Pathology of Pancreatic Cysts: From Cytology to Molecular

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Disclosure:

Dr. Swanson owns stock and serves on the Scientific Advisory Board for Cogen Bioscience

Dr. Swanson lectures for the France Foundation/ASCP/ACCC (CME/AMA) on biomarker testing in colorectal carcinoma

Objectives

Pancreatic Cystic Lesions(PCL)

- Brief Overview
- Classification
  - Pathology (Cytology and Surgical Pathology)
- Molecular
- Research (if time allows ☺)
- Multidisciplinary approach

Pancreatic Cystic Lesions

A common clinical conundrum

- Pancreatic cyst prevalence of 2.5%
- Increases with age
- M=F
- Up to 40% of patients age >70 have a PCL
- Increased utilization of cross-sectional imaging leading to increased detection of PCL

Am J Gastroenterol, 2013; 108: 1546-1550
Am J Gastroenterol 2010: 105:2079-2084

Pancreatic Cystic Lesions

A common clinical conundrum

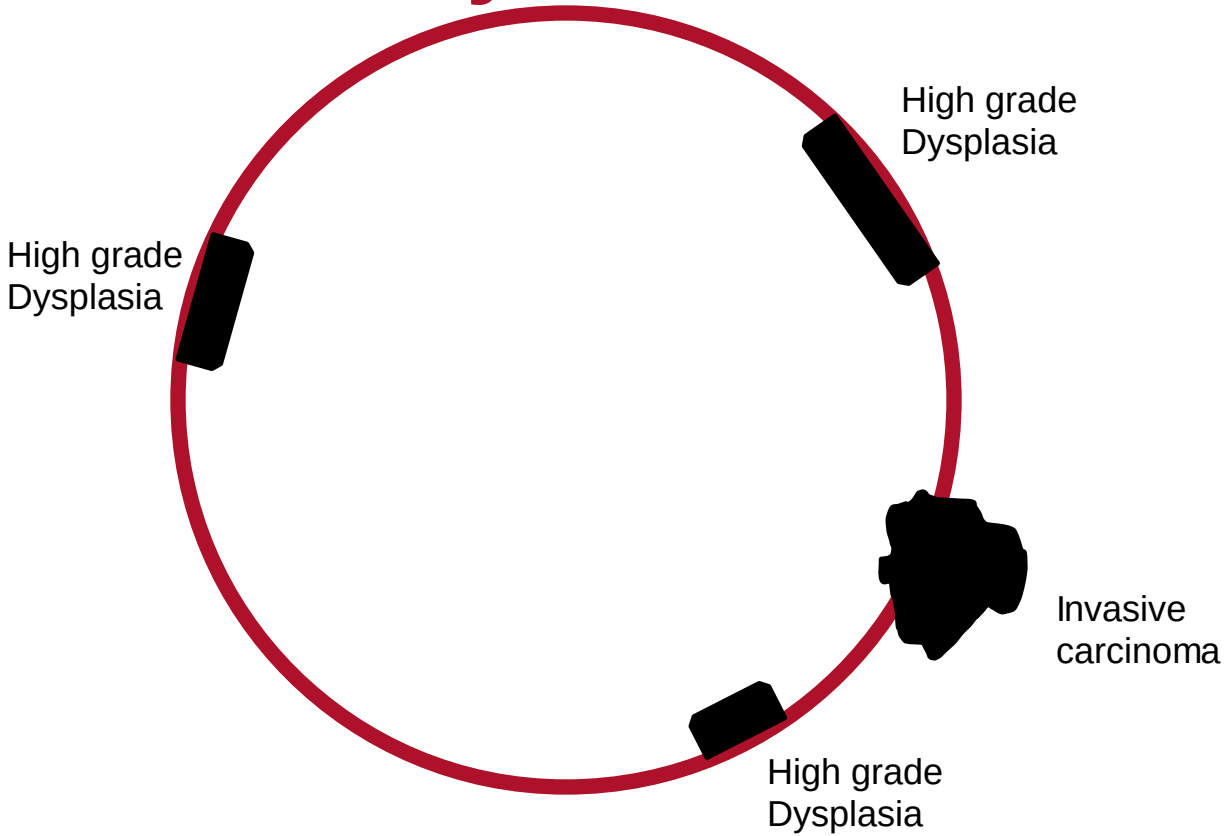
Many cysts are indeterminate at detection

About 50% of PCLs are mucinous (IPMN or MCN)

Many PCLs are benign or are unlikely to become cancer

→But some PCLs DO harbor cancer

Dysplasia/Carcinoma in Pancreatic Cysts



Pancreatic Cystic Lesions

Common:

- Intraductal papillary mucinous neoplasm (IPMN)
- Mucinous cystic neoplasm(MCN)
- Serous cystadenoma(SCA)
- Pseudocyst

Less common:

- Cystic pancreatic ductal adenocarcinoma
- Solid pseudopapillary neoplasm(SPN)
- Cystic pancreatic neuroendocrine tumor(PNET)

