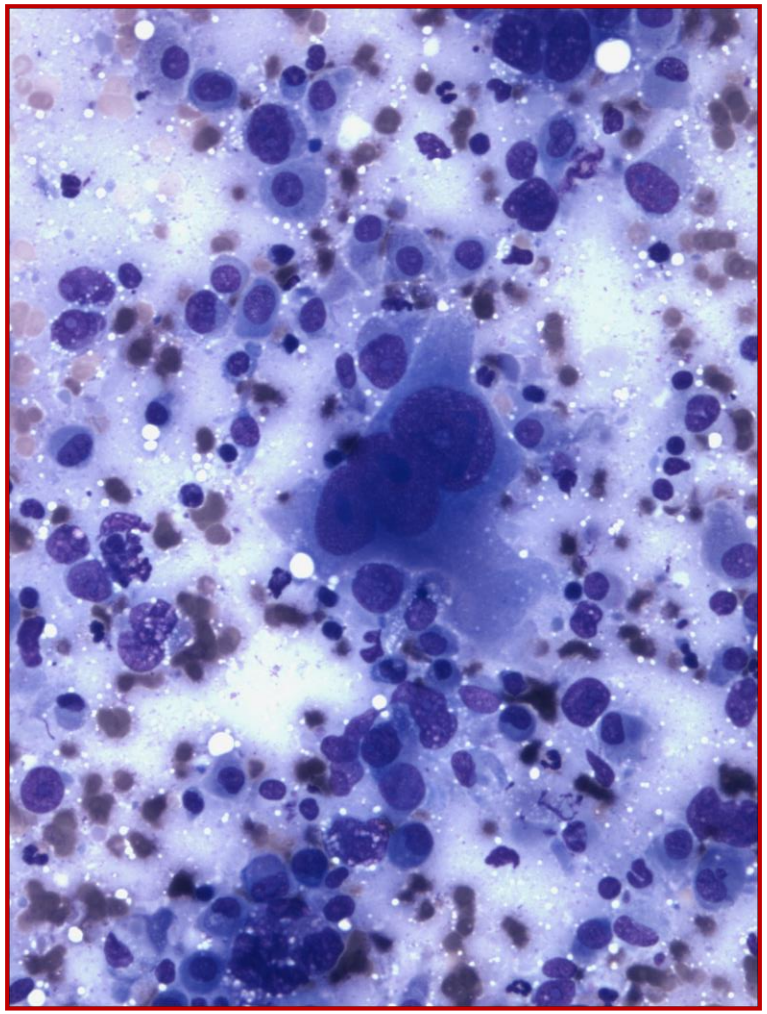


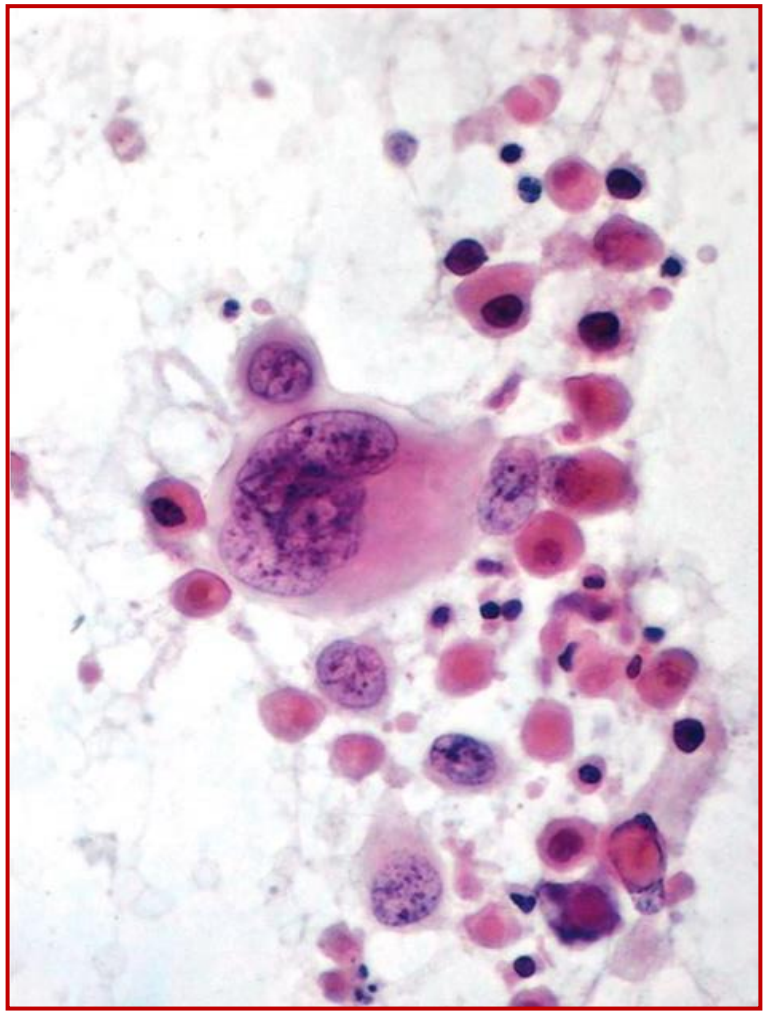


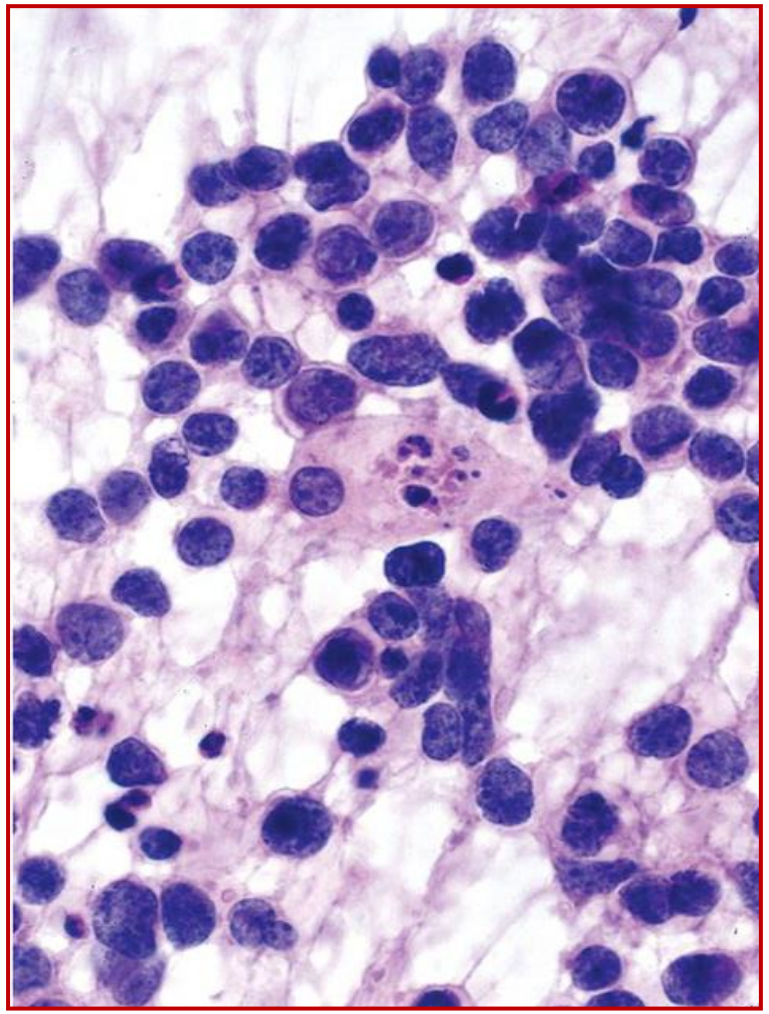
Adenocarcinoma vs Chronic Pancreatitis

	Adenocarcinoma	Chronic Pancreatitis
Cell type	Predominantly ductal cells	Mixed ductal, acinar, and islet cells
Architecture	Monolayered sheets with disorganization, nuclear crowding or overlapping	Monolayered sheets with uniformly spaced nuclei
Single cells	Scattered	Rare or no
Nuclei	Abnormally shaped nuclei with irregular nuclear contours	Round to oval nuclei with smooth nuclear contours
Chromatin	Chromatin clearing or coarse	Even chromatin
Nucleoli	Prominent nucleoli	Small nucleoli

Variants of Ductal Adenocarcinoma

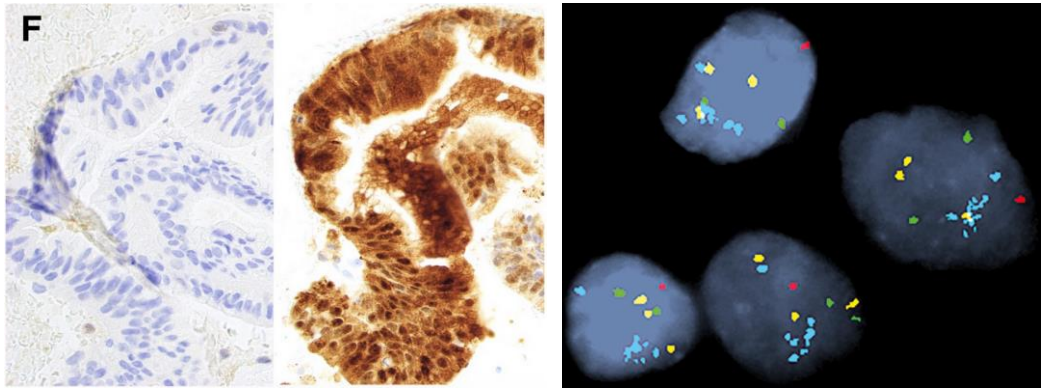


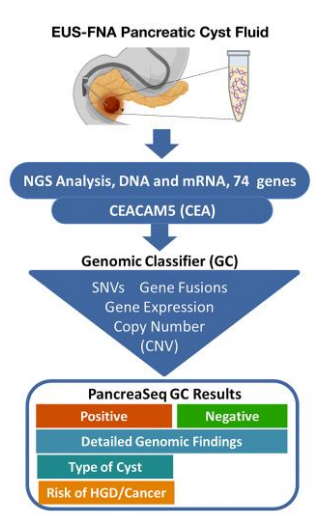


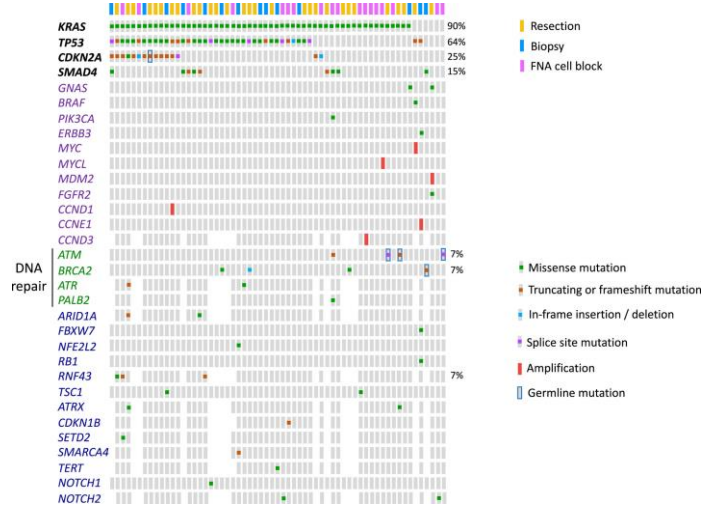


Ancillary Testing

- Molecular-derived testing
  - Immunocytochemistry: SMAD4, p53, S100P, IMP3, mesothelin, p16
  - Fluorescence in situ hybridization (FISH): UroVysion (3, 7, 17, 9p), PB-FISH (1, 7, 8, 9p)
  - Polymerase chain reaction (PCR): *KRAS, GNAS*
  - Next generation sequencing (NGS)
  - LOH or microRNA







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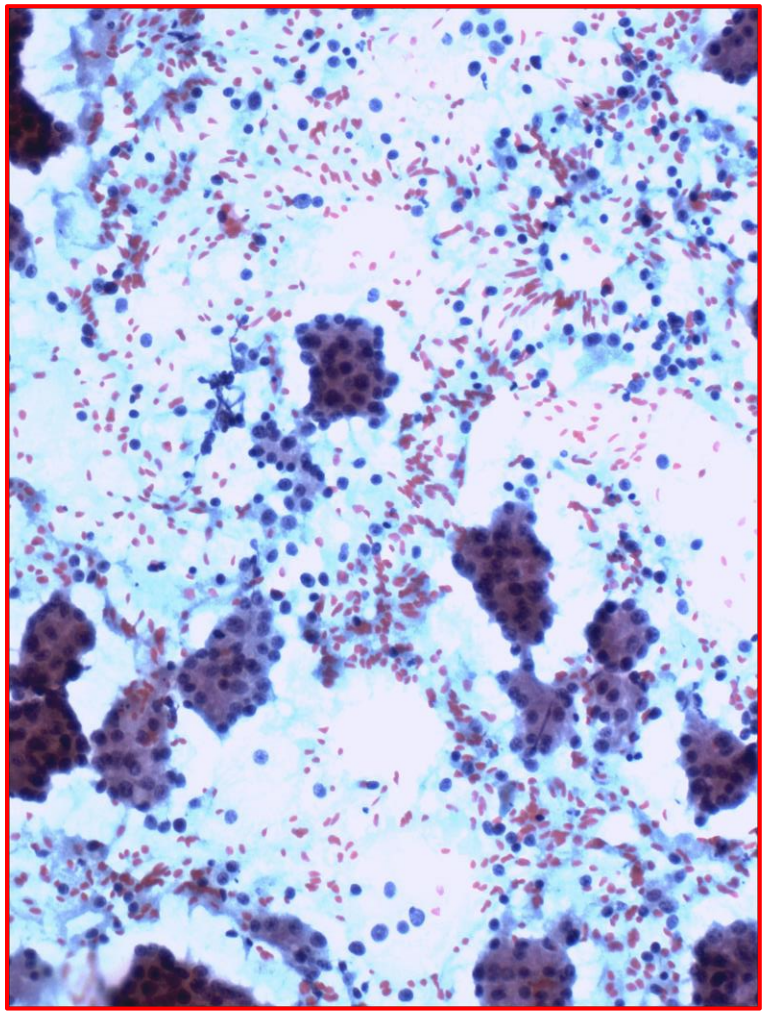
Molecular Testing

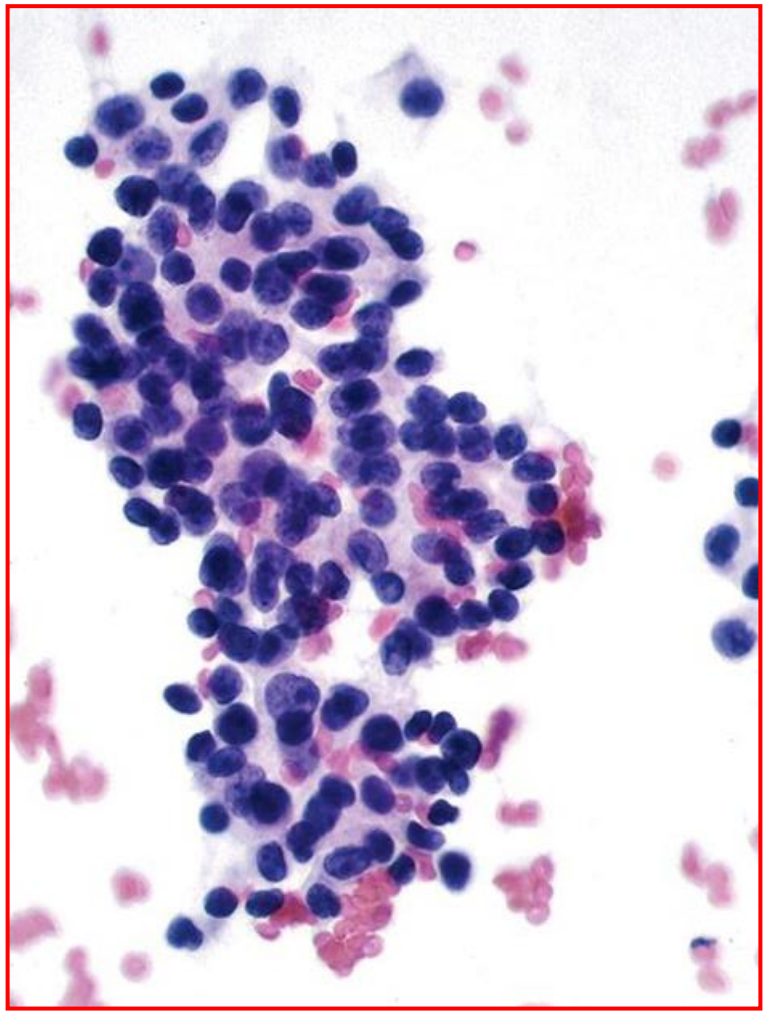
Marker	Purpose	Diagnostic finding	Utility
KRAS mutation	Identification of adenocarcinoma	Mutation present	Insufficient specificity for malignancy to warrant usage
SMAD4	Identification of adenocarcinoma	Mutation present [IHC shows loss of staining]	Supports the diagnosis of adenocarcinoma
FISH	Identification of adenocarcinoma	Presence of copy number abnormalities in CEP3, CEP7, CEP17 and abnormalities of band 9p21 favor malignancy	Most reliable test for confirming adenocarcinoma in conjunction with routine cytology
Mesothelin	Identification of malignancy	Overexpression of mesothelin by IHC	Supports the diagnosis of adenocarcinoma
Loss of heterozygosity	Identification of adenocarcinoma	Losses of chromosome arms 3p, 6Qp and 10pq along with gains of 5q, 12q, 18q, and 20q supports a diagnosis adenocarcinoma	Clinical importance to be determined
microRNAs	Identification of adenocarcinoma	Presence of miRNA including miR-21 and mi-155 supports a diagnosis of adenocarcinoma	Clinical utility to be determined

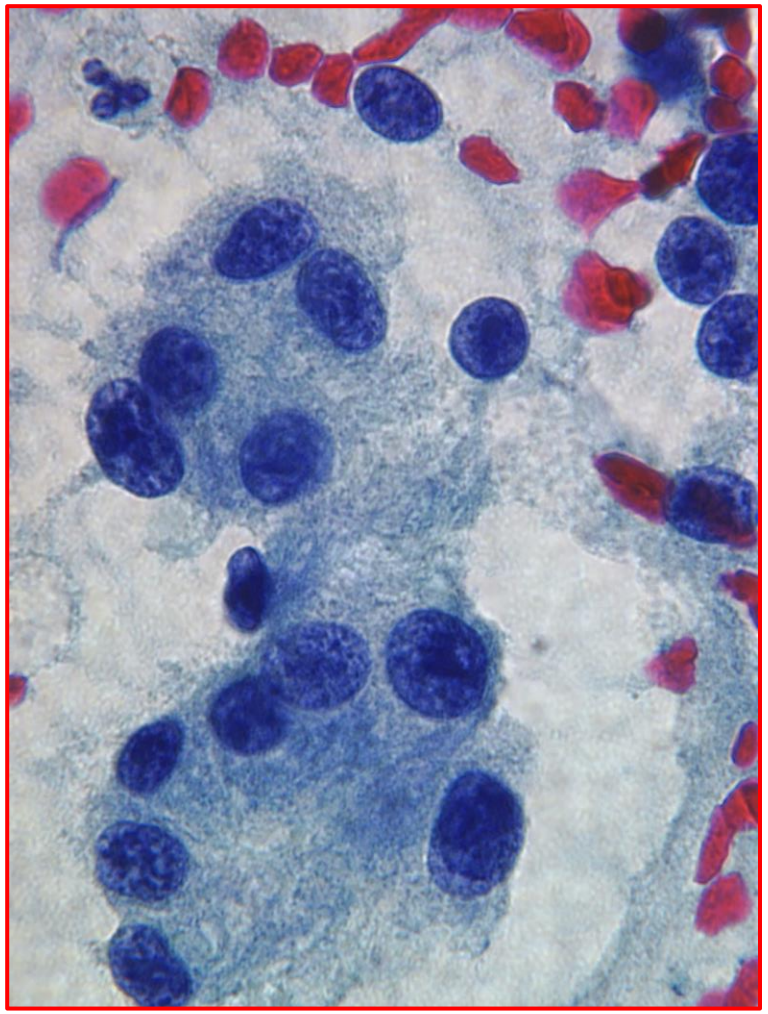
Acinar Cell Carcinoma

- Hypercellular smears
- Cell clusters with trabecular and acinar architectures
- Naked nuclei
- Modest granular cytoplasm
- Eccentrically located large nuclei with coarsely clumped chromatin and prominent nucleoli
- Trypsin, chymotrypsin, BCL10, neuroendocrine makers +/-