Management

Treatment decisions

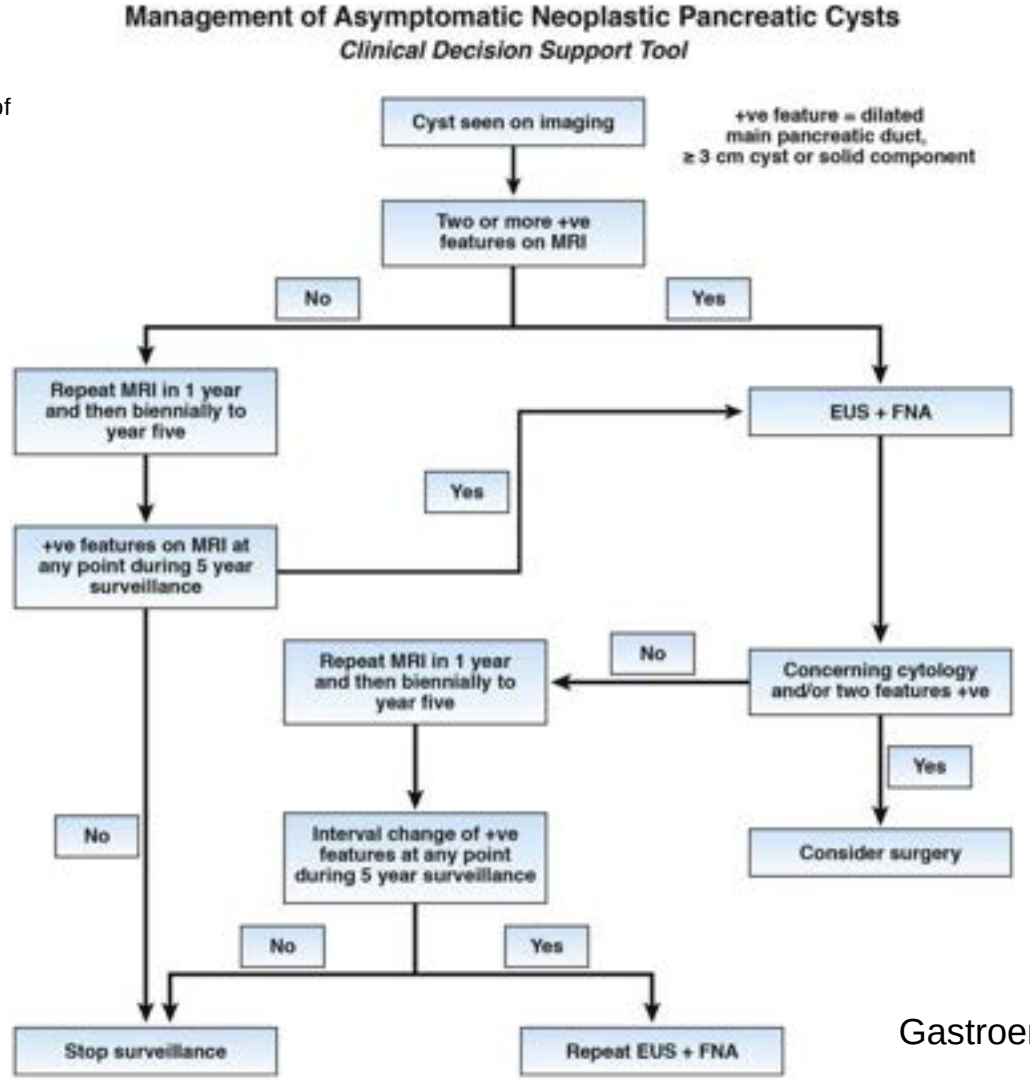
- MRI
- Observation with MRCP
- EUS with aspiration/biopsy
- Resection

Multidisciplinary approach

Pathology:

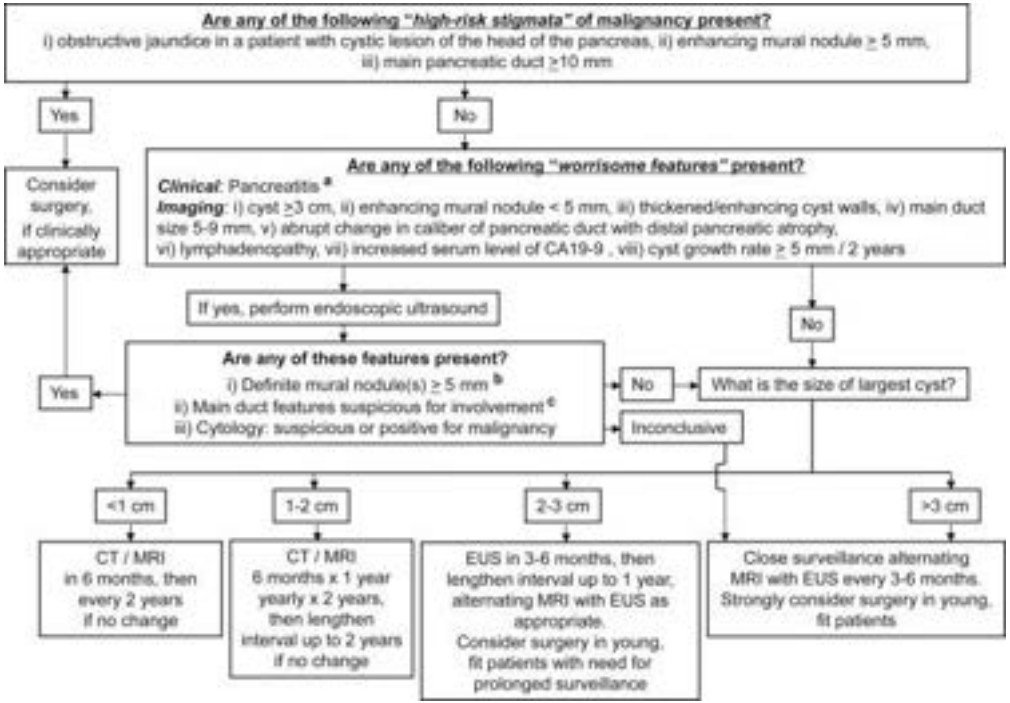
- Surgical Pathology: Definitive/Gold Standard: but not useful for pre-surgery decision making
- Cytology: Obtained from EUS-FNA, low sensitivity but very high specificity
- Molecular (Next generation sequencing (NGS) of DNA): Emerging technology with ability to be both highly sensitive and specific

American Gastroenterological Association Institute Guideline on the Diagnosis and Management of Asymptomatic Neoplastic Pancreatic Cysts



Gastroenterology 2015: 148:819-822

IAP/Fukuoka Guidelines for IPMN



Pancreatology 2017 Sep-Oct;17(5):738-753

IPMN Cytology

EUS: Often see mucin coming out of the ampulla of Vater

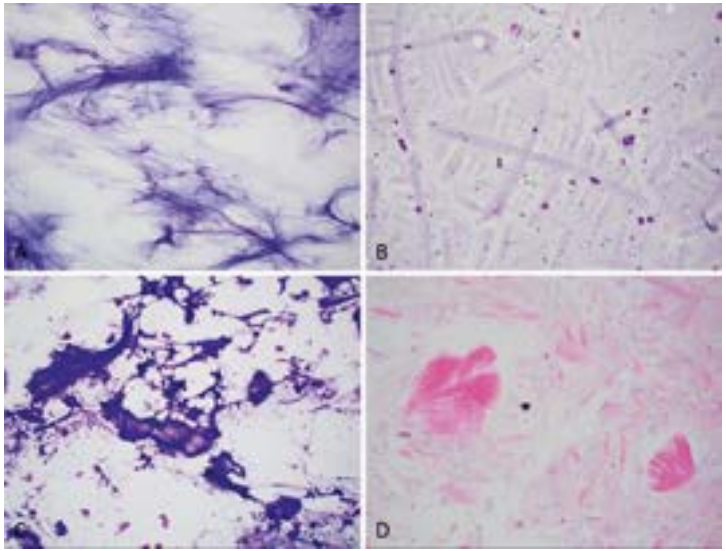
Cyst fluid analysis: Elevated CEA >192-200 ng/mL