Acinar Cell Carcinoma



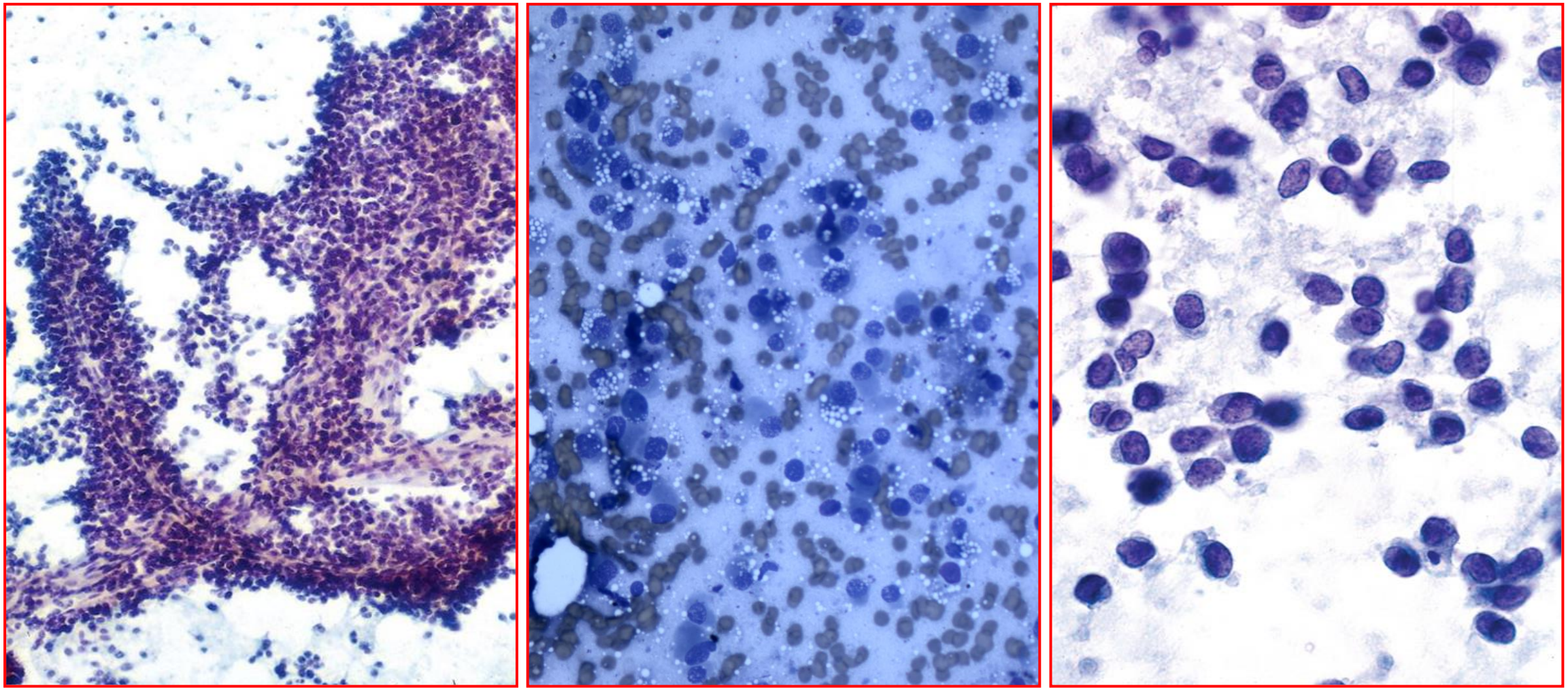




Neuroendocrine Tumor

- High cellularity
- Dyscohesive clusters with prominent vasculatures and rosette-like arrangement
- Monotonous single cells
- Basophilic, wispy, and ill-defined cytoplasm; cytoplasmic vacuoles
- Round to oval, eccentrically placed nuclei with fine stippled chromatin and small nucleoli
- Pleomorphism variably present
- Rarely binucleation or multinucleation

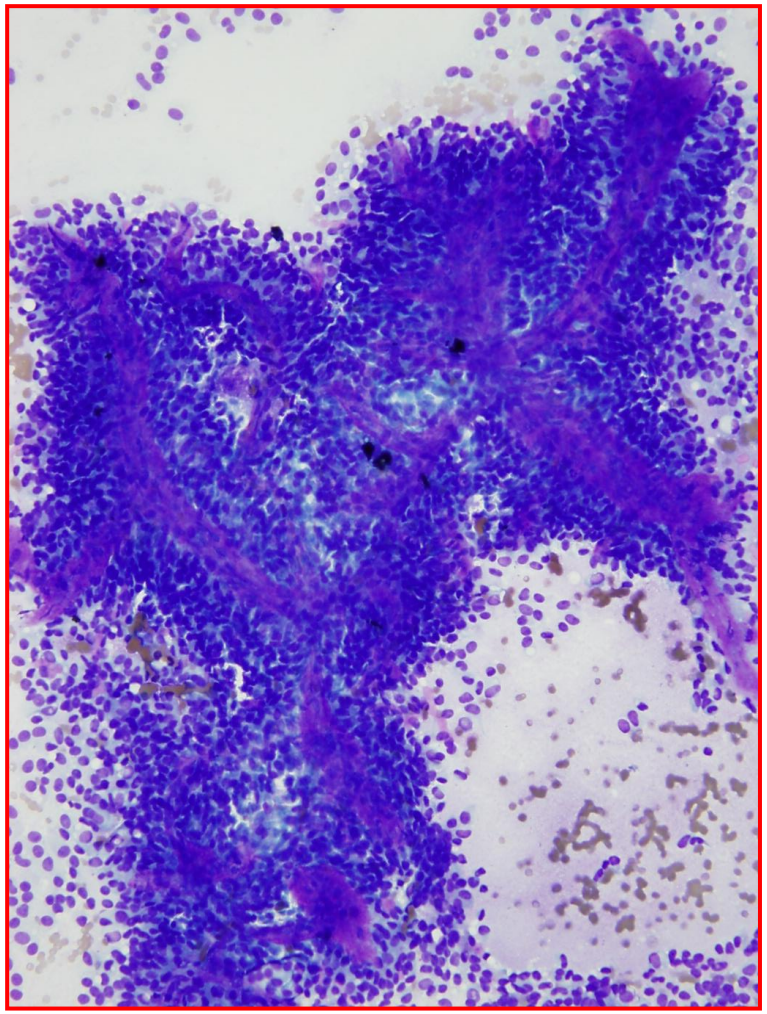
Neuroendocrine Tumor

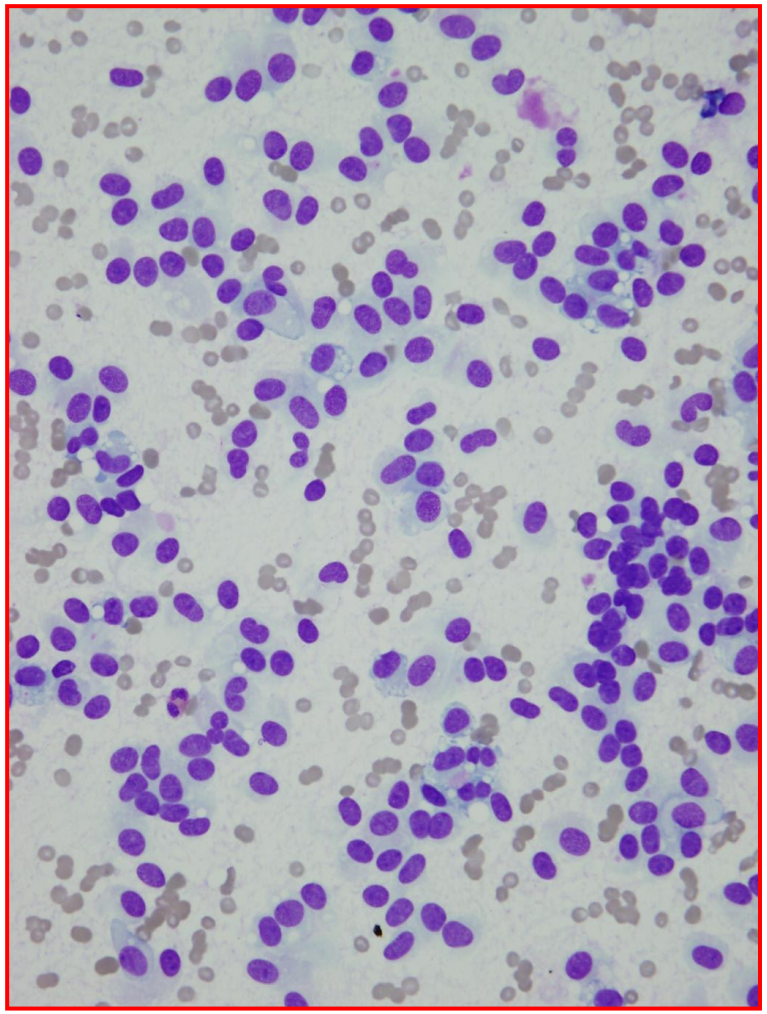


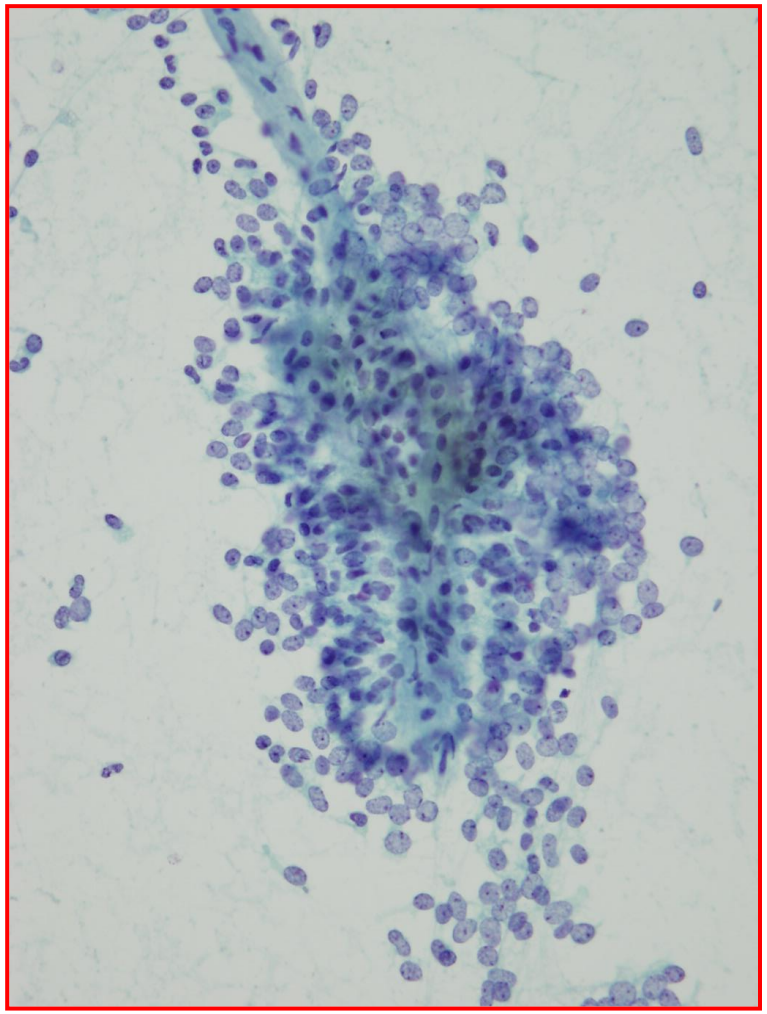
Solid Pseudopapillary Neoplasm

- Hypercellular
- Straight or branching papillary structures
- Monomorphic epithelial cells
- Round to oval nuclei with fine chromatin, nuclear indentation or grooves, and small nucleoli
- Scant and ill-defined amphophilic cytoplasm
- Hyaline globules, necrosis and macrophages
- Beta-catenin (nuclear)

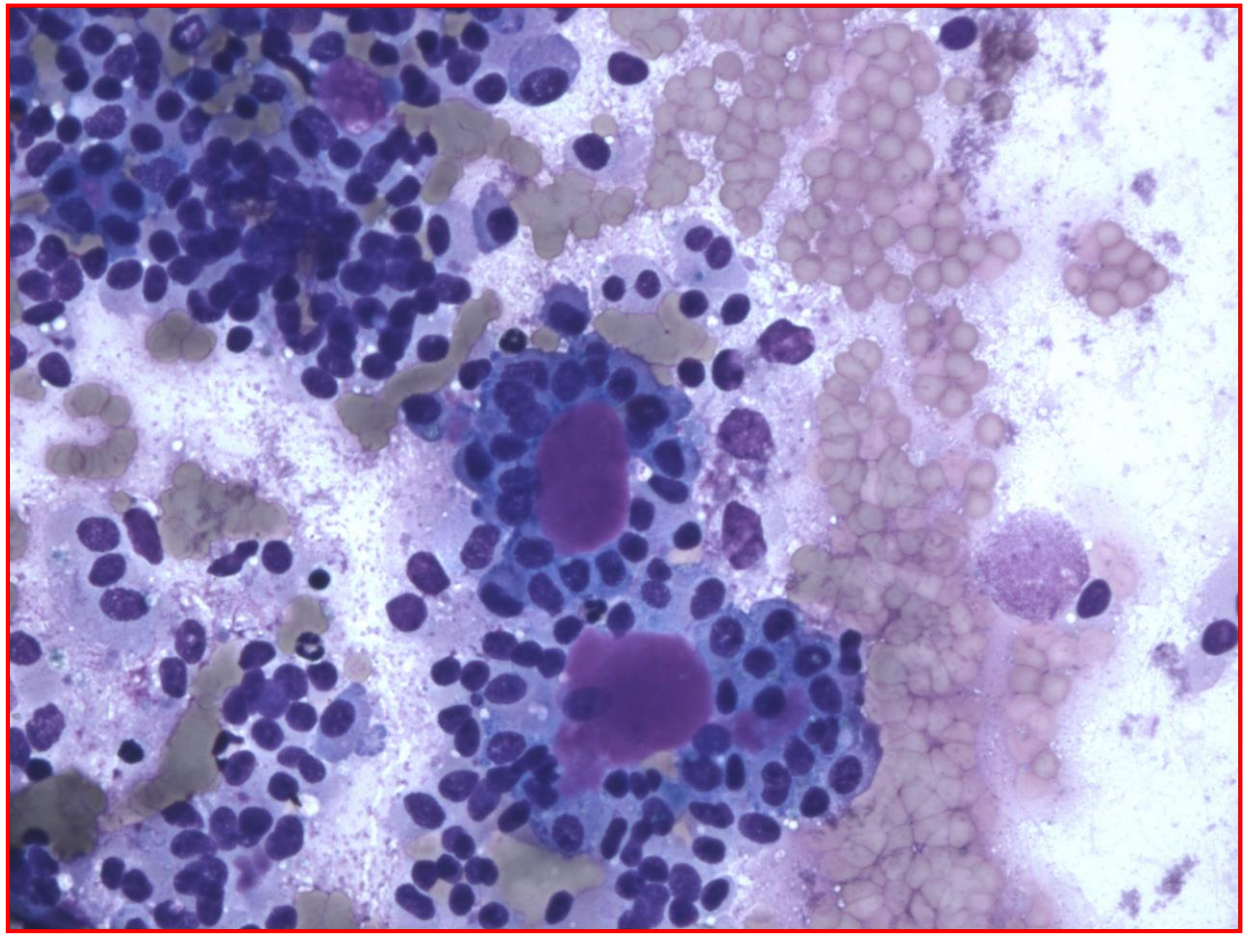
Solid Pseudopapillary Neoplasm

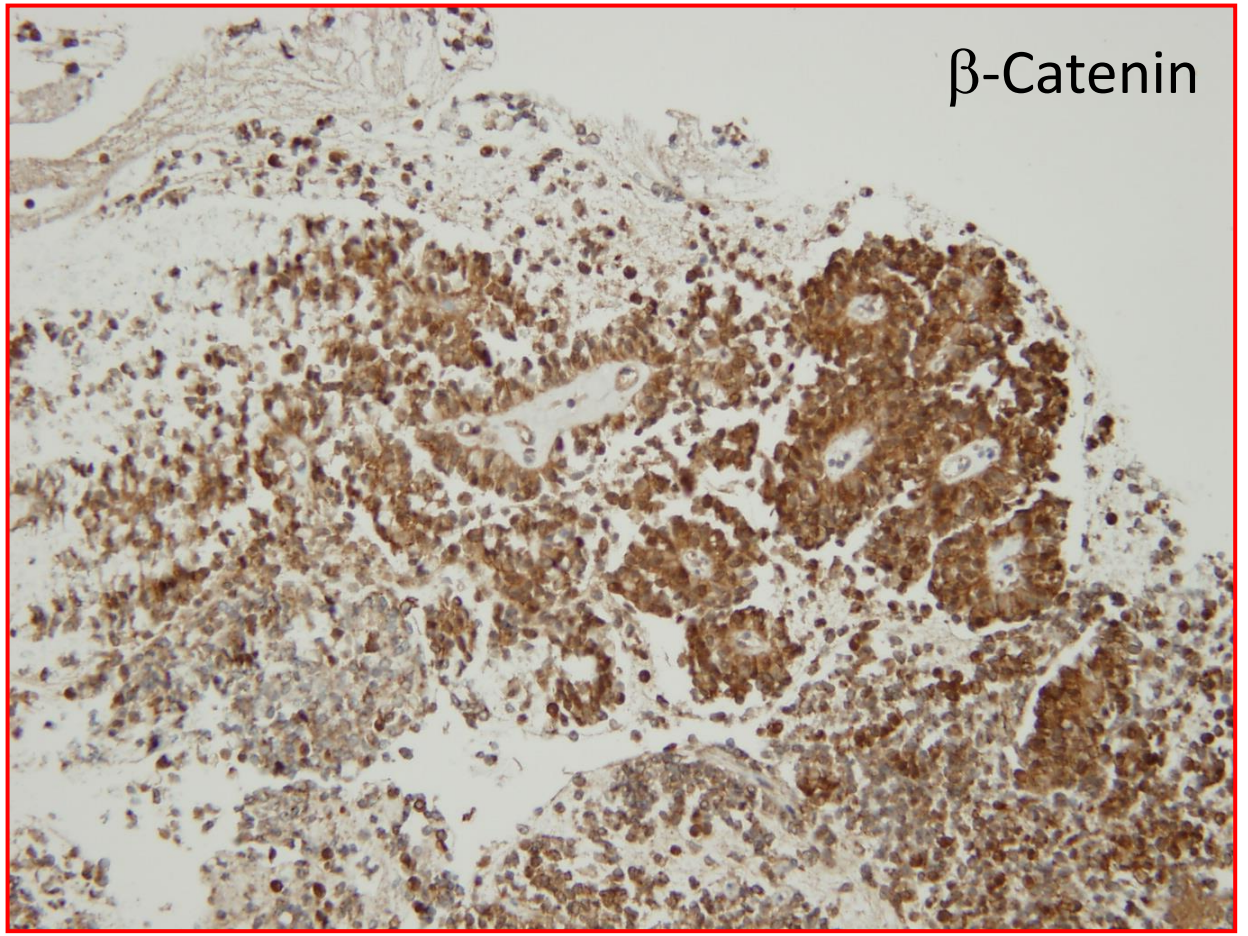






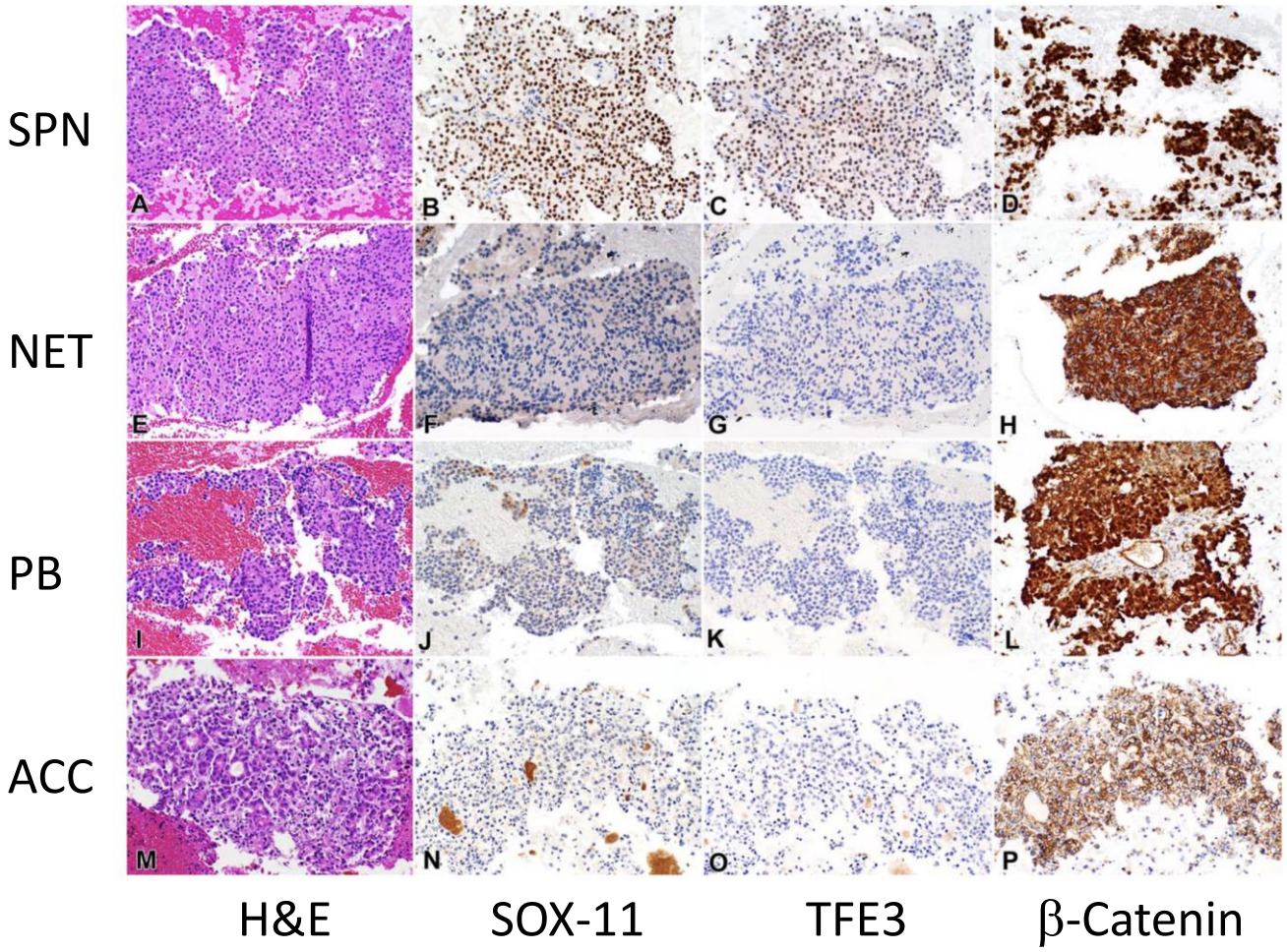
Solid Pseudopapillary Neoplasm





Solid Pseudopapillary Neoplasm

SOX-11 and TFE3 as Diagnostic Markers for Solid Pseudopapillary Neoplasms of the Pancreas



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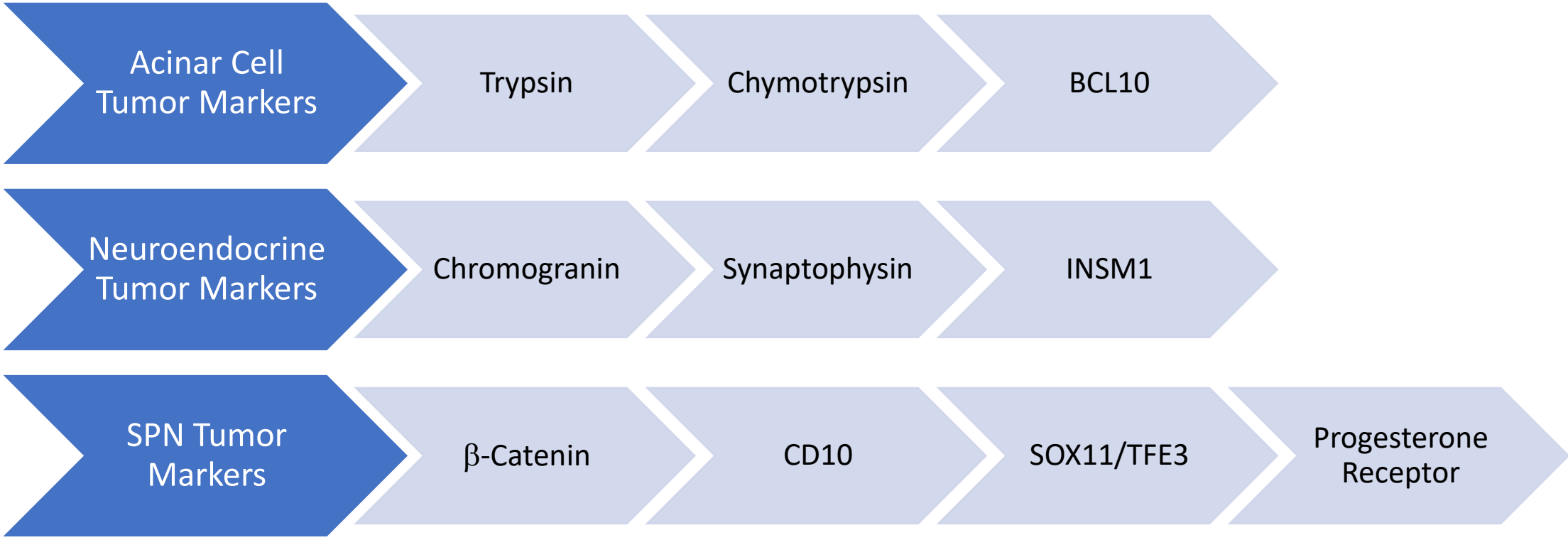
Immunocytochemistry



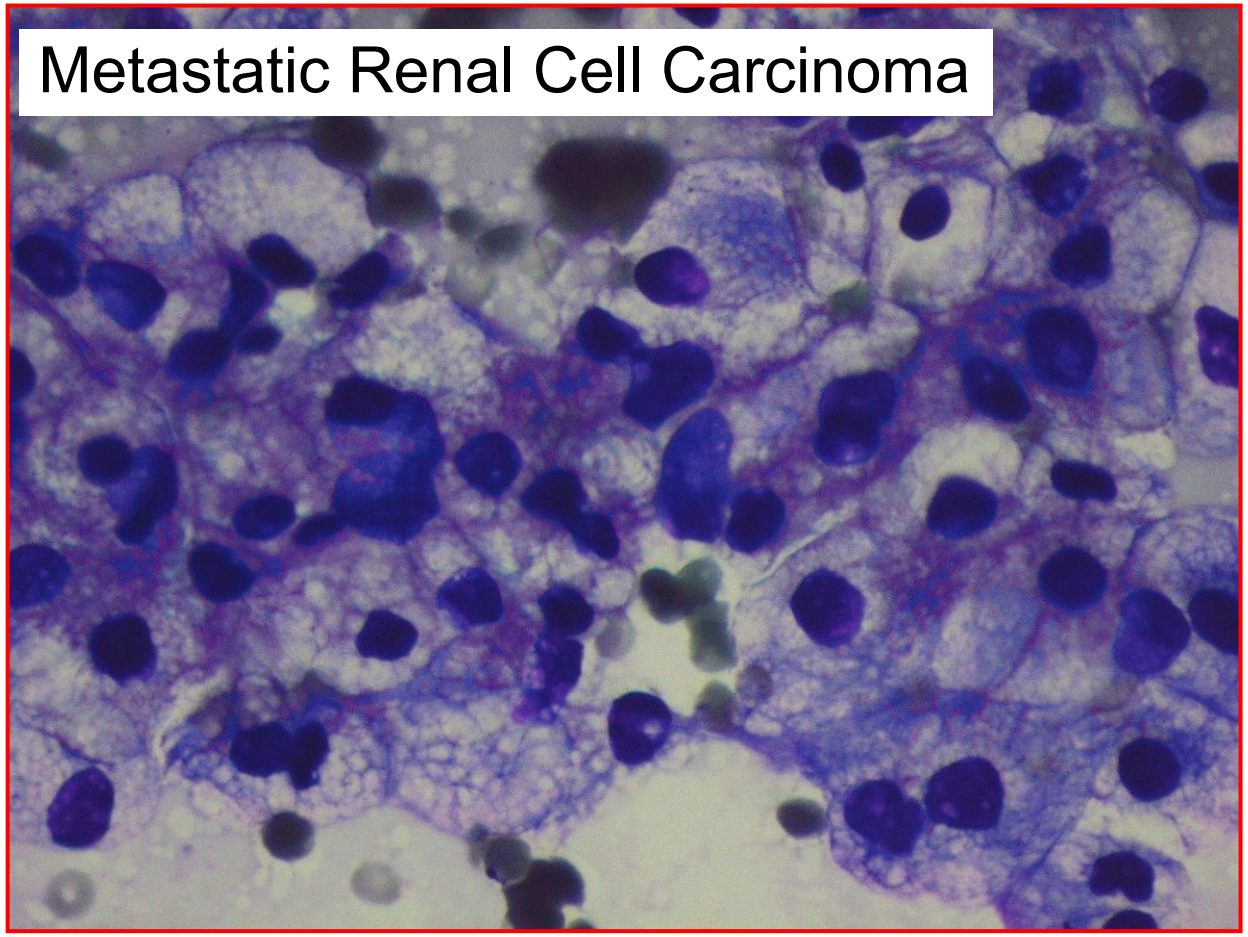
Immunocytochemistry

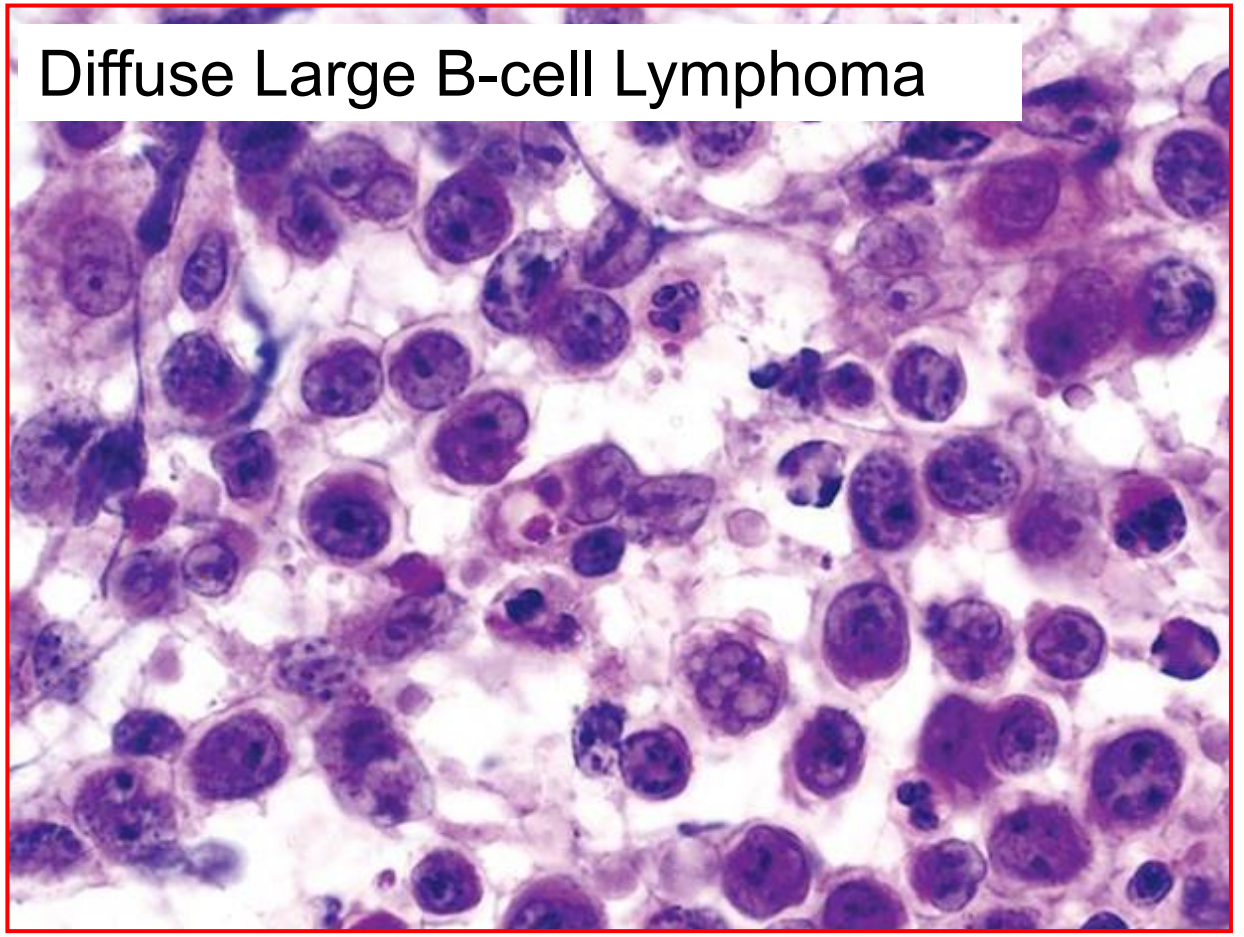
	Acinar Cell Carcinoma	Neuroendocrine Neoplasm	Solid Pseudopapillary Tumor	Pancreato-blastoma
Cytokeratin	+	+	-	+ (f)
Vimentin	-	-	+	-
Chromogranin	-	+	-	-/+
Synaptophysin	-/+	+	-/+	-/+
CD10	-	-	+	+ (f)
Beta-catenin (nuclear)	-	-	+	+
BCL10	+	-	-	+
SOX11 or TEF3	-	-	+	-
Trypsin or chymotrypsin	+	-	-	+
Alpha 1-antitrypsin or alpha 1-antichymotrypsin	+	-	+	+

Secondary Pancreatic Tumors

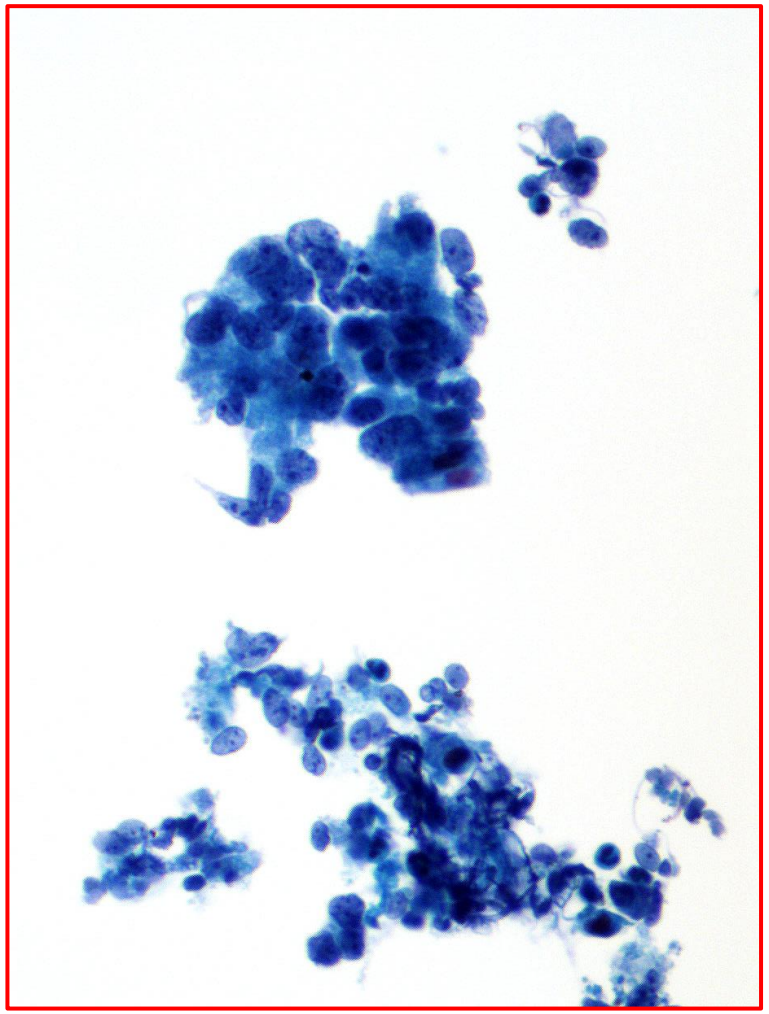
- Metastases represent up to 4% of pancreatic tumors
- Metastatic carcinomas
  - Lung and kidney are the most common primaries
  - Others include breast, stomach and skin
- Metastatic non-carcinomas
  - Sarcomas, melanoma
- Lymphoproliferative disorders
  - Mostly secondary involvement
  - Non-Hodgkin lymphomas, plasmacytoma

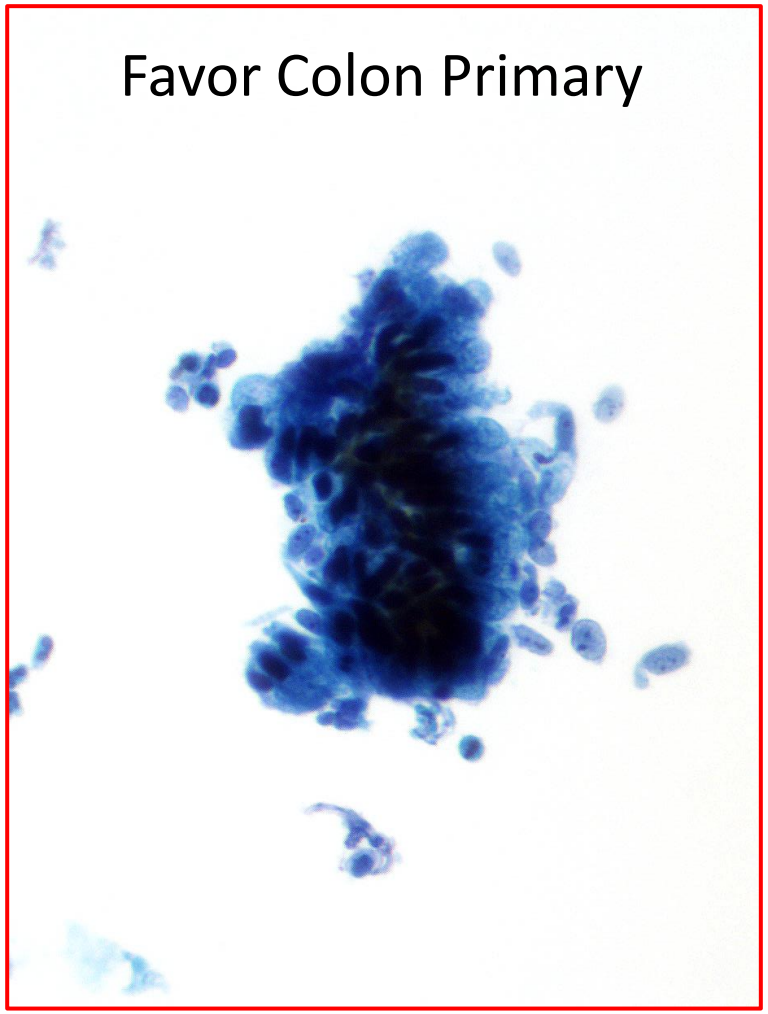
Secondary Tumors Involving Pancreas

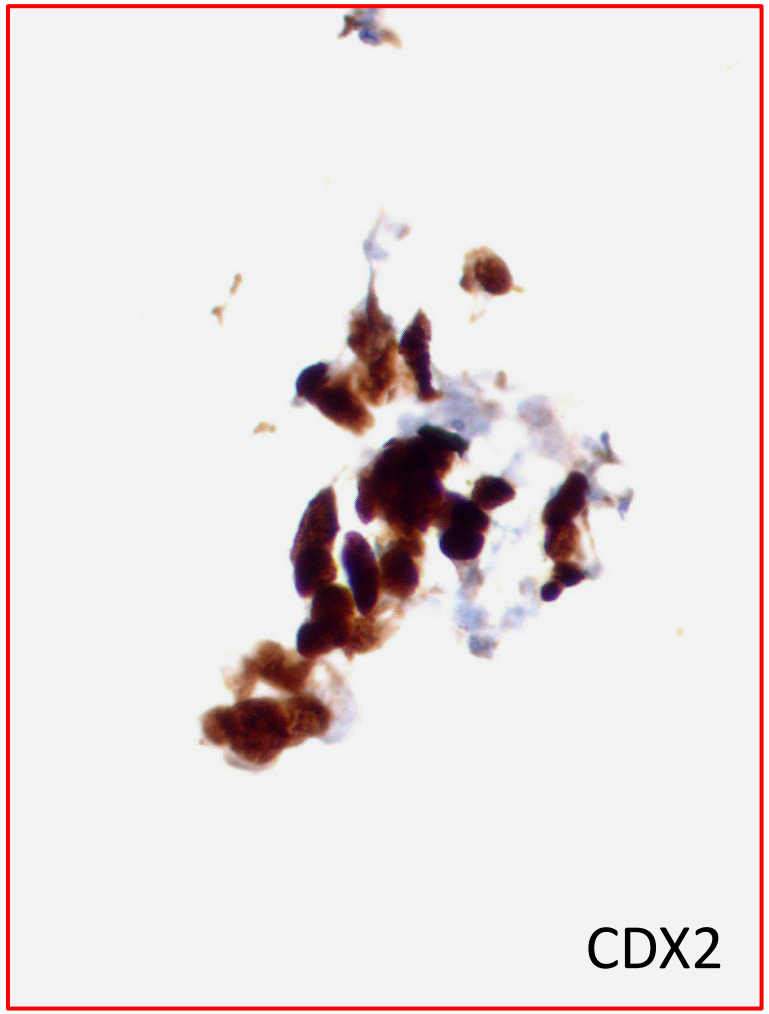




Secondary Tumors Involving Pancreas

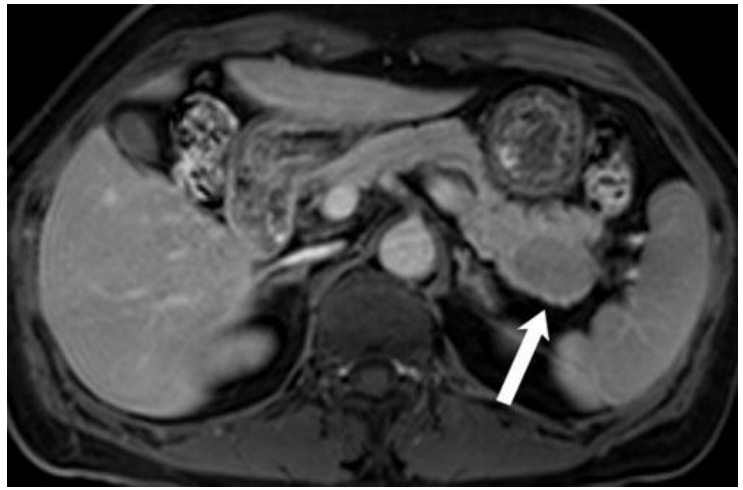


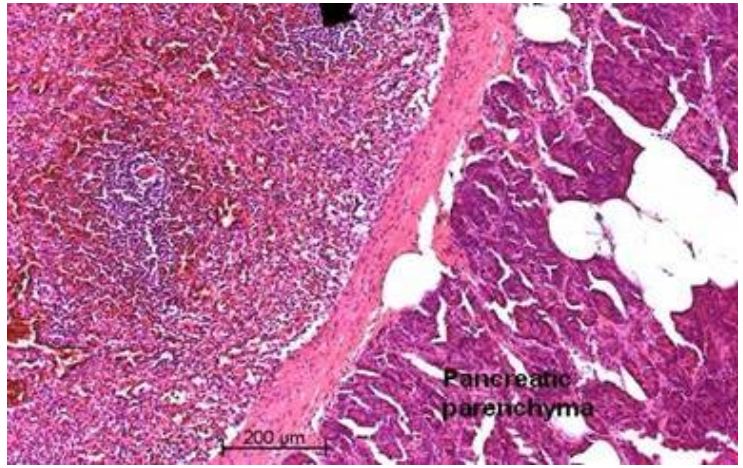




Accessory Spleen (Splenule)

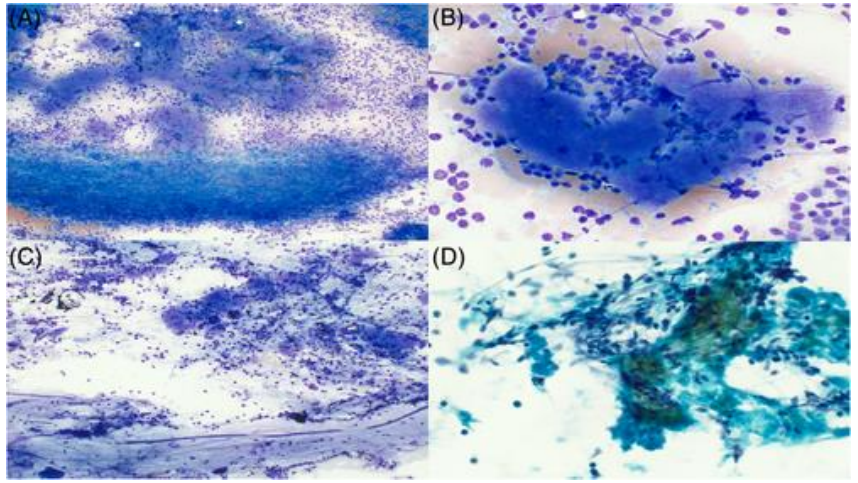
- Found in the soft tissues of the splenic hilum or the pancreatic parenchyma, mostly in the tail
- Intrapancreatic splenule is typically a small (< 30 mm), solid, round, well-demarcated mass
- Typical splenic parenchyma, including both red and white pulps.