Cytology: thick mucin (fan-like or fern-like), papillary clusters of cells with mucinous epithelium and/or goblet cells

Epithelium isn't always present

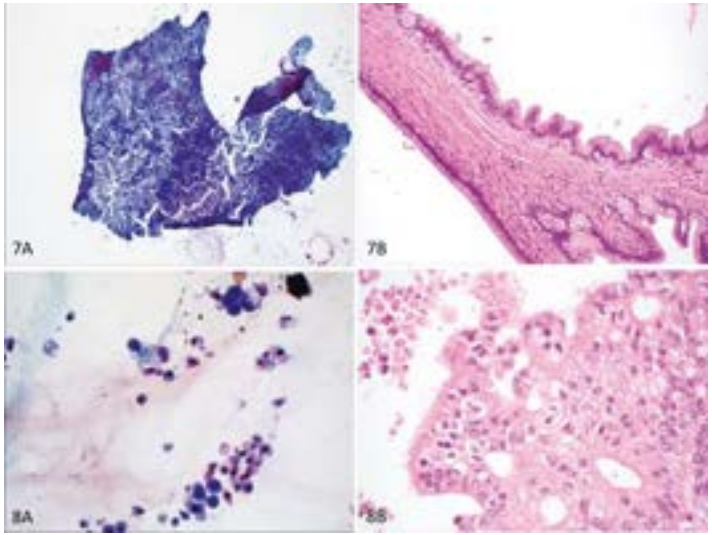
When epithelium present, pathologist should try and grade as low or high-grade (but we aren’t very good at this!)

IPMN Cytology
Mucin



From Abdelkader et al. "Cystic Lesions of the Pancreas: Differential Diagnosis and Cytologic-Histologic Correlation. Arch Pathol Lab Med. 2019;144(1):47-61. doi:10.5858/arpa.2019-0308-RA

IPMN Cytology



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IPMN Surgical Pathology

Cyst lined by mucinous epithelium forming papillary structures

4 recognized histologic subtypes: gastric, pancreaticobiliary, intestinal, and oncocytic

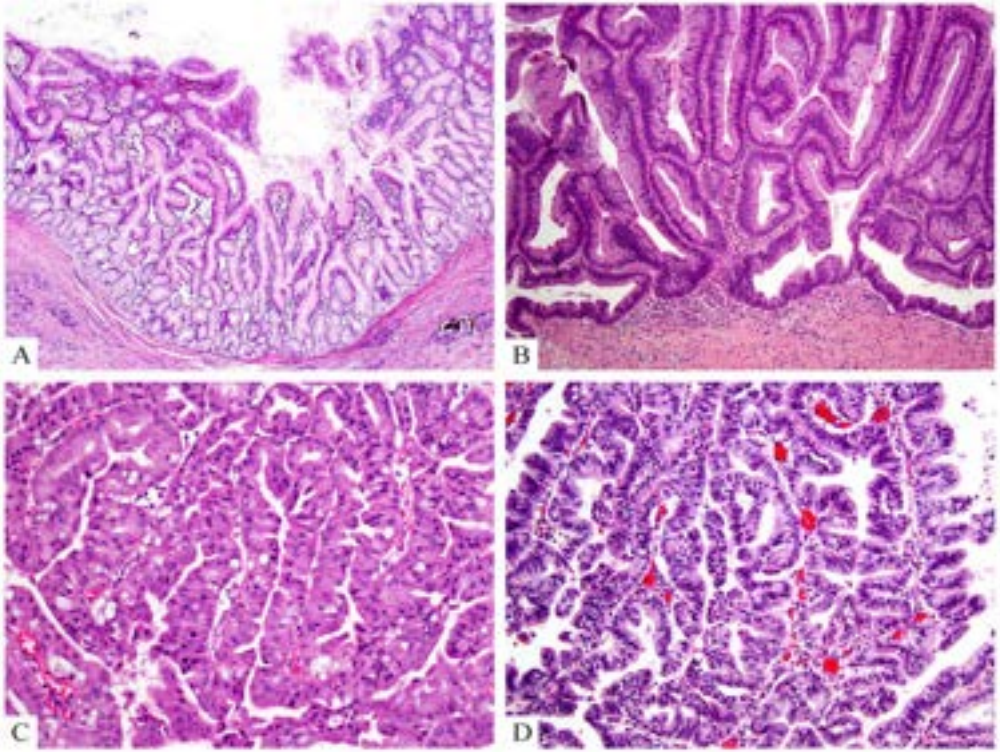
Pathologist should grade as low- or high-grade, as well as +/- invasive carcinoma

Side-branch IPMN: more likely to be low-grade dysplasia

Main duct IPMN: more likely to have high-grade dysplasia and invasive carcinoma

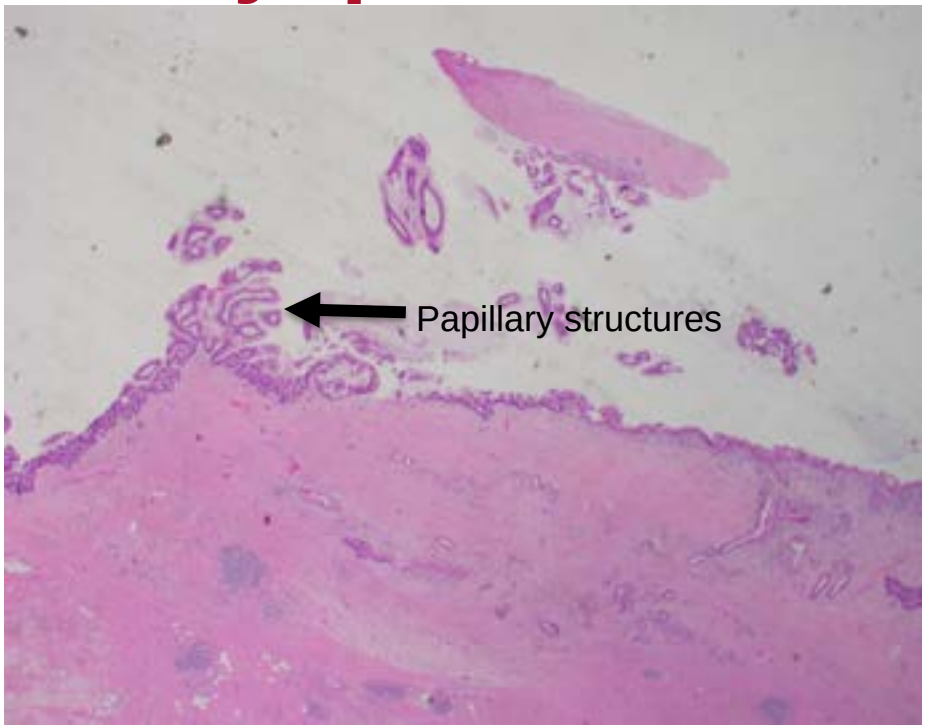
Molecular: Often have mutations in *GNAS* and/or *BRAF (Class II or III)*

IPMN Subtypes

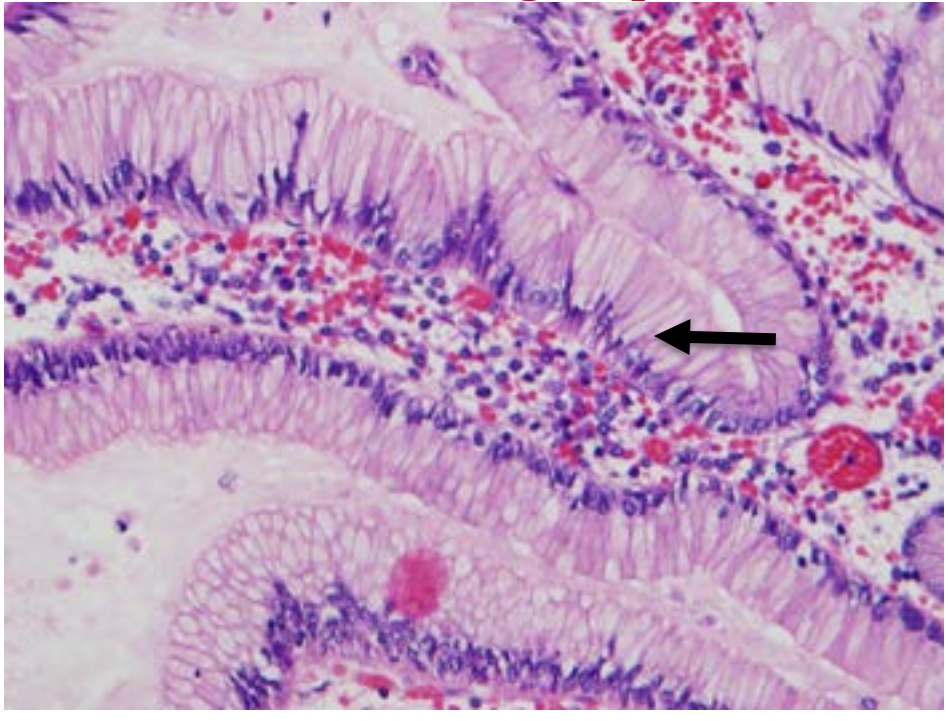


From Adsay et al. "Pathologic Evaluation and Reporting of Intraductal Papillary Mucinous Neoplasms of the Pancreas and Other Tumoral Intraepithelial Neoplasms of Pancreatobiliary Tract: Recommendations of Verona Consensus Meeting."Ann Surg. 2016 Jan;263(1):162-77

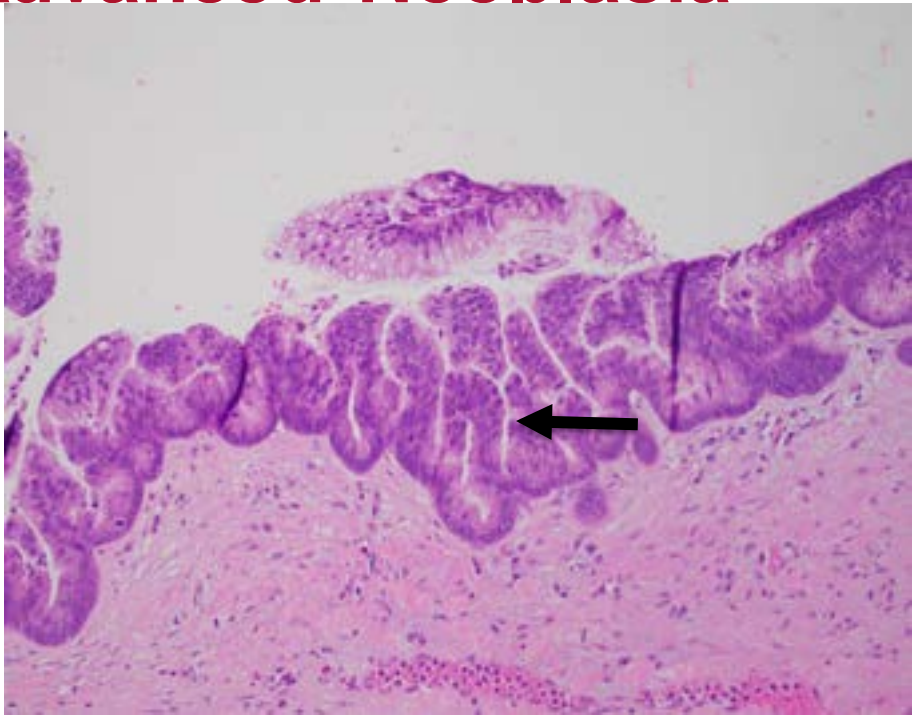
IPMN
Side Branch
Low-Grade Dysplasia



IPMN
Side Branch
Low-Grade Dysplasia



IPMN Main Duct
High-Grade Dysplasia
aka “Advanced Neoplasia”