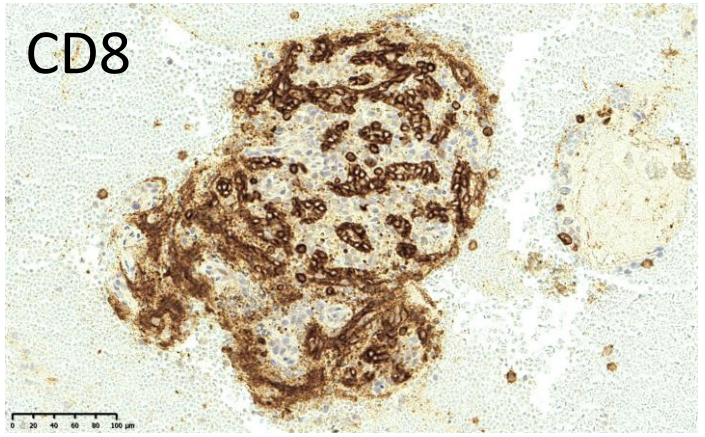




Accessory Spleen (Splenule)

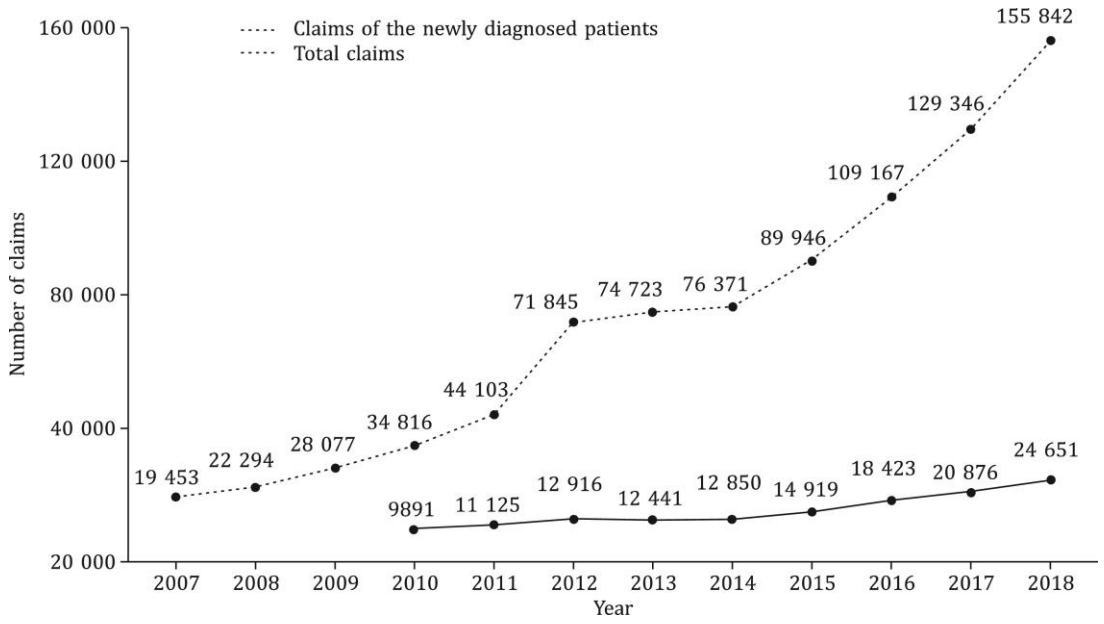
- Predominantly small lymphocytes
- Dispersed single cells with cohesive lymphoid tissue fragments
- Large platelet aggregates
- No tangible-body macrophages
- Small sinusoidal vascular structures
- CD8 highlighting sinusoidal lining (littoral) cells





Pancreatic Cysts: Prevalence

- Majority of pancreatic cysts are atypically asymptomatic lesions.
- Pancreatic cysts are increasingly detected incidentally due to factors including ubiquitous use of abdominal imaging studies and an increasing population of elderly individuals.
- The prevalence of asymptomatic pancreatic cysts in general population is likely about 1%-3%, but cysts may be found in 2.4%-19.6% of all patients examined by abdominal imaging.



The total number of claims including those in newly diagnosed patients during the study period. The total claims for five pancreatic cyst codes were rapidly increased from 19 453 to 155 842 between 2007 and 2018. After a washout period for three years, the incidence of newly diagnosed pancreatic cysts also increased from 9891 to 24 651 between 2010 and 2018.

- *Elta GH, et al. Am J Gastroenterol 2018; 113:464-479.*
- *Park J, et al. Hepatobiliary Pancreat Dis Int. 2023; 22:294-301.*

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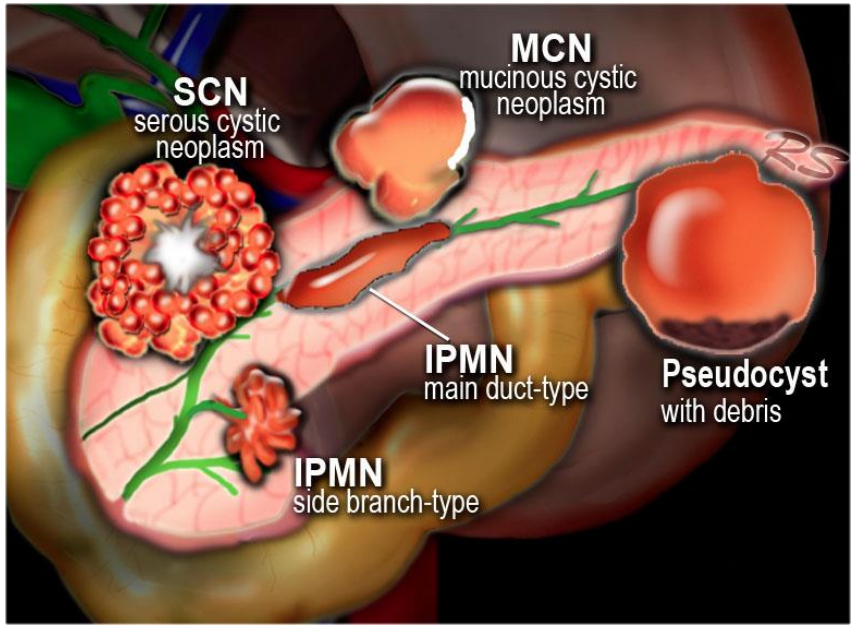
Common Pancreatic Cystic Lesions

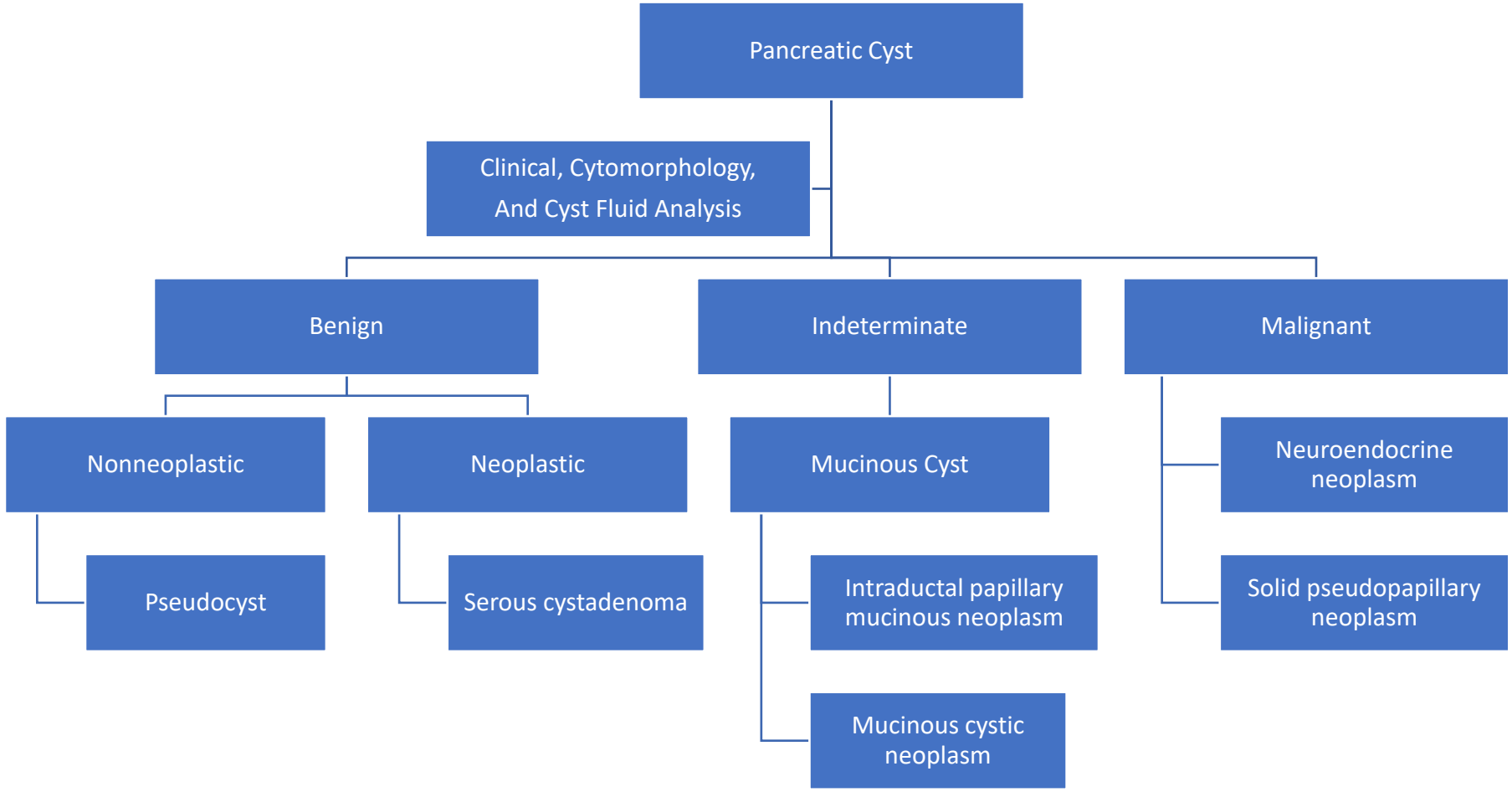
- Non-neoplastic cysts
  - Pseudocyst
- Neoplastic cysts
  - Serous cystadenoma
  - Mucinous cystic neoplasm
  - Intraductal papillary mucinous neoplasm
  - Cystic neuroendocrine neoplasm
  - Solid pseudopapillary neoplasm



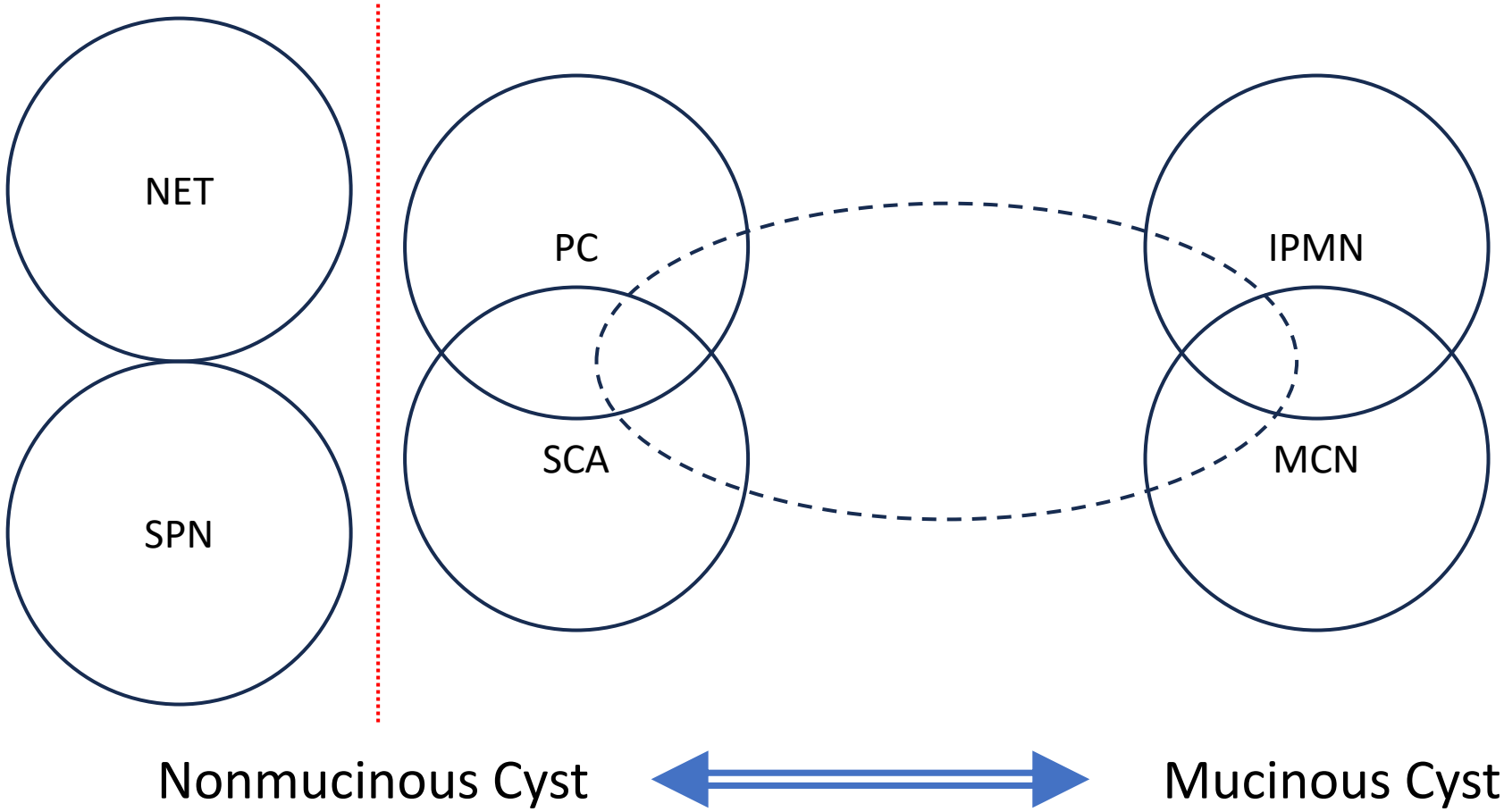
Evaluation of Pancreatic Cysts

- Cystic pancreatic lesions may have different biological behaviors.
  - Serous cyst most likely follows a benign course while mucinous lesion has the potential to progress.
- Accurate preoperative diagnosis is essential for proper management.
  - A patient with a cyst suspected to be benign without malignant potential may be managed expectantly.
  - A patient with a cyst suspected to be malignant may be managed surgically.

Diagnostic Approach for Cystic Lesions



Diagnostic Considerations



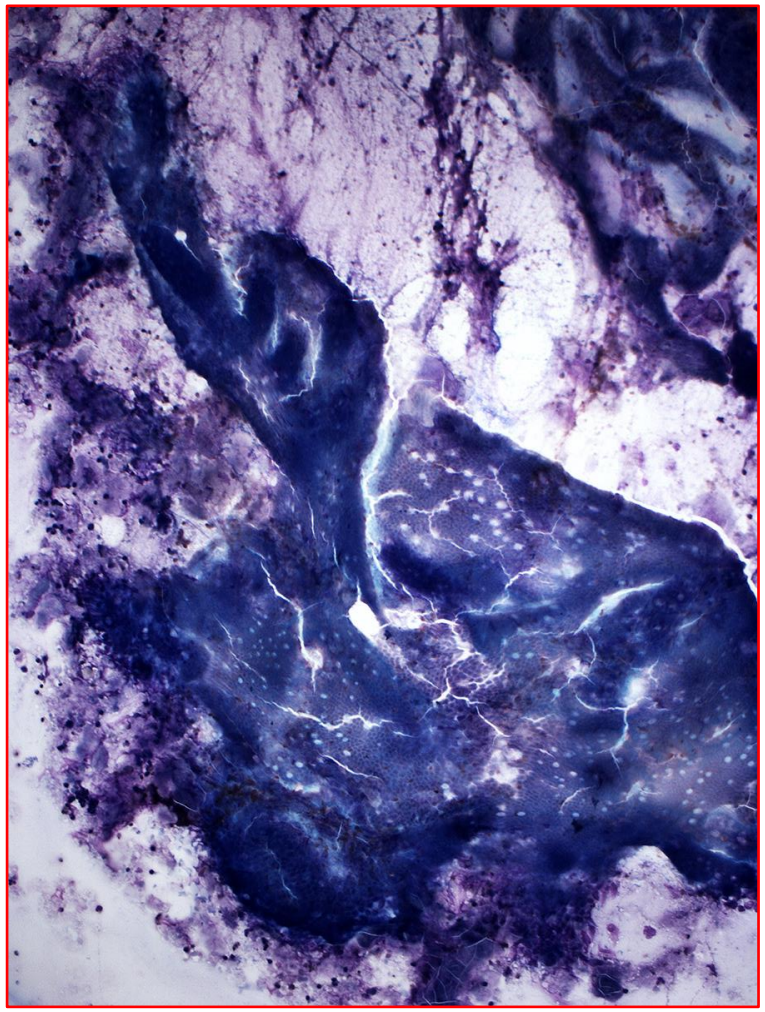
Diagnostic Considerations

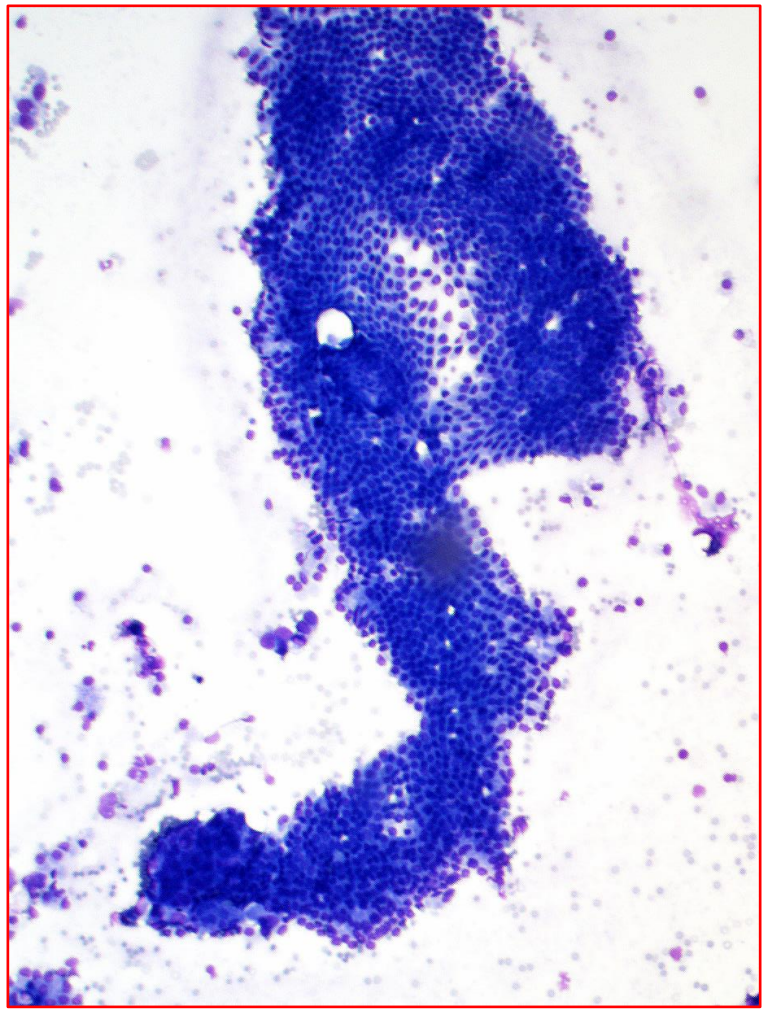
Step 1: Cytomorphologic Analysis

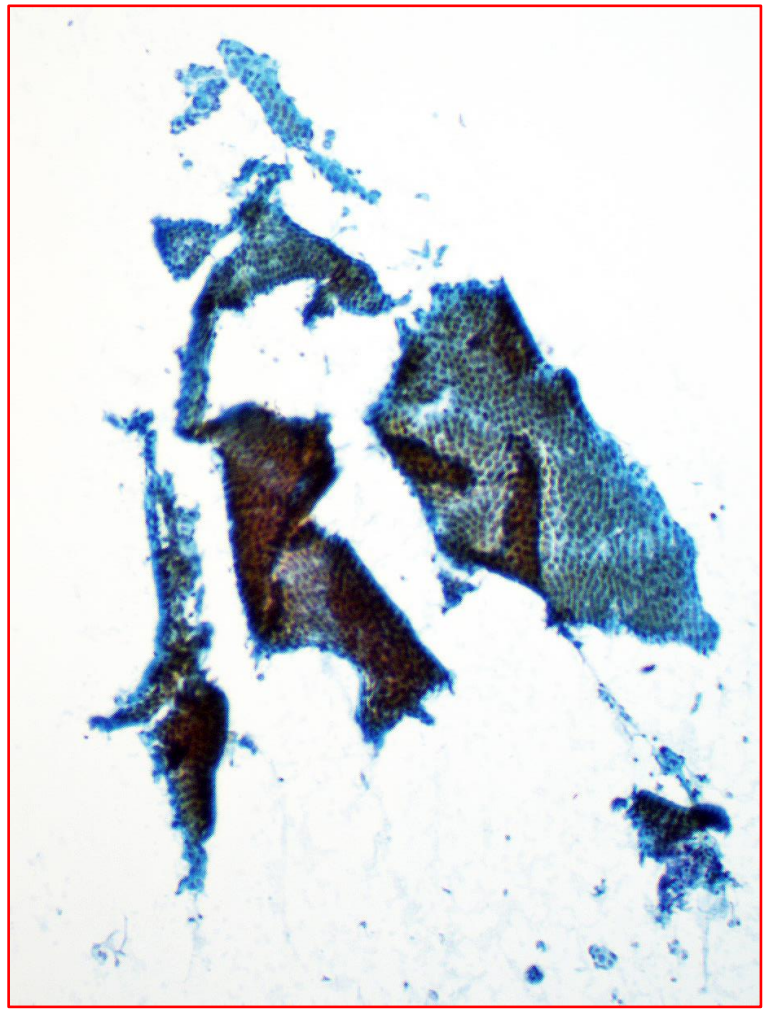
Diagnostic Challenges

- Cytology evaluation alone has relatively low sensitivity and specificity due to:
  - Scant cellularity
  - Bland or overlapping cytomorphology
  - Gastrointestinal contaminants
  - Absence of mucinous epithelial cells
  - Failure in recognition of background mucin, especially on liquid-based preparations

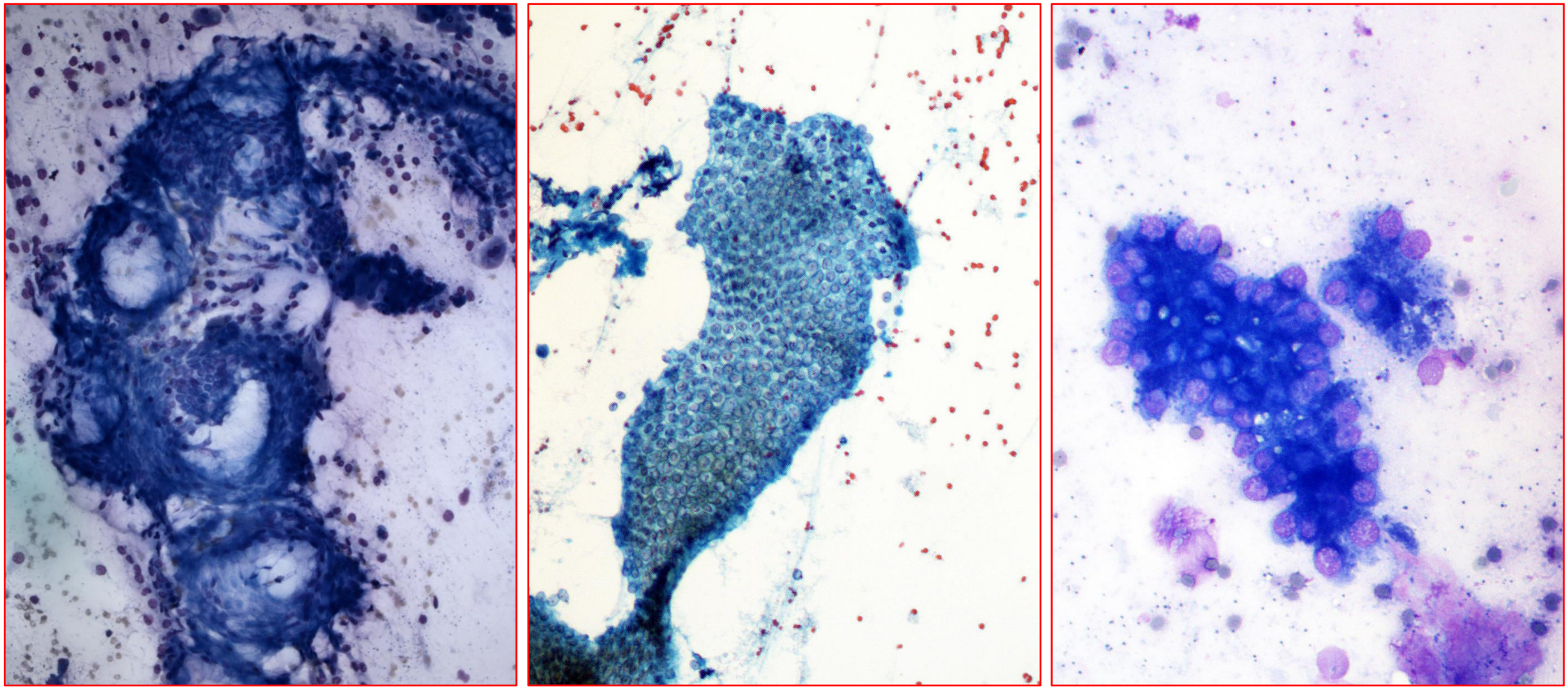
Duodenal Epithelial Contaminants





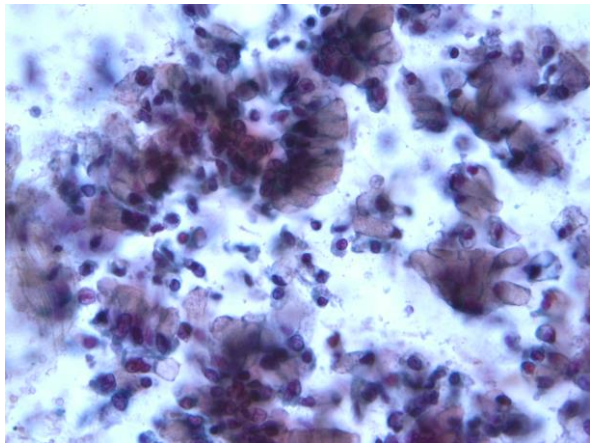


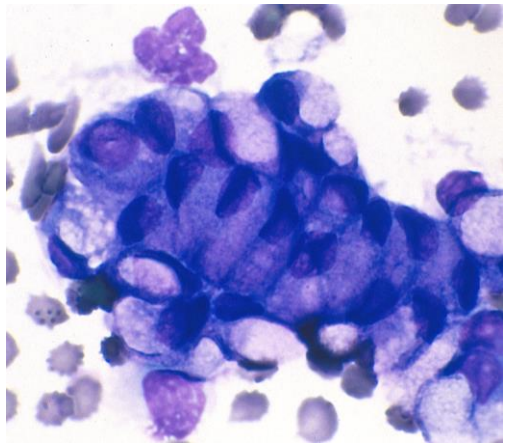
Gastric Epithelial Contaminant

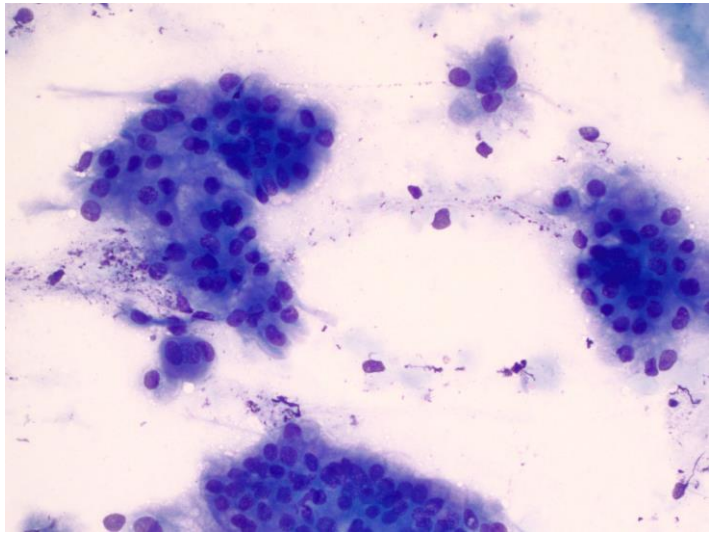


Identification of Mucinous Epithelial Cells

- Mucinous epithelial cells are the cells containing intracytoplasmic mucin.
- Mucinous epithelial cells may be seen as signet-ring cells.
- Some mucinous cysts may not have mucinous epithelial cells.
- Mucinous epithelial cells may represent gastrointestinal contaminants.

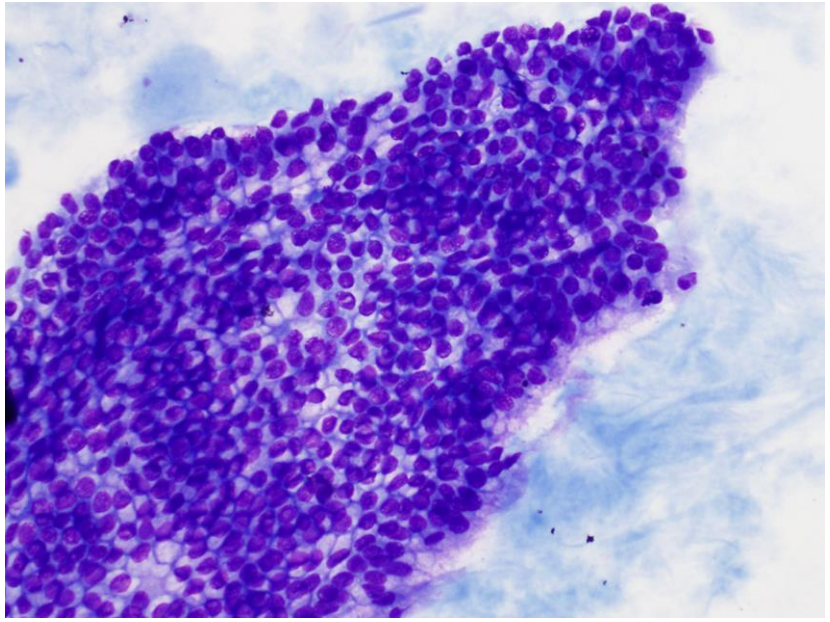


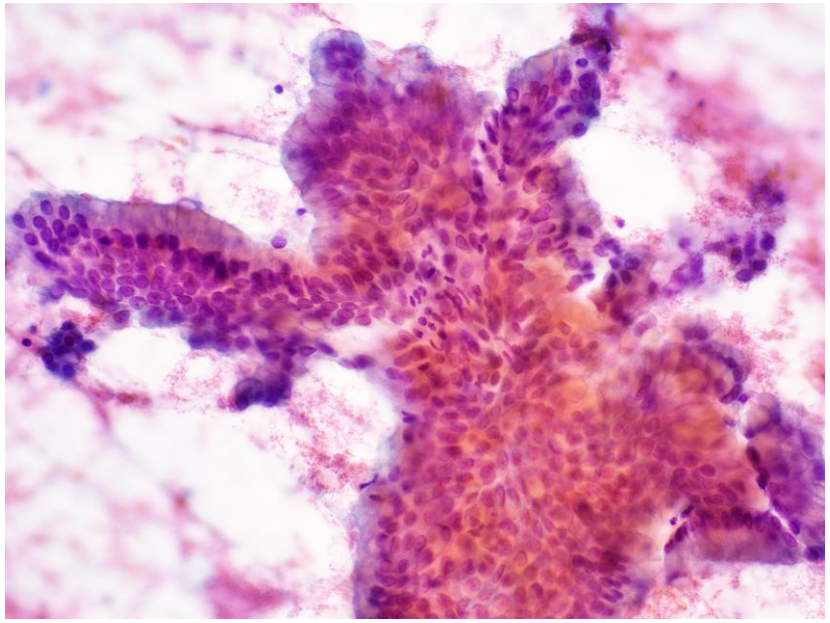




Identification of Mucinous Epithelial Cells

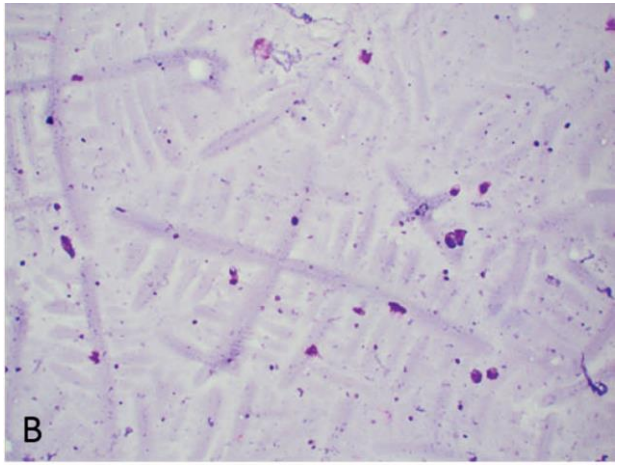
- Low-grade neoplastic epithelial cells have evenly spaced nuclei with apical cytoplasmic mucin, nearly identical to gastric foveolar epithelium.
- Neoplastic epithelium shows nuclear crowding, mild nuclear enlargement, mild to moderate cytopathological disorganization, and papillary fragments.





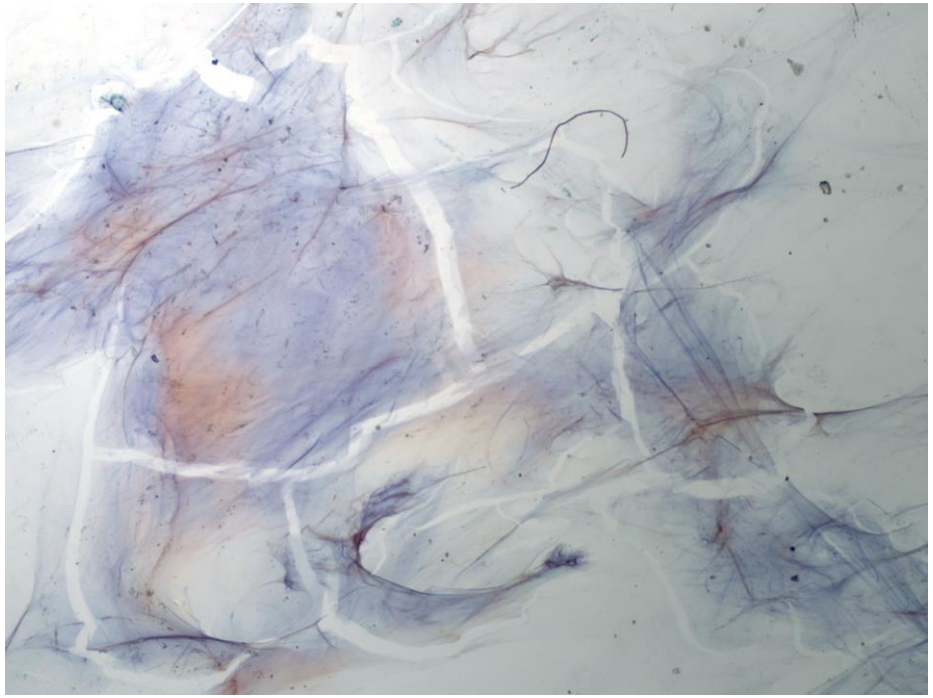
Identification of Mucin

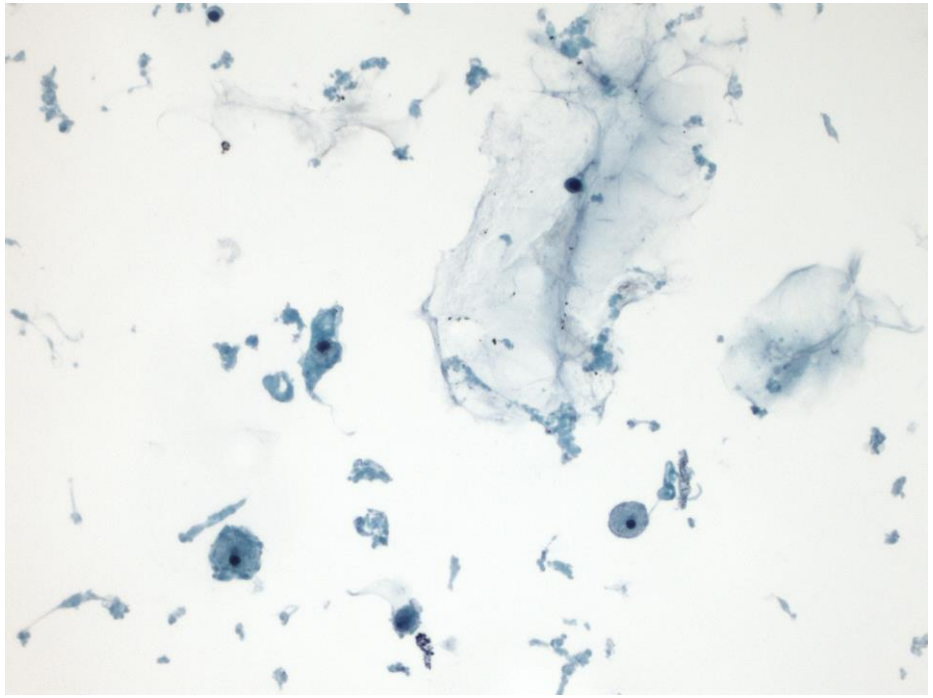
- Thick mucin has a high predictive value for mucin (ferning sign).
- Watery mucin may be representative of gastrointestinal contaminant.
- Mucin may be underappreciated on liquid-based preparation.
- “String sign” has a high specificity but low sensitivity for a mucinous cyst.



Identification of Mucin

- Thick mucin is diagnostic even without mucinous epithelium.
- Thin, wispy mucin is indeterminate for origin.





Diagnostic Considerations

Step 2: Cyst Fluid Analysis and Molecular Testing

CEA and Amylase Levels

- Cyst fluid CEA level is useful for distinguishing mucinous from non-mucinous lesions.
- The reported sensitivity and specificity are variable.
- With the CEA cut-off point > 192 ng/ml, the sensitivity and specificity are 75% and 84% for mucinous cyst.
- Amylase level in cyst fluid is helpful for the exclusion diagnosis of pancreatic pseudocyst (cutoff point < 250 units/L).