IPMN with Invasive Adenocarcinoma aka “Advanced Neoplasia”

Surgical Pathology

Grossly identified as a solid nodule in cyst wall

Need to extensively sample IPMN to find cancer

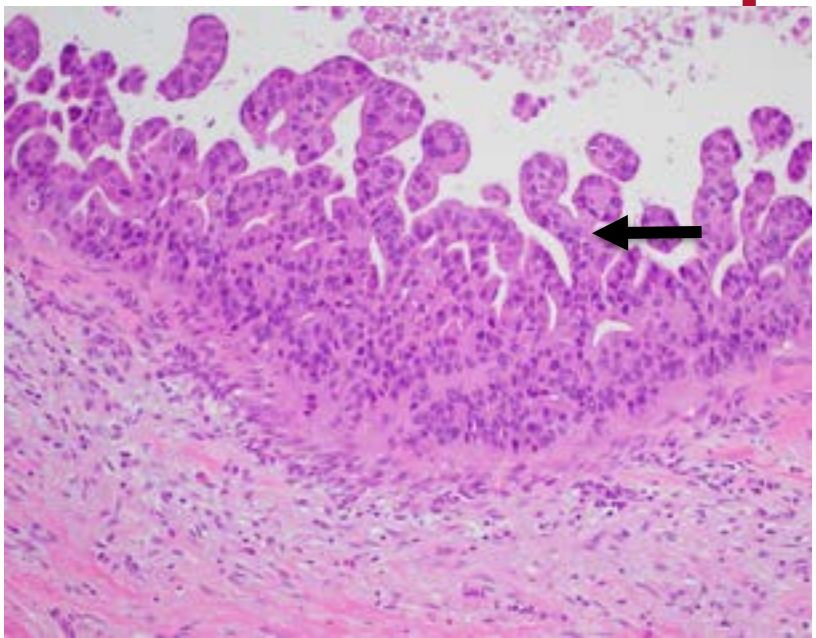
Haphazard growth-low power

Variation in nuclear size

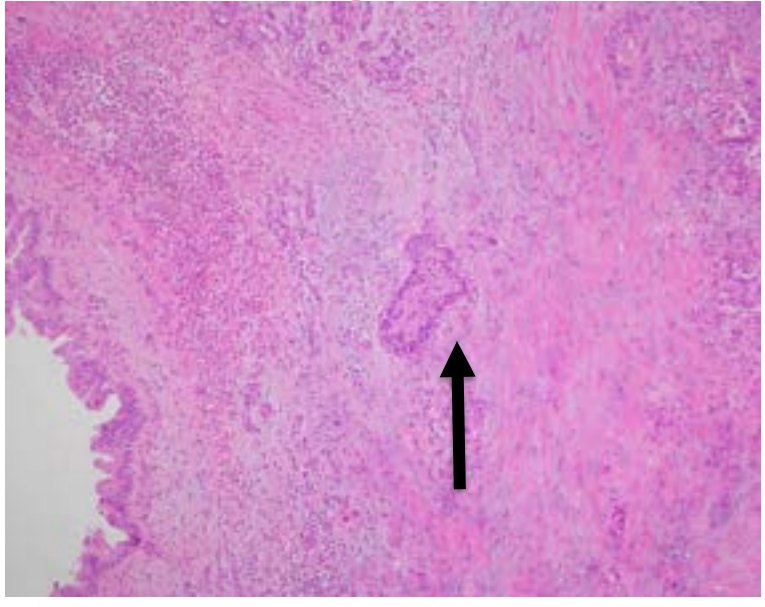
Perineural/perivascular invasion

Desmoplasia
Molecular: Often have mutations in *GNAS* and/or *BRAF*
Advanced neoplasia (HGD or invasive carcinoma): mutations in *TP53*, *SMAD4* and *mTOR*

IPMN with Invasive Adenocarcinoma & High Grade Dysplasia aka “Advanced Neoplasia”