Glucose Level

Intracystic Glucose and Carcinoembryonic Antigen in Differentiating Histologically Confirmed Pancreatic Mucinous Neoplastic Cysts

Zachary L. Smith, DO[1,2,*], Sagarika Satyavada, MD[2,*], Roberto Simons-Linares, MD[3], Shaffer R.S. Mok, MD, MBS[2,4], Bélen Martinez Moreno, MD[5], José Ramón Aparicio, MD[5] and Prabhleen Chahal, MD[3]

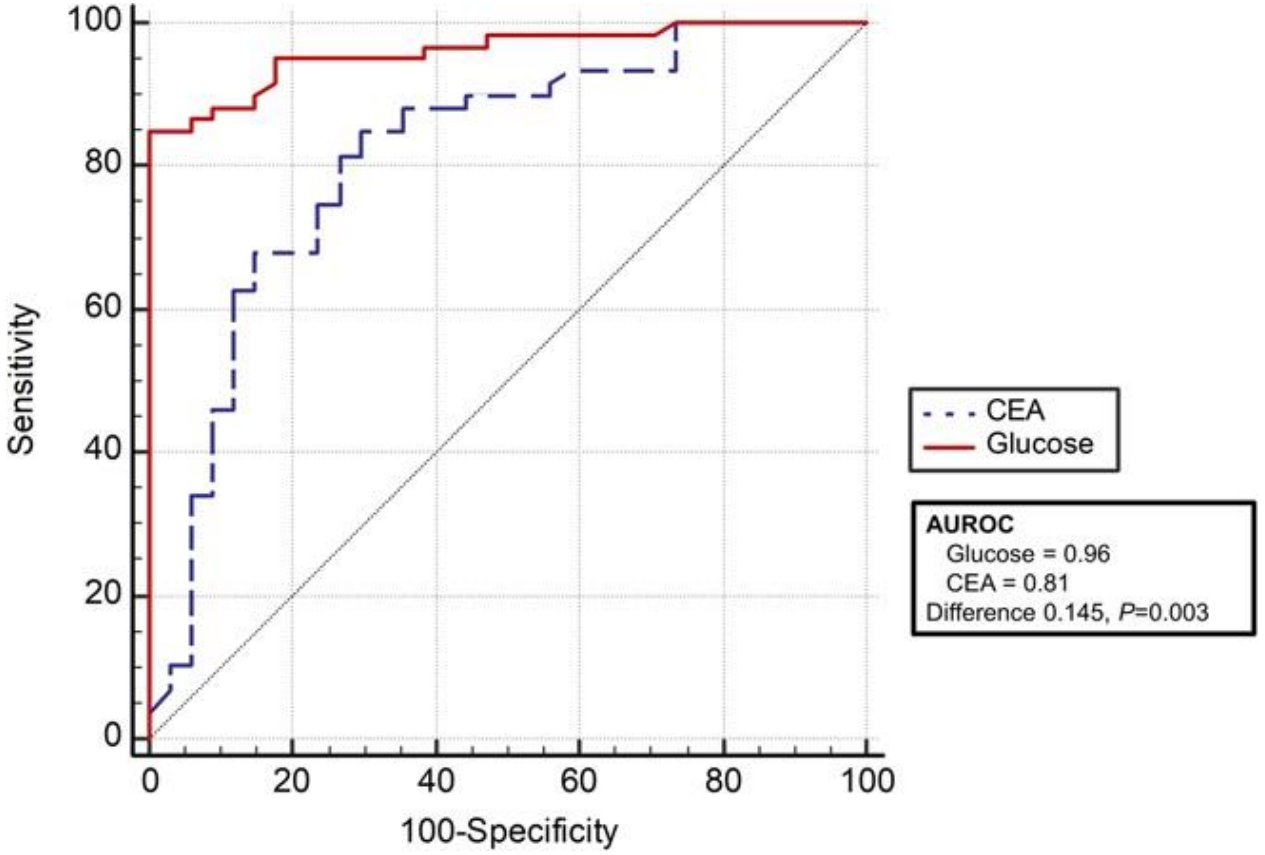
INTRODUCTION: **Differentiating mucinous neoplastic pancreatic cysts (MNPC) from cysts without malignant potential can be challenging. Guidelines recommend using fluid carcinoembryonic antigen (CEA) to differentiate MNPC; however, its sensitivity and specificity vary widely. Intracystic glucose concentration has shown promise in differentiating MNPC, but data are limited to frozen specimens and cohorts of patients without histologic diagnoses. This study aimed to compare glucose and CEA concentrations in differentiating MNPC using fresh fluid obtained from cysts with confirmatory histologic diagnoses.**

METHODS: **This multicenter cohort study consisted of patients undergoing endoscopic ultrasound–guided fine-needle aspiration (EUS-FNA) for pancreatic cysts during January 2013–May 2020. Patients were included if the cyst exhibited a histologic diagnosis and if both CEA and glucose were analyzed from fresh fluid. Receiver operating curve (ROC) characteristics were analyzed, and various diagnostic parameters were compared.**

RESULTS: **Ninety-three patients, of whom 59 presented with MNPC, met the eligibility criteria. The area under the receiver operating curve (AUROC) was 0.96 for glucose and 0.81 for CEA (difference 0.145, $P = 0.003$). A CEA concentration of ≥192 ng/mL had sensitivity of 62.7% and specificity of 88.2% in differentiating MNPC, whereas glucose concentration of ≤25 mg/dL had sensitivity and specificity of 88.1% and 91.2%, respectively.**

DISCUSSION: **Intracystic glucose is superior to CEA concentration for differentiating MNPC when analyzed from freshly obtained fluid of cysts with histologic diagnoses. The advantage of glucose is augmented by its low cost and ease of implementation, and therefore, its widespread adoption should come without barriers. Glucose has supplanted CEA as the best fluid biomarker in differentiating MNPC.**

Am J Gastroenterol 2022;117:478–485. https://doi.org/10.14309/ajg.0000000000001623



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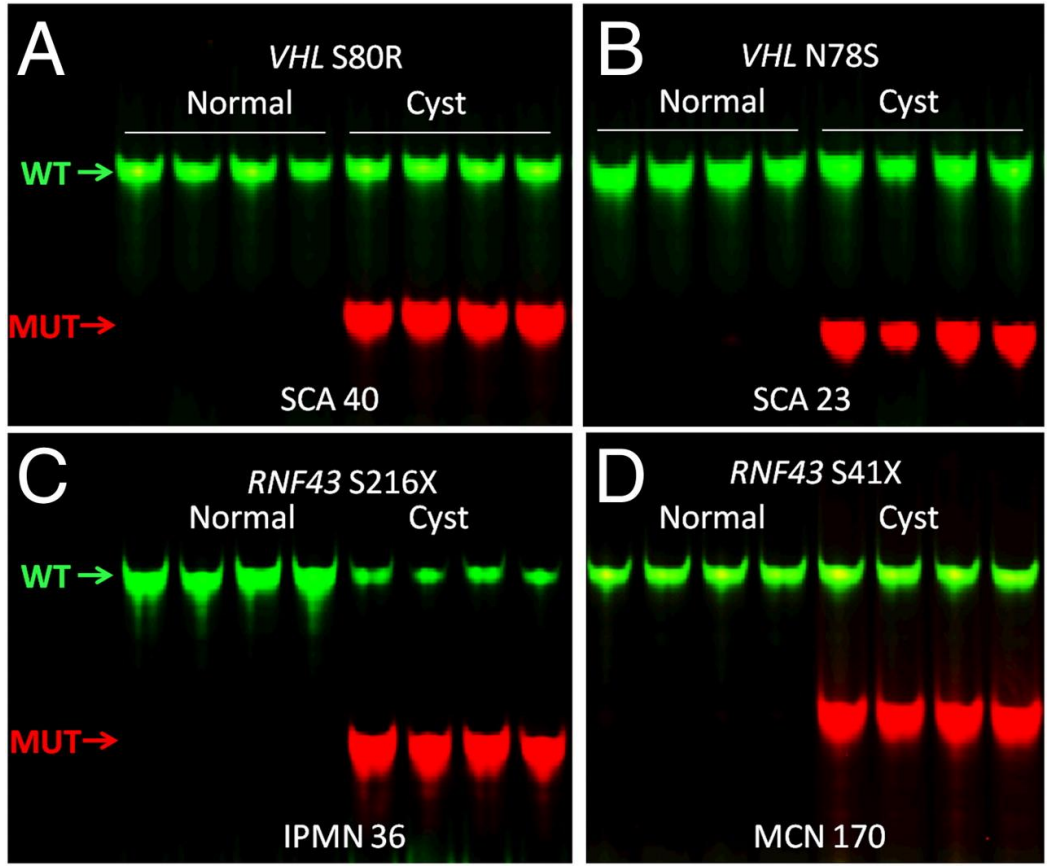
Cyst Fluid Analysis

Entities	CEA Level (Cutoff: 192 ng/ml)	Amylase Level (Cutoff: 250 U/L)	Glucose Level (cutoff: 50 mg/dl)
Pseudocyst	Not elevated	High level, >1000s U/L	> 50 mg/dl
Serous cystadenoma	Not elevated	Low level, <1000 U/L	> 50 mg/dl
Intraductal papillary mucinous neoplasm	Usually elevated	Variable	=< 50 mg/dl
Mucinous cystic neoplasm	Usually elevated	Usually not elevated	=< 50 mg/dl
Cystic neuroendocrine tumor	Not elevated	Low level, <500 U/L	N/A
Solid pseudopapillary neoplasm	Not elevated	Low level, <500 U/L	N/A

- A low CEA does not exclude a mucinous cystic lesion; CEA levels do not distinguish between benign and malignant cysts. Glucose level may help the distinction of mucinous from non-mucinous cysts.

Molecular Testing



Cyst type	Sample ID	*VHL*	*RNF43*	*KRAS*	*GNAS*	*CTNNB1*
SCA	SCA 14	LOH	None	None	None	None
SCA	SCA 23	LOH, p.N78S	None	None	None	None
SCA	SCA 27	LOH	None	None	None	None
SCA	SCA 29	LOH	None	None	None	None
SCA	SCA 35	LOH, p.W117L	None	None	None	None
SCA	SCA 37	LOH	None	None	None	None
SCA	SCA 38	p.C162W	None	None	None	None
SCA	SCA 40	LOH, p.S80R	None	None	None	None
IPMN	IPMN 4	None	None	None	p.R201C	None
IPMN	IPMN 11	None	LOH, p.R145X	p.G12D	p.R201C	None
IPMN	IPMN 12	None	LOH, p.Y177X	None	p.R201C	None
IPMN	IPMN 20	None	p.Q152X	p.G12D	p.R201C	None
IPMN	IPMN 21	None	LOH, p.R371X	p.G12D	p.R201H	None
IPMN	IPMN 26	None	None	p.G12R	None	None
IPMN	IPMN 36	None	LOH, p.S216X	p.G12R	None	None
IPMN	IPMN 41	None	p.R113X	None	None	None
MCN	MCN 158	None	None	None	None	None
MCN	MCN 162	None	None	p.G12V	None	None
MCN	MCN 163	None	None	p.G13D	None	None
MCN	MCN 164	None	None	p.G12V	None	None
MCN	MCN 166	None	p.R371X	p.G12D	None	None
MCN	MCN 168	None	LOH, p.R127P	p.G12V	None	None
MCN	MCN 169	None	LOH	None	None	None
MCN	MCN 170	None	p.S41X	p.G12V	None	None
SPN	SPN 2	None	None	None	None	p.G34R
SPN	SPN 4	None	None	None	None	p.S33C
SPN	SPN 5	None	None	None	None	p.D32H
SPN	SPN 6	None	None	None	None	p.D32A
SPN	SPN 8	None	None	None	None	p.S37F
SPN	SPN 12	None	None	None	None	p.G34V
SPN	SPN 17	None	None	None	None	p.G34R
SPN	SPN 19	None	None	None	None	p.D32N

- *Wu J, et al. Proc Natl Acad Sci U S A. 2011; 108(52):21188-21193.*

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Molecular Testing

KRAS Test Meta Analysis

Subgroup	Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Malignant	Shoedel et al, 2006	2	2	2	10	0.50 (0.07-0.93)	0.89 (0.52-0.98)
	Sreenarasimhaiah et al, 2009	2	0	4	0	0.33 (0.04-0.78)	0.50 (0.00-1.00)
	Sawhney et al, 2009	1	1	4	13	0.20 (0.01-0.72)	0.93 (0.66-1.00)
	Mertz et al, 2011	0	4	0	6	0.50 (0.00-1.00)	0.60 (0.26-0.88)
	Toll et al, 2010	1	0	0	1	1.00 (0.03-1.00)	1.00 (0.03-1.00)
	Panarelli et al, 2012	0	1	1	2	0.00 (0.00-0.98)	0.67 (0.09-0.99)
	Nikiforova et al, 2013	7	46	24	65	0.23 (0.10-0.41)	0.59 (0.49-0.68)
	Kung et al, 2014	2	0	3	1	0.40 (0.05-0.85)	1.00 (0.03-1.00)
	Jones et al, 2015	4	4	2	0	0.67 (0.22-0.96)	0.00 (0.00-0.60)
	Singhi et al, 2016	10	7	3	21	0.77 (0.46-0.95)	0.75 (0.55-0.89)
	Singhi et al, 2017	16	31	12	43	0.57 (0.37-0.76)	0.58 (0.46-0.69)
	Winner et al, 2015	4	12	9	15	0.31 (0.09-0.61)	0.56 (0.35-0.75)
Malignant + Mucinous	Shoedel et al, 2006	4	0	12	0	0.25 (0.07-0.52)	0.50 (0.00-1.00)
	Sreenarasimhaiah et al, 2009	2	0	4	0	0.33 (0.04-0.78)	0.50 (0.00-1.00)
	Sawhney et al, 2009	2	0	15	2	0.12 (0.01-0.36)	1.00 (0.16-1.00)
	Mertz et al, 2011	3	1	4	2	0.43 (0.10-0.82)	0.67 (0.09-0.99)
	Toll et al, 2010	1	0	0	1	1.00 (0.03-1.00)	1.00 (0.03-1.00)
	Panarelli et al, 2012	1	0	3	0	0.25 (0.01-0.81)	0.50 (0.00-1.00
	Nikiforova et al, 2013	53	0	63	26	0.46 (0.36-0.55)	1.00 (0.87-1.00)
	Kung et al, 2014	2	0	3	1	0.40 (0.05-0.85)	1.00 (0.03-1.00)
	Jones et al, 2015	8	0	2	0	0.80 (0.44-0.97)	0.50 (0.00-1.00)
	Singhi et al, 2016	17	0	9	15	0.65 (0.44-0.83)	1.00 (0.78-1.00)
	Singhi et al, 2017	47	0	28	27	0.63 (0.51-0.74)	1.00 (0.87-1.00)
	Al-Haddad et al, 2014	16	0	26	6	0.38 (0.24-0.54)	1.00 (0.54-1.00)
	Kadayifci et al, 2016	50	0	70	27	0.42 (0.33-0.51)	1.00 (0.87-1.00)

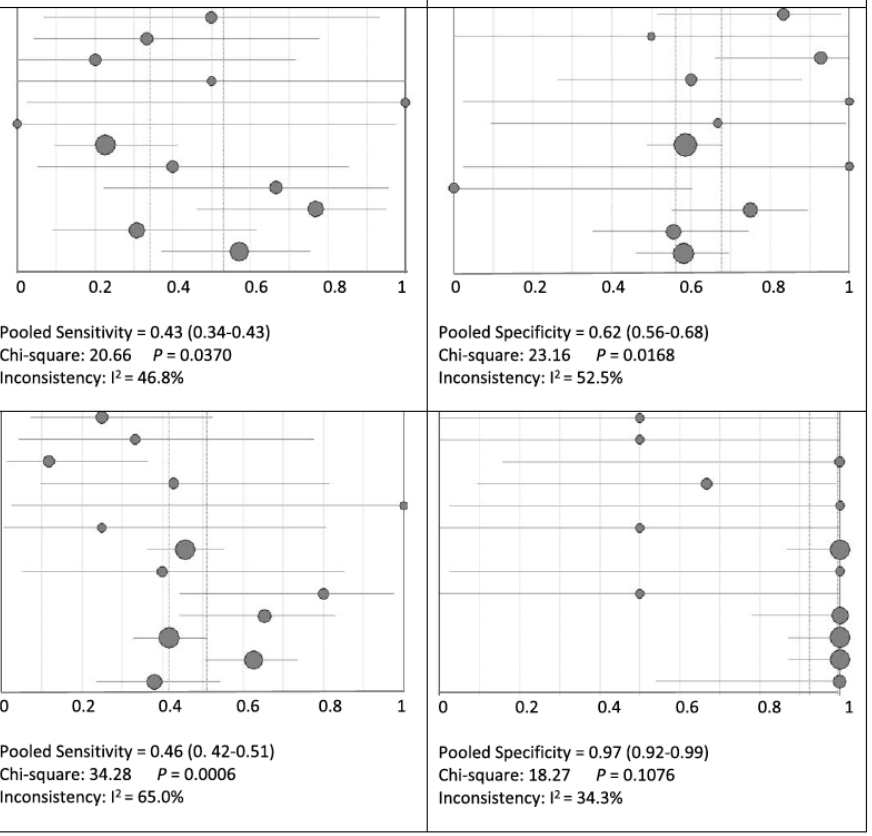

FIGURE 3. Forest plots of the included studies for *KRAS*. Between brackets are the 95% CIs of the sensitivity and specificity. The figure shows the estimated sensitivity of the study (circle) and its 95% CI (horizontal line). The area of the circle reflects the weight that the study contributes to the meta-analysis.

- *Faias S, et al. Pancreas. 2019; 48(6):749-758.*

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Molecular Testing

Marker	Purpose	Diagnostic finding	Utility
DNA analysis	Separation of benign from malignant cysts	Aneuploid and tetraploid results favor malignancy	Does not significantly improve diagnostic accuracy over routine cytology in majority of studies
KRAS mutation	Identification of mucinous cystic lesions	Presence of *KRAS* mutation supports diagnosis of a mucinous cyst	Distinguishes mucinous from nonmucinous cysts
VHL gene mutation	Identification of serous cystadenoma	Mutation present	Support the diagnosis of serous cystadeoma
CTNNB1 (beta-catenin) mutation	Identification of solid pseudopapillary neoplasm	Mutation present	Supports the diagnosis of solid pseudopapillary neoplasm
GNAS mutation	Identification of IPMN	Mutation present	Supports the diagnosis of IPMN
RNF43 mutations	Identification of cystic mucinous lesions	Mutation present	Distinguishes mucinous from nonmucinous cysts

Intraductal Papillary Mucinous Neoplasm

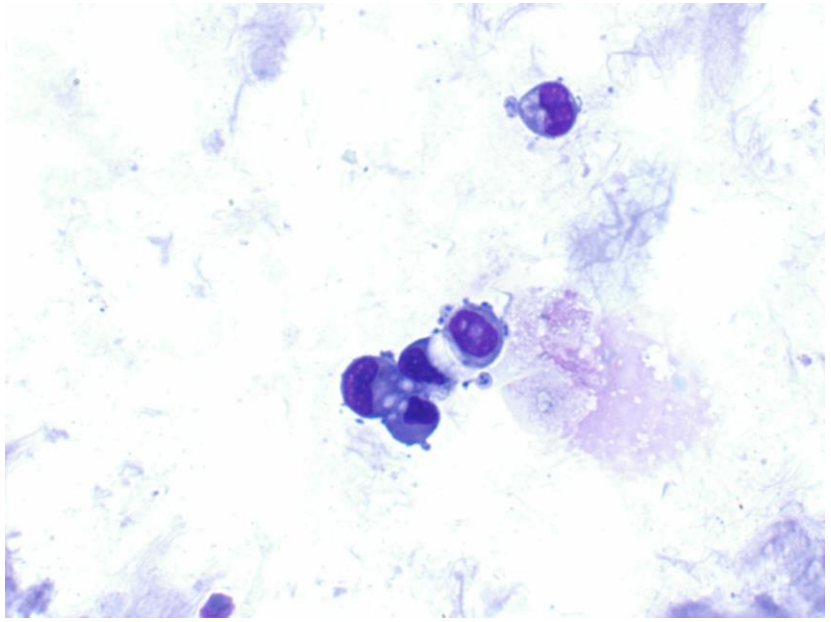
- The primary differential diagnosis for low-grade intraductal papillary mucinous neoplasm (IPMN) is gastrointestinal contamination.
- Ancillary tests are often critical for the diagnosis of a low-grade mucinous cyst because of the pauci-cellularity and thin mucin of most branch duct IPMNs.
- Cyst fluid CEA levels > 192 ng/mL are approximately 80% accurate for a mucin-producing neoplasm and have been shown to be the best test for classifying a cyst as mucinous.
- Molecular assays performed on cyst fluid or supernatant material can identify mutations correlating with mucinous neoplasia, such as *KRAS*, *GNAS*, and *RNF43* mutations, which are seen in as many as 75%, 60%, and 70% of IPMNs, respectively.

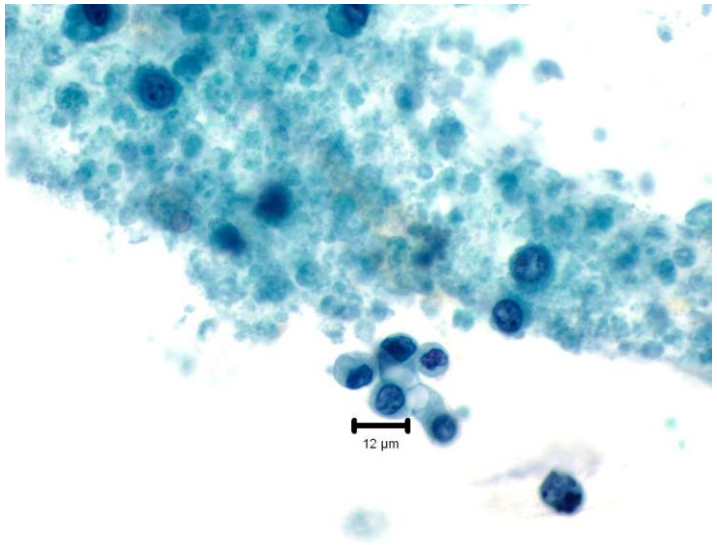
Diagnostic Considerations

Step 3: Grading Mucinous Neoplasm

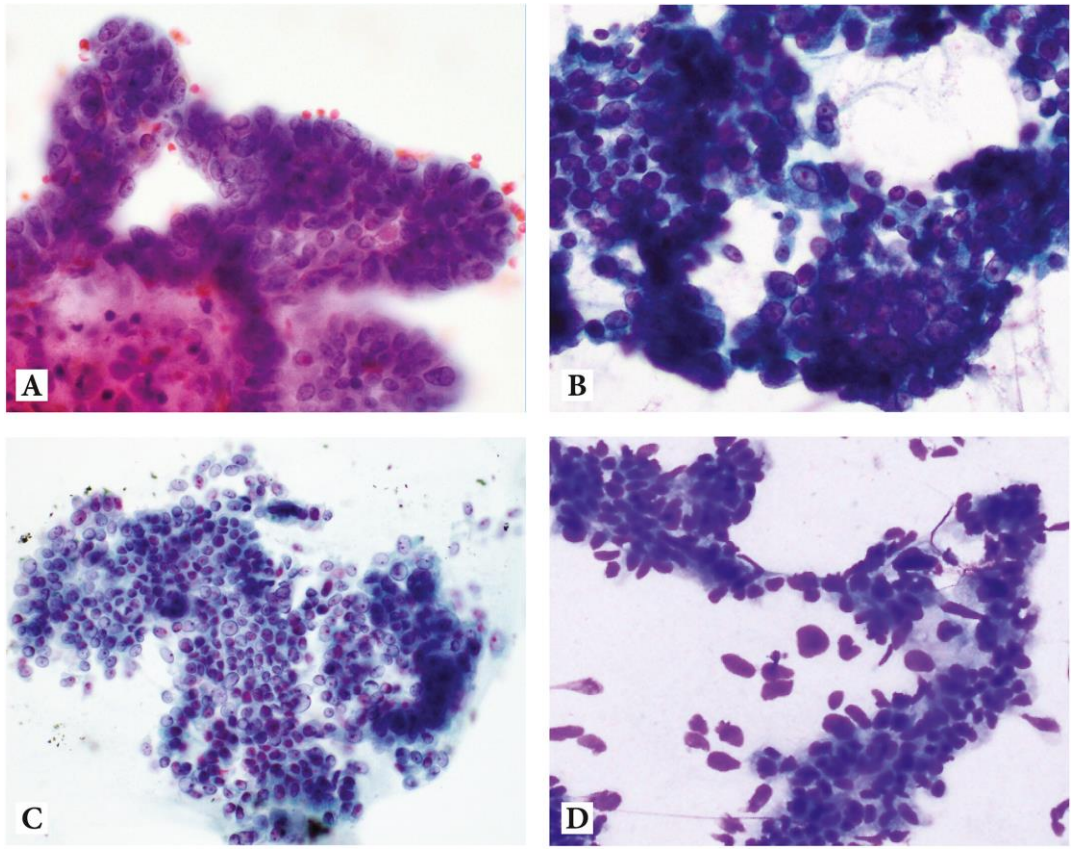
High-Grade Dysplasia

- Defined as a cell smaller than a 12 µm duodenal enterocyte with a high N:C ratio and abnormal chromatin, which can be hypochromatic or hyperchromatic, with or without a background of cellular necrosis.





High-Grade Dysplasia



- Three-dimensional architecture
- High N:C ratio
- Moderate nuclear membrane abnormalities
- Nuclear hyperchromasia
- Loss of nuclear polarity
- > 4:1 nuclear size variation
- Karyorrhexis
- Necrosis

Greater than or equal to four of eight select high-grade features was present in 36% of high-grade MN with sensitivity 37% and 98% specificity.

- *Sigel C, et al. Cancer Cytopathol. 2023; 131(5):325-336.*

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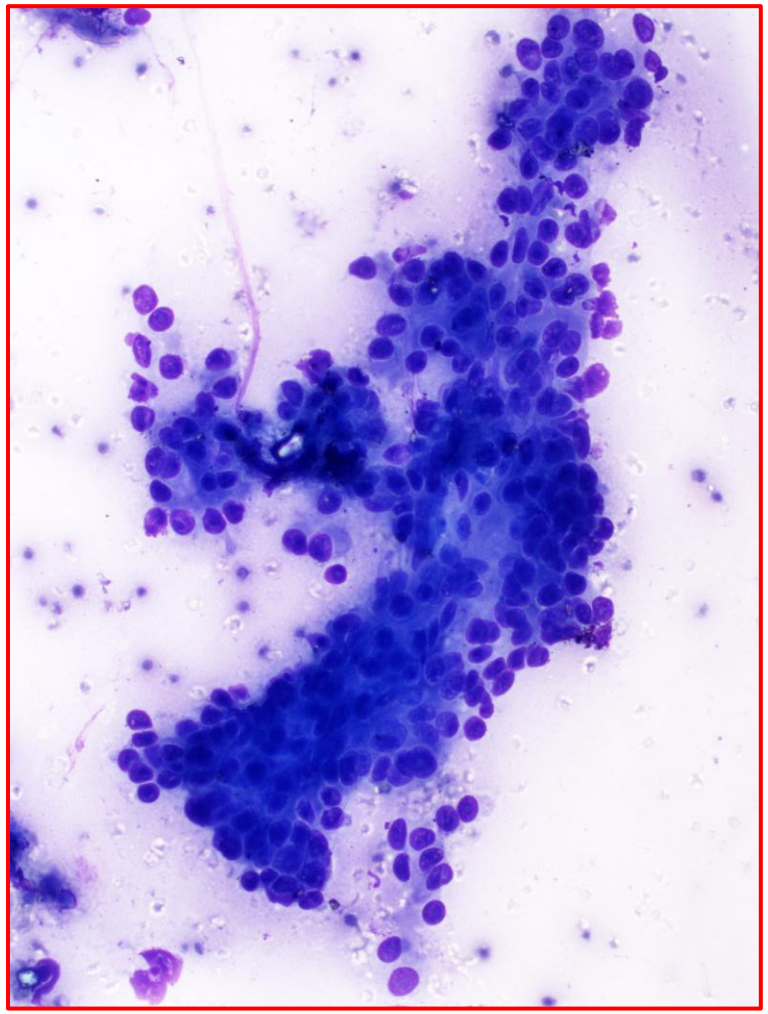
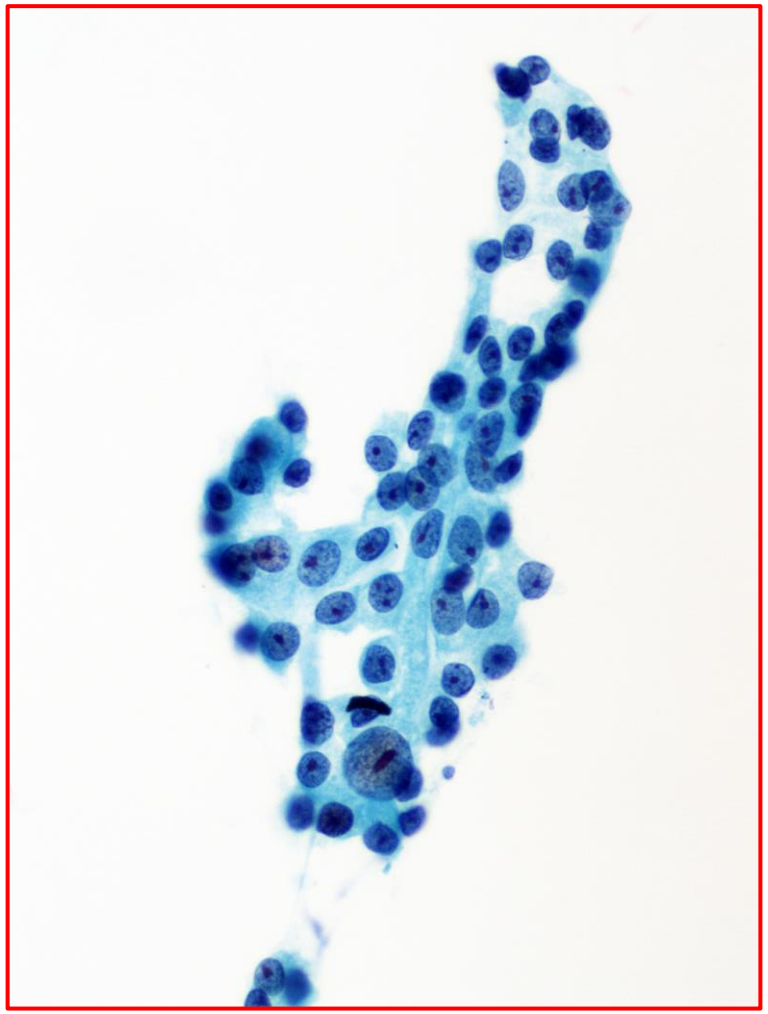
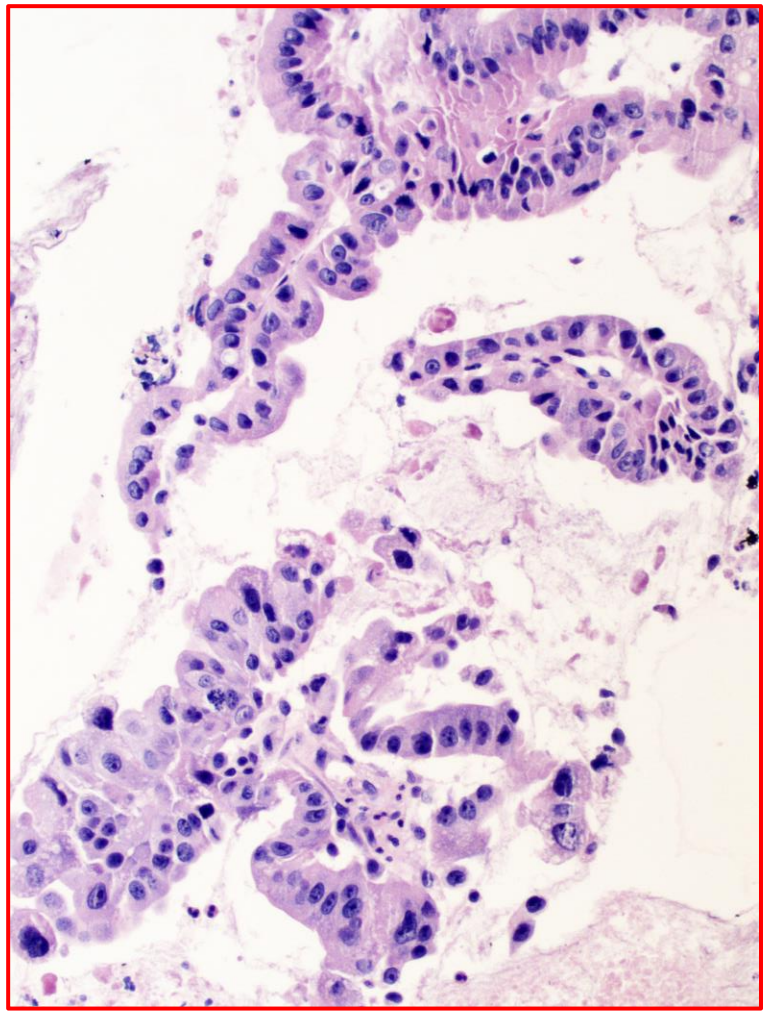
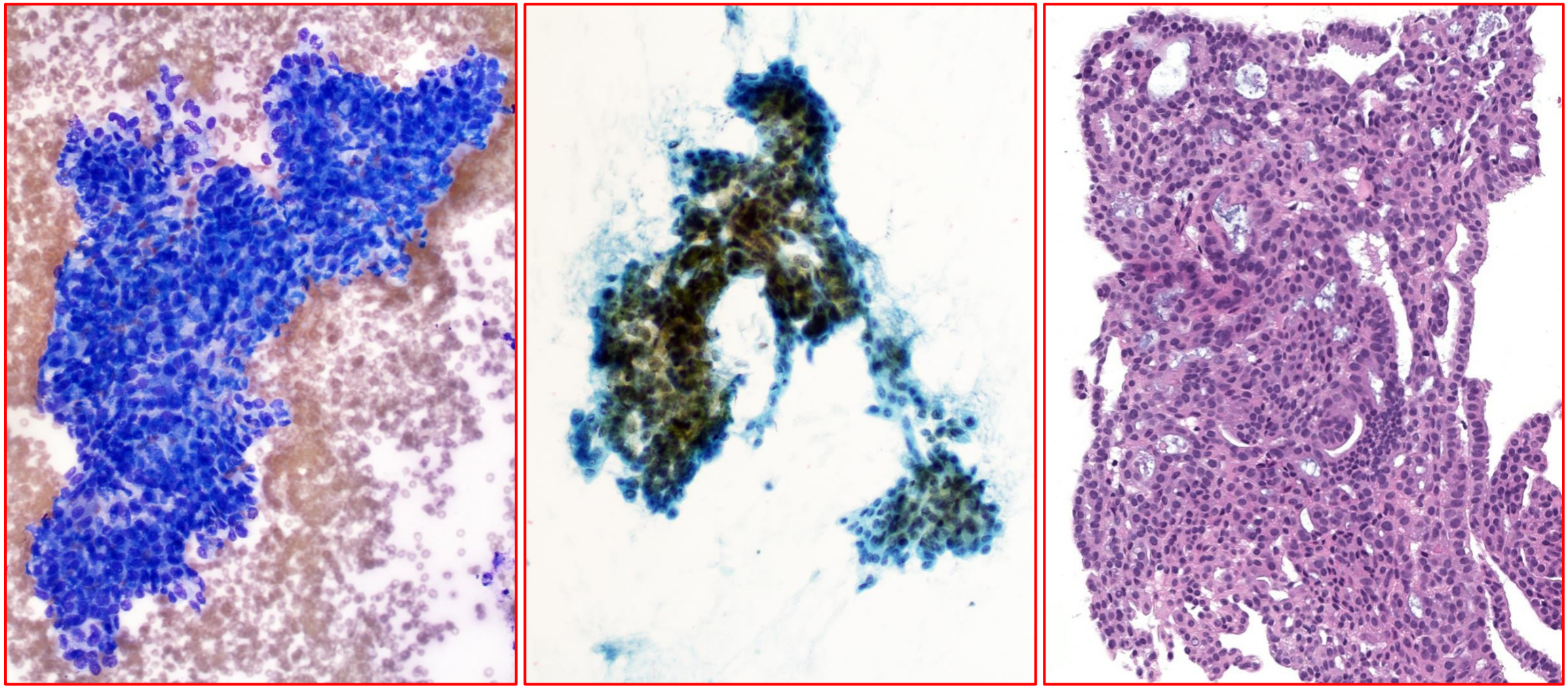
Intraductal Oncocytic Papillary Neoplasm







Intraductal Tubulopapillary Neoplasm



High-Grade Dysplasia

- Cytopathology is the best test for the detection of high-grade dysplasia, because CEA does not correlate with grade and molecular analysis is not yet the standard of care in the evaluation of cyst fluids.
- Genetic analysis can contribute to the assessment of risk by detecting mutations, which occur late in the progression to malignancy, including the *TP53* mutation and deletions in *CDKN2A* (*P16*) and *SMAD4*.
- Additional mutations in *PIK3CA* and *PTEN* are sensitive and specific for intraductal papillary mucinous neoplasm (IPMN) with either high-grade dysplasia or invasive carcinoma.

Pancreaticobiliary Neoplasm, High-Grade

Table 4 Sensitivities and specificities of molecular testing and other diagnostic modalities based on 102 surgically resected PCs

Parameter	Sensitivity (95% CI)	Specificity (95% CI)
IPMNs		
KRAS and/or *GNAS* mutations	100% (0.92 to 1.00)	96% (0.84 to 0.99)
Presence of multiple cysts	54% (0.40 to 0.67)	72% (0.56 to 0.84)
Increased fluid viscosity	82% (0.69 to 0.91)	80% (0.66 to 0.90)
Elevated CEA*	57% (0.40 to 0.73)	70% (0.53 to 0.83)
IPMNs with advanced neoplasia		
TP53, *PIK3CA* and/or *PTEN* alterations	88% (0.62 to 0.98)	95% (0.88 to 0.98)
KRAS and/or *GNAS* mutations with *TP53*, *PIK3CA* and/or *PTEN* alterations	88% (0.62 to 0.98)	97% (0.89 to 0.99)
GNAS MAF >55% or *TP53*/*PIK3CA*/*PTEN* MAFs at least equal to *KRAS*/*GNAS* MAFs	100% (0.77 to 1.00)	100% (0.95 to 1.00)
Main pancreatic duct dilatation	47% (0.24 to 0.71)	74% (0.63 to 0.83)
Presence of a mural nodule	35% (0.15 to 0.61)	94% (0.86 to 0.98)
Malignant cytopathology†	35% (0.15 to 0.61)	97% (0.91 to 1.00)
IPMNs and MCNs		
KRAS and/or *GNAS* mutations	89% (0.79 to 0.95)	100% (0.88 to 1.00)
Increased fluid viscosity	77% (0.65 to 0.86)	89% (0.73 to 0.96)
Elevated CEA*	57% (0.42 to 0.71)	80% (0.61 to 0.92)
IPMNs and MCNs with advanced neoplasia		
TP53, *PIK3CA* and/or *PTEN* alterations	79% (0.54 to 0.93)	95% (0.88 to 0.98)
KRAS and/or *GNAS* mutations with *TP53*, *PIK3CA* and/or *PTEN* alterations	79% (0.54 to 0.93)	96% (0.89 to 0.99)
GNAS MAF >55% or *TP53*/*PIK3CA*/*PTEN* MAFs at least equal to *KRAS*/*GNAS* MAFs	89% (0.66 to 0.98)	100% (0.95 to 1.00)
Main pancreatic duct dilatation	42% (0.21 to 0.66)	74% (0.63 to 0.82)
Presence of a mural nodule	32% (0.14 to 0.57)	94% (0.86 to 0.98)
Malignant cytopathology†	32% (0.13 to 0.57)	98% (0.91 to 1.00)