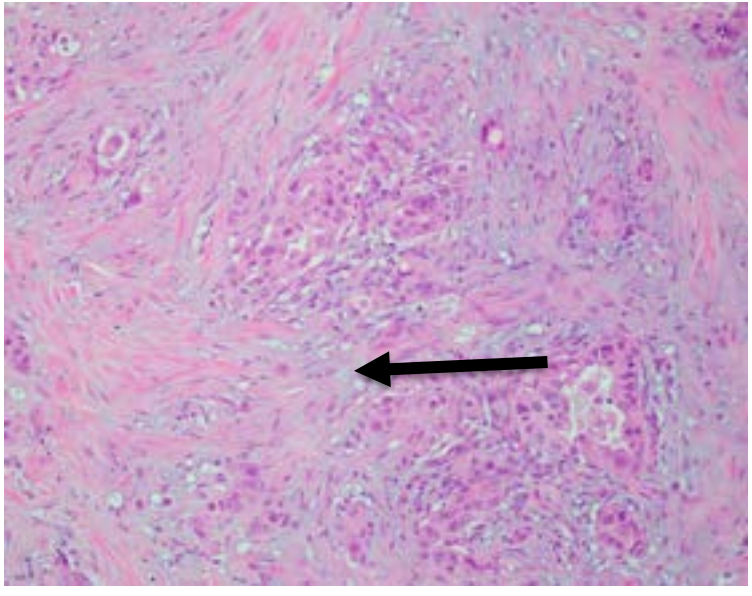


IPMN with Invasive Adenocarcinoma “Advanced Neoplasia”
Haphazard growth

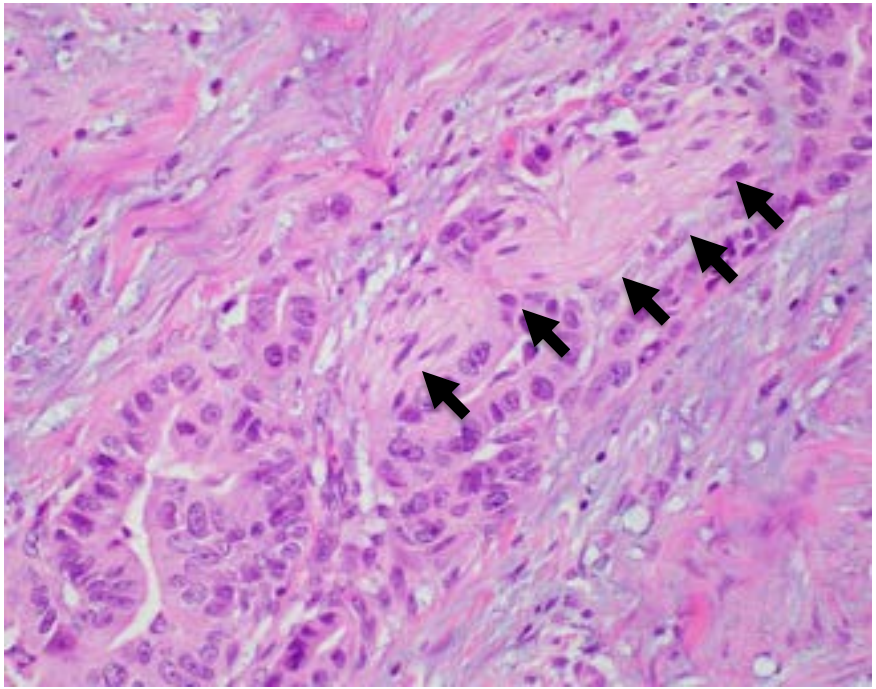


IPMN with Invasive Adenocarcinoma “Advanced Neoplasia”

Desmoplasia



IPMN with Invasive Adenocarcinoma Perineural Invasion



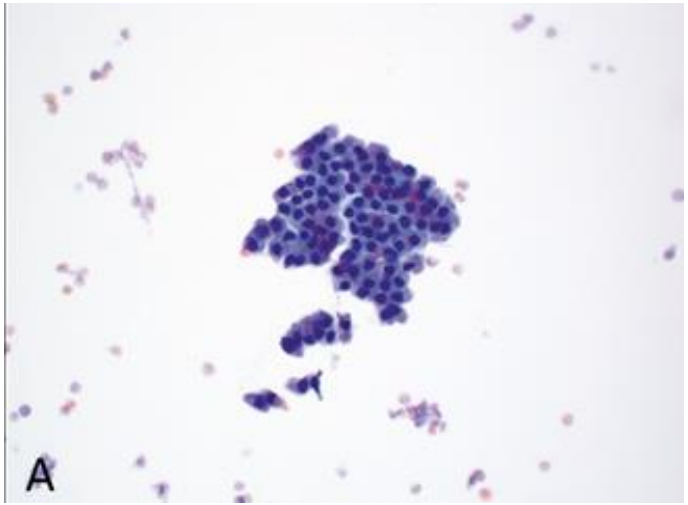
Mucinous Cystic Neoplasm Cytology

Cyst fluid analysis: CEA >192-200 ng/mL

Cytology: Low-grade MCN: Bland mucin cells arranged in a honeycomb arrangement;

High-grade: more complex architecture, more prominent nucleoli

Mucinous Cystic Neoplasm Cytology



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Mucinous Cystic Neoplasm Surgical Pathology

Gross exam: Cyst does not communicate with pancreatic duct

Lined by mucinous epithelium

Epithelium graded as low or high-grade dysplasia

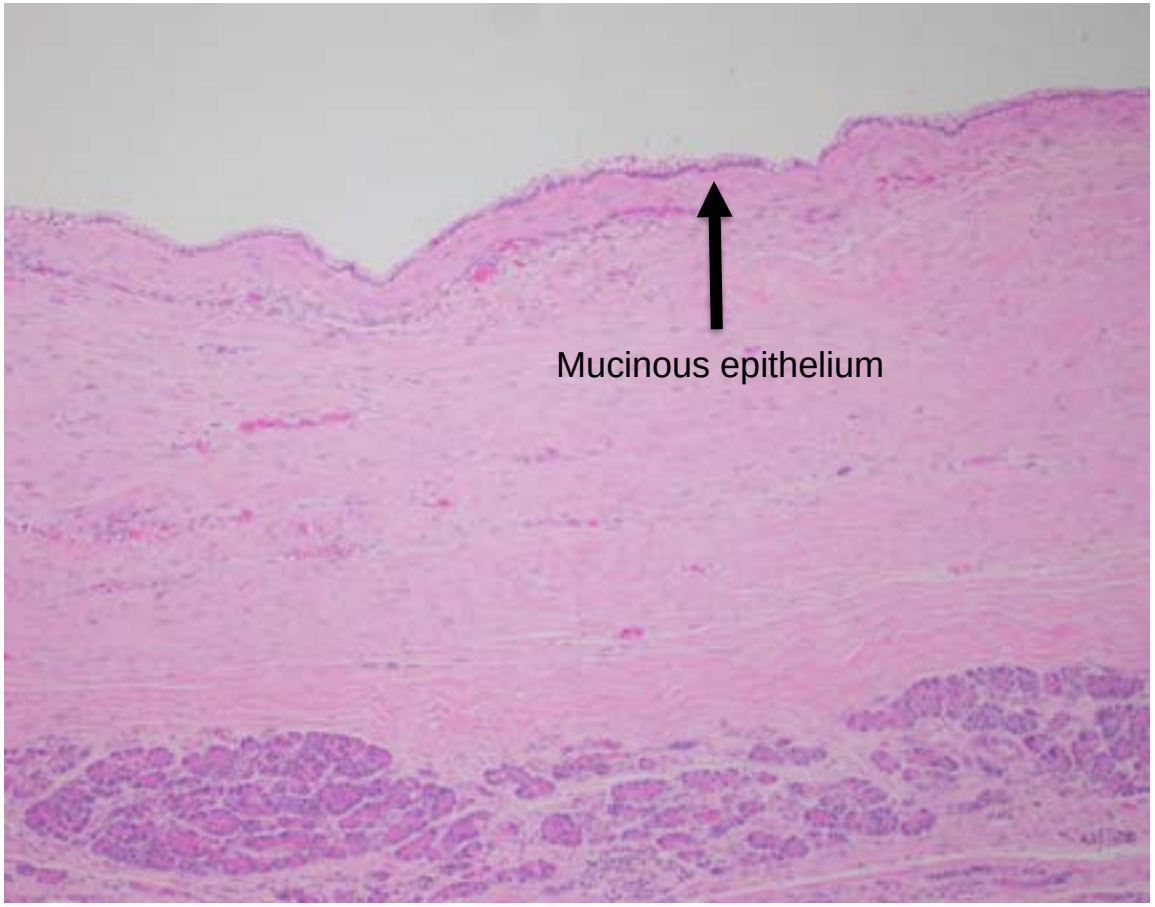
Invasive carcinoma must be excluded: sampling

Must have ovarian-type stroma: ER +, PR +, CD10 +, etc.

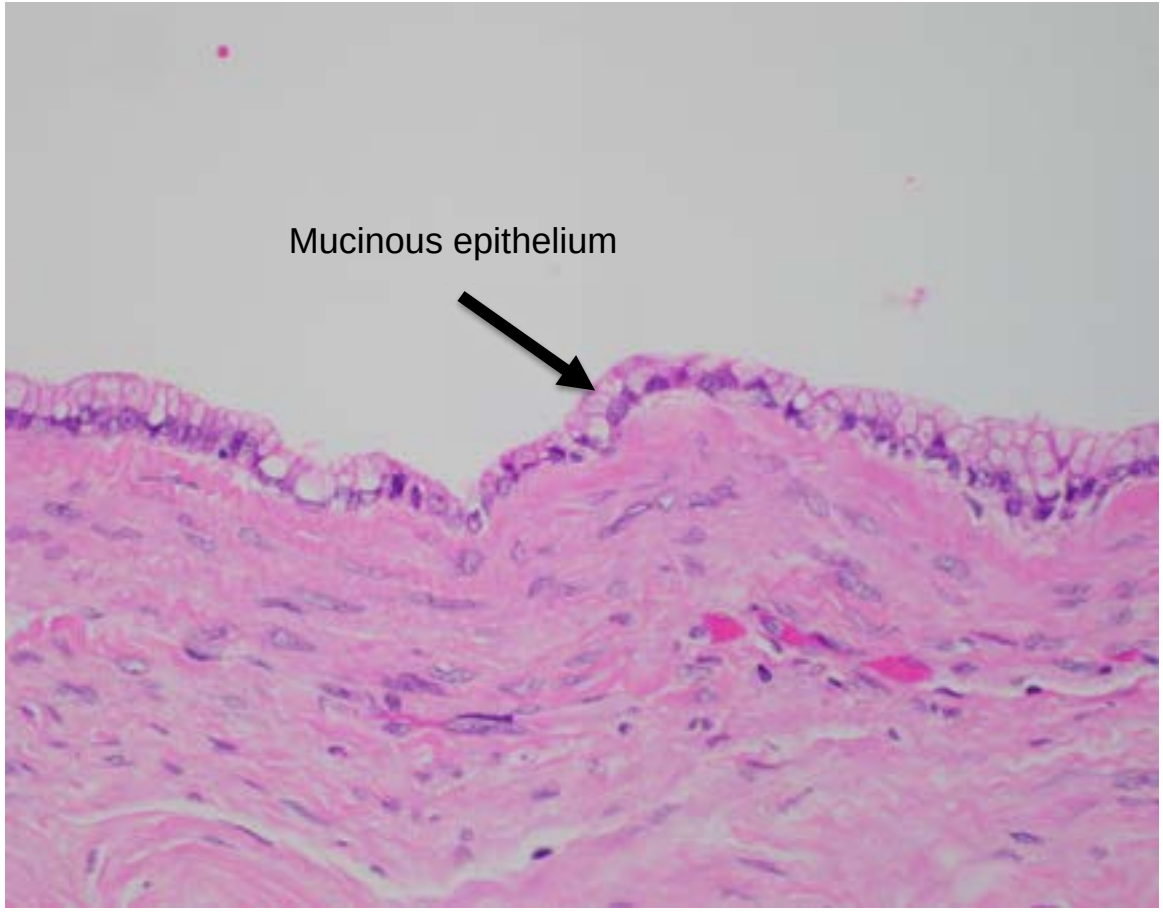
Some areas of the wall may be fibrotic/hyalinized

Molecular: Mutations in *KRAS* (*MAPK*)