- *Singhi AD, et al. Gut 2018; 67:2131-2141.*

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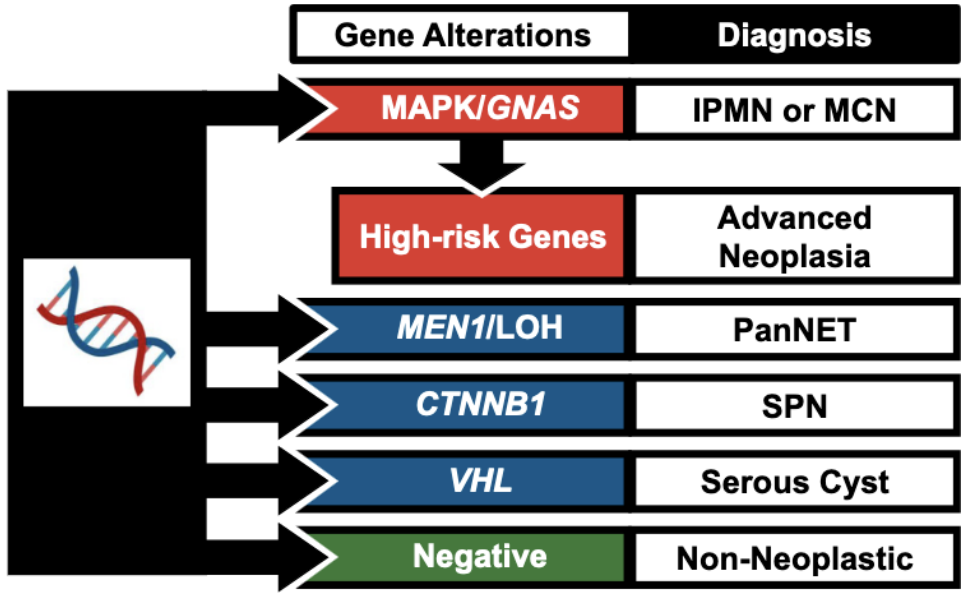
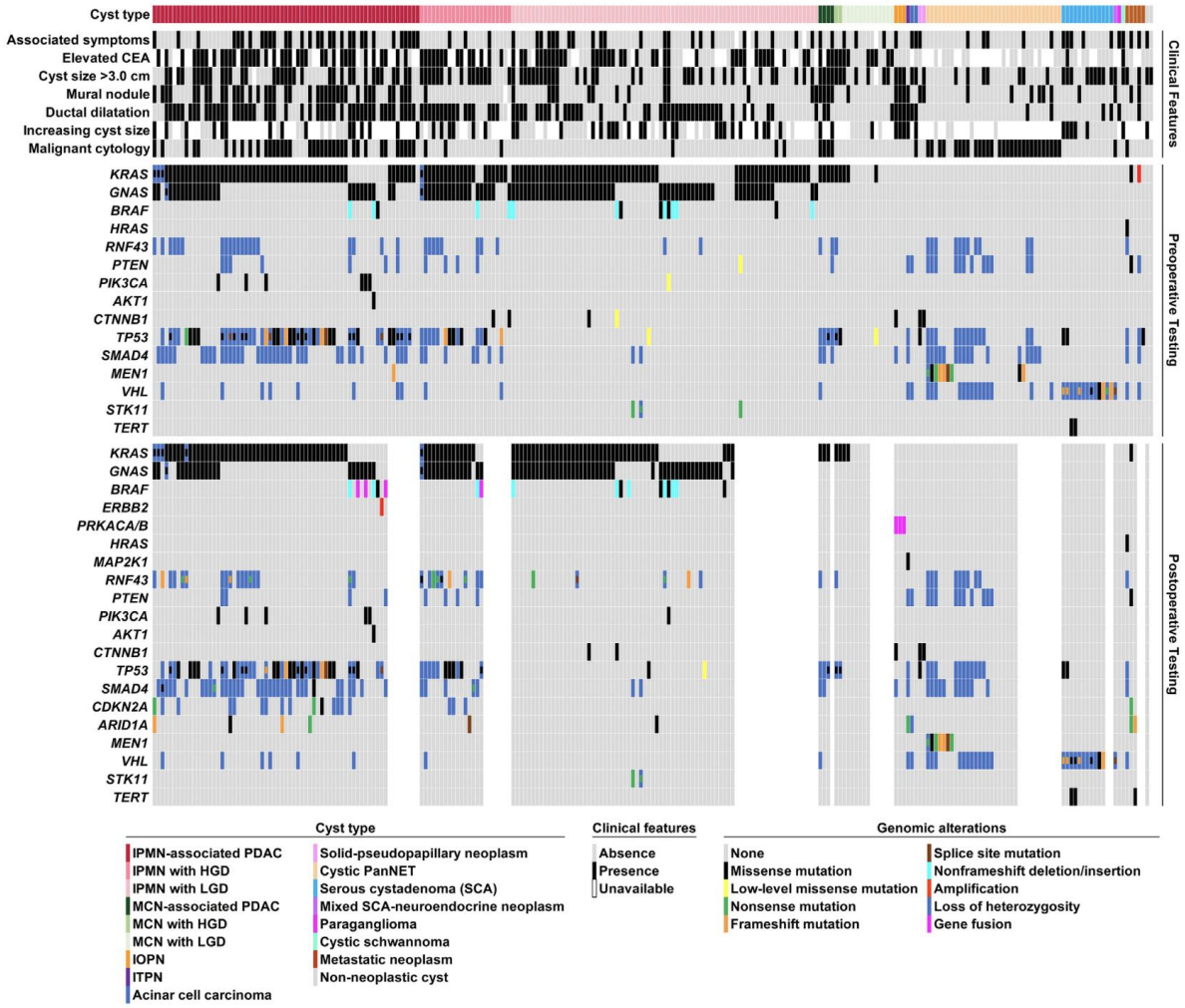
Pancreaticobiliary Neoplasm, High-Grade





- *Paniccia A, et al. Gastroenterology. 2023; 164:117-133.*

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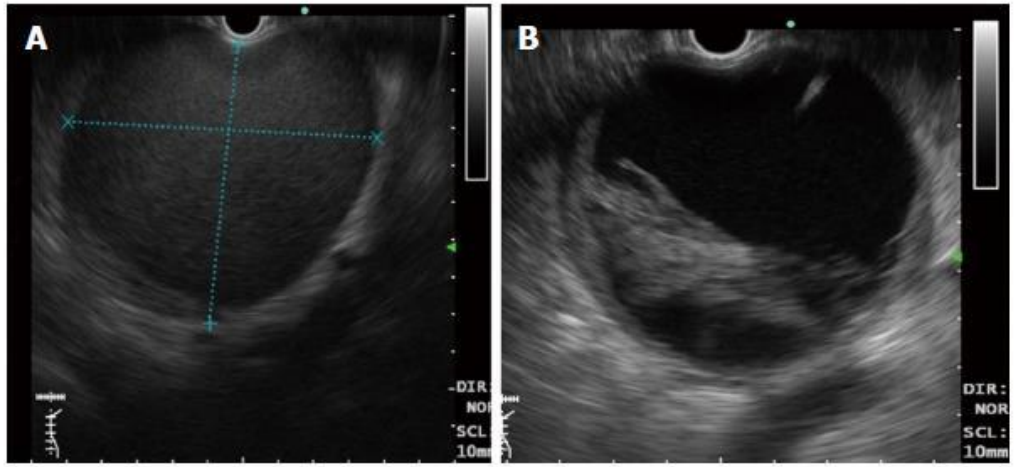
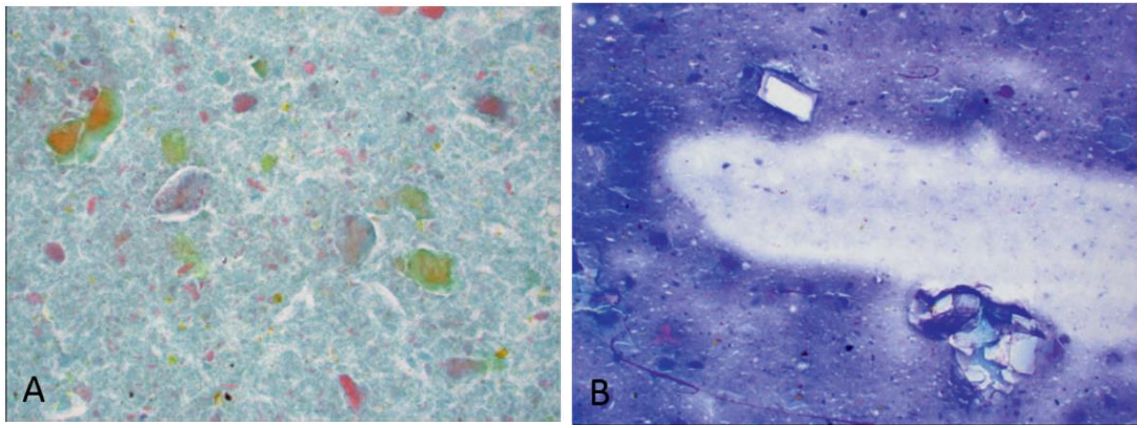
Diagnostic Considerations

Step 4: Recognition of Non-mucinous Lesions

Pseudocyst

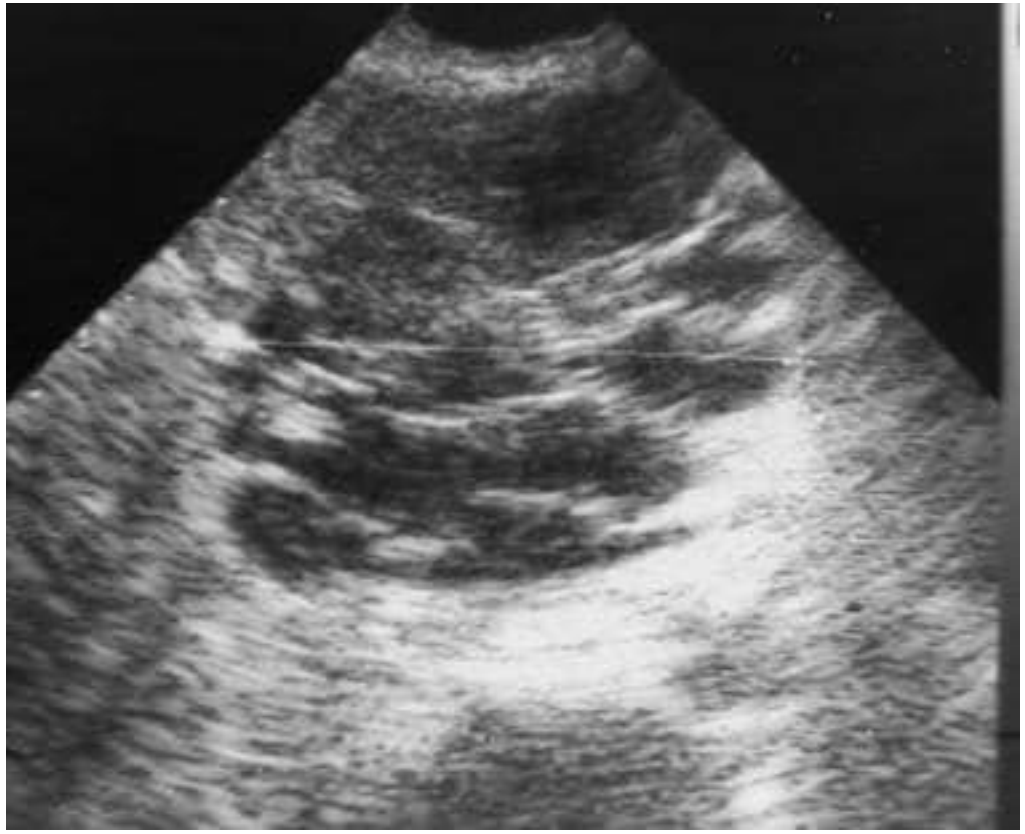
- Diagnosis of pseudocysts is based primarily on the patient's history and imaging findings.
- Aspirates show abundant inflammatory cells and histiocytes.
- Pseudocyst may show yellow pigment and cholesterol crystals.





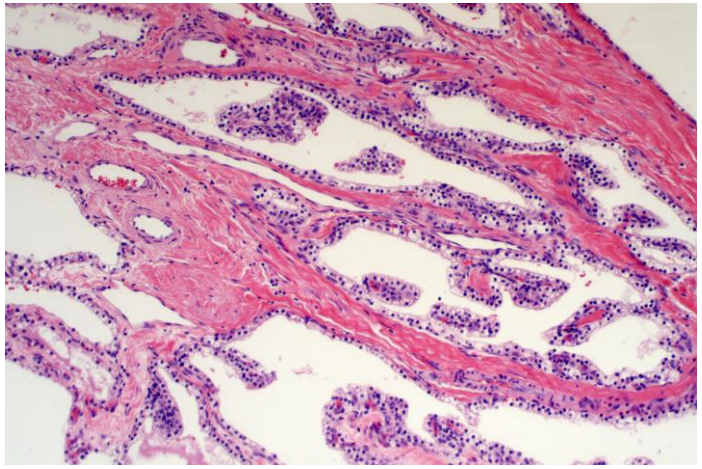
Serous Cystadenoma

- The most common benign pancreatic cystic neoplasm
- Female > male, ~ 60yrs
- May be associated with von Hippel–Lindau syndrome (90% VHL develop this tumor)
- Large microcystic mass with characteristic “soap bubble” pattern on ultrasound



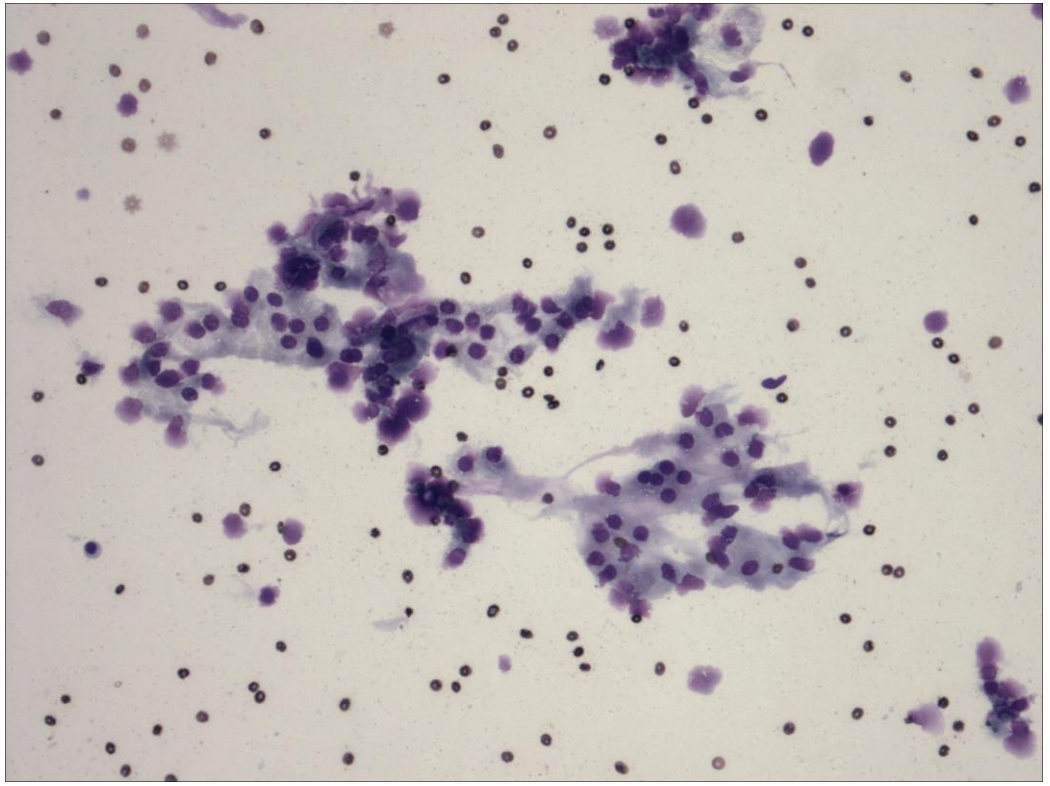
Serous Cystadenoma

- Well circumscribed cystic lesion with a central stellate scar and radiating hyalinized, vascular septa
- Microcystic, macrocystic or oligocystic
- Cysts are lined by low cuboidal cells with clear cytoplasm and central round to oval nuclei with indistinct nucleoli



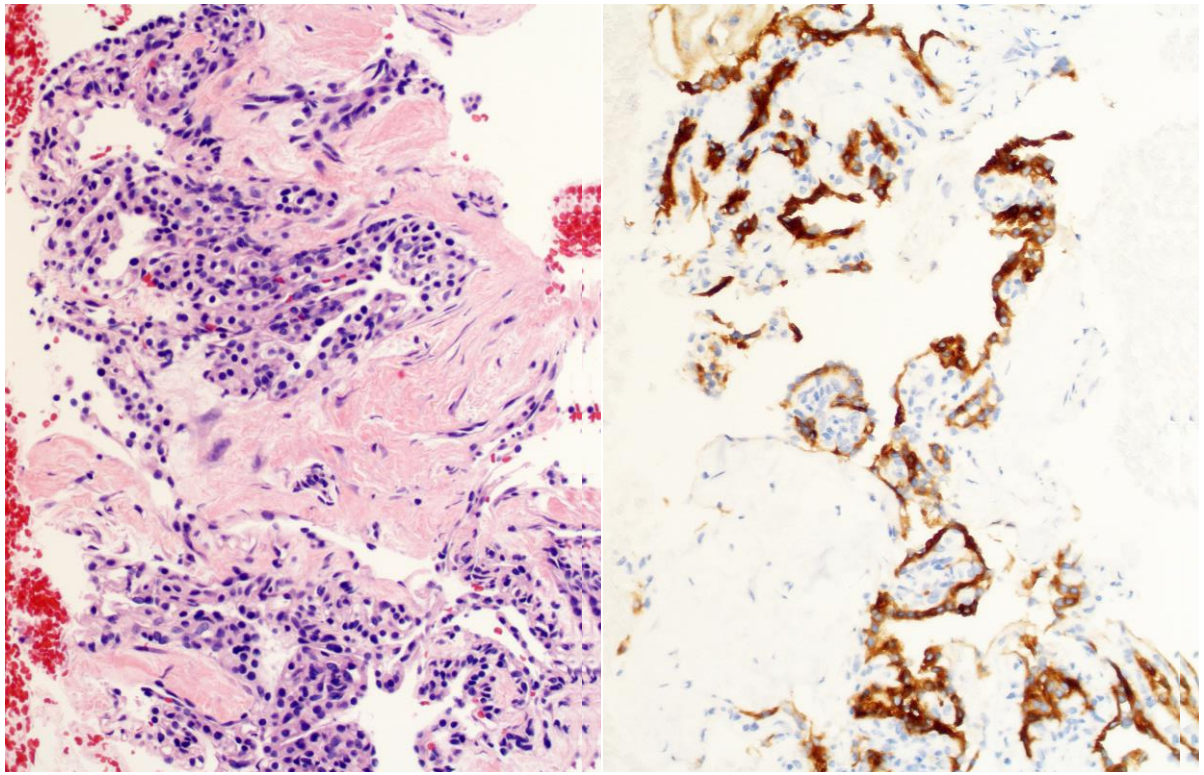
Serous Cystadenoma

- Sparsely cellular
- Loose or monolayered sheets
- Bland cuboidal cells with indistinct cell borders and granular or clear cytoplasm
- Round nuclei, inconspicuous nucleoli
- Hemosiderin-laden macrophages
- Clean or granular background without extracellular mucin

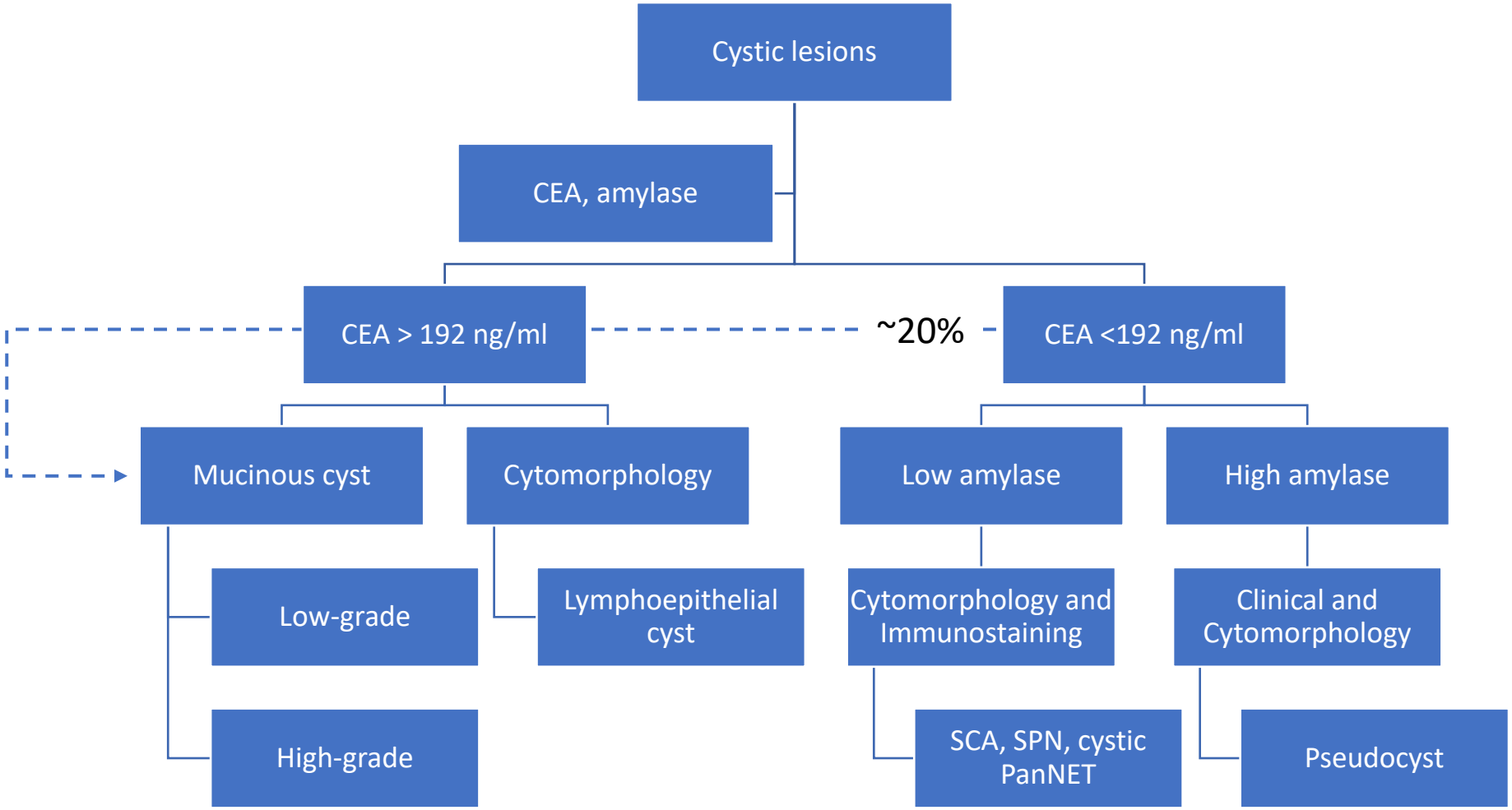


Serous Cystadenoma

- Low CEA (<192 ng/ml)
- Low amylase level (< 250 U/L)
- High glucose level (> 50 mg/dL)
- No *KRAS*/*GNAS* mutations
- *VHL* mutations or loss of heterozygosity
- Better yield by FNB
- Inhibin immunocytochemistry



Diagnostic Assessment



Summary

- WHO Reporting System consists of a seven-tiered diagnostic categories with corresponding risks for malignancy and management recommendation.
- Solid pancreatic lesions should be diagnosed based on cytomorphology in junction with immunocytochemistry and/or molecular testing.
- Cystic lesions of the pancreas should be assessed with a multimodal approach which combines clinical/imaging findings, cyst fluid analysis, and molecular testing.

Thank You!

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