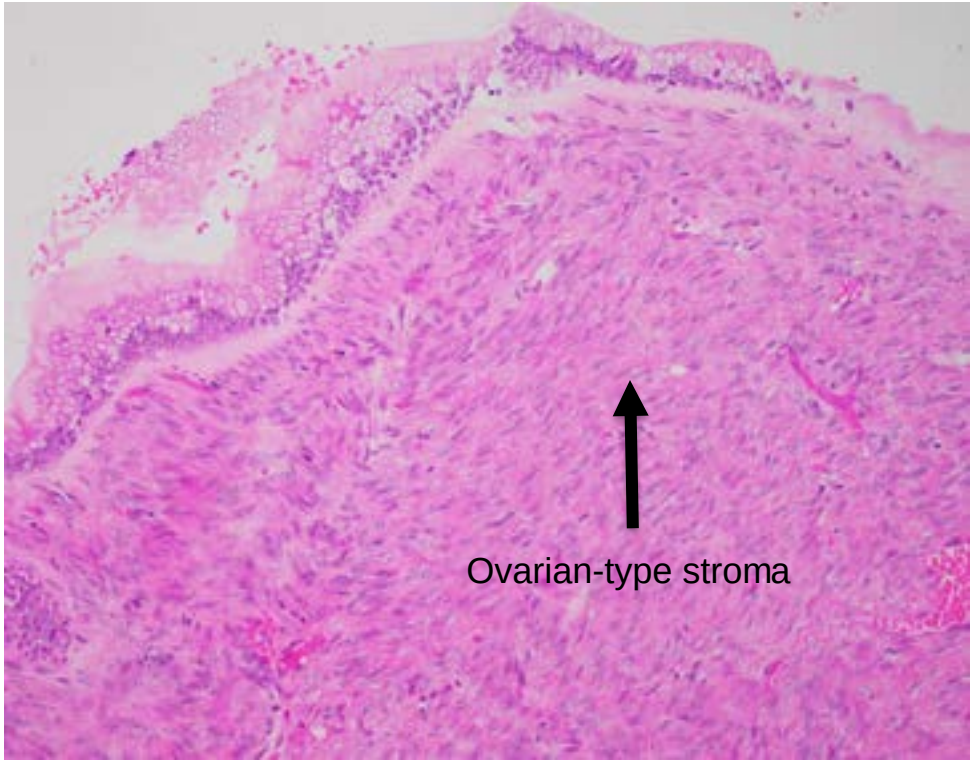
Mucinous Cystic Neoplasm



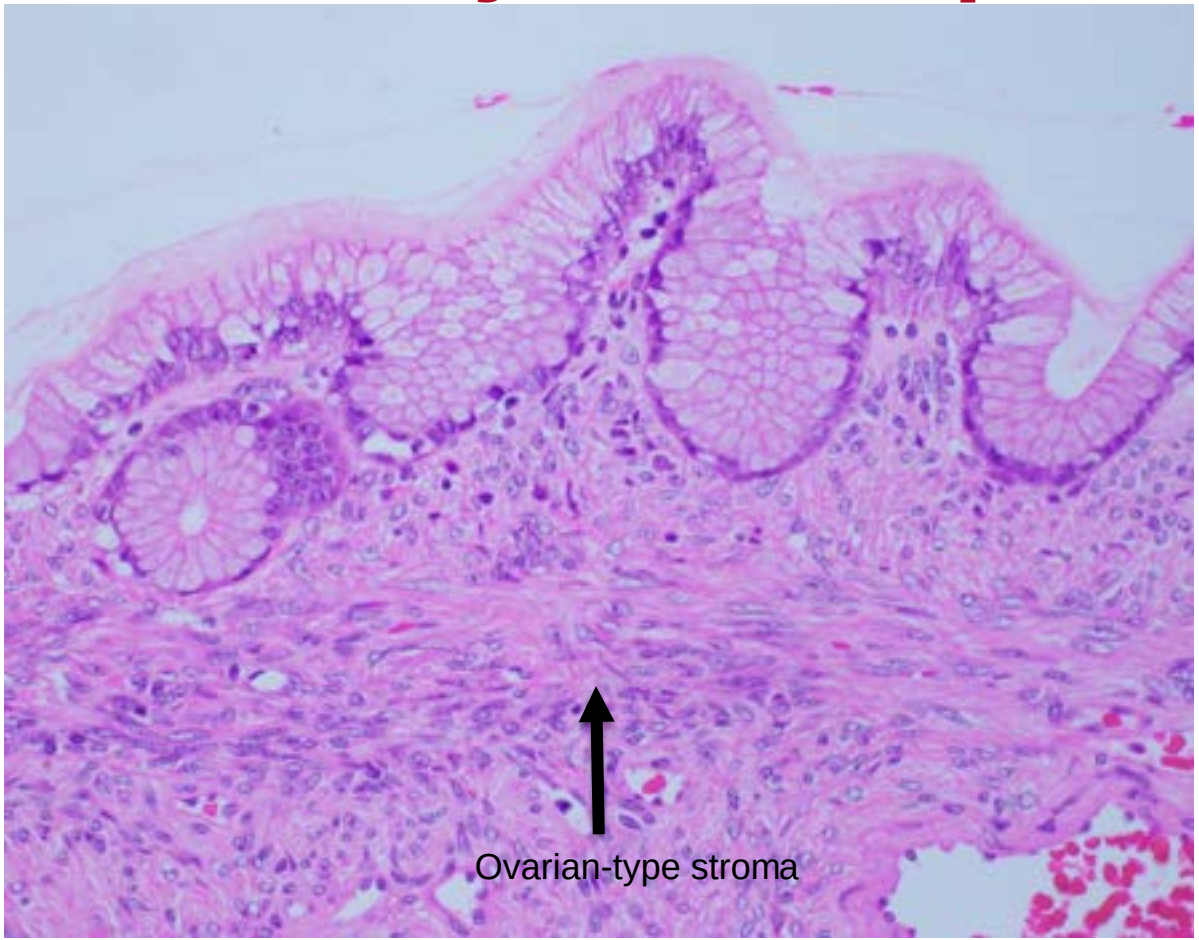
Mucinous Cystic Neoplasm



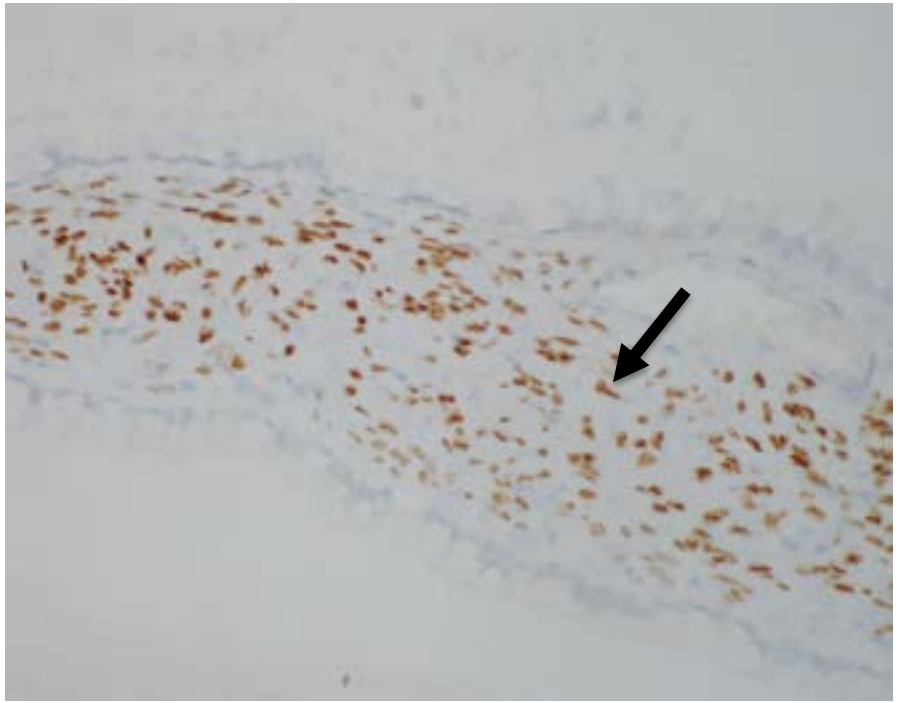
Mucinous Cystic Neoplasm



Mucinous Cystic Neoplasm



Mucinous Cystic Neoplasm
Progesterone Receptor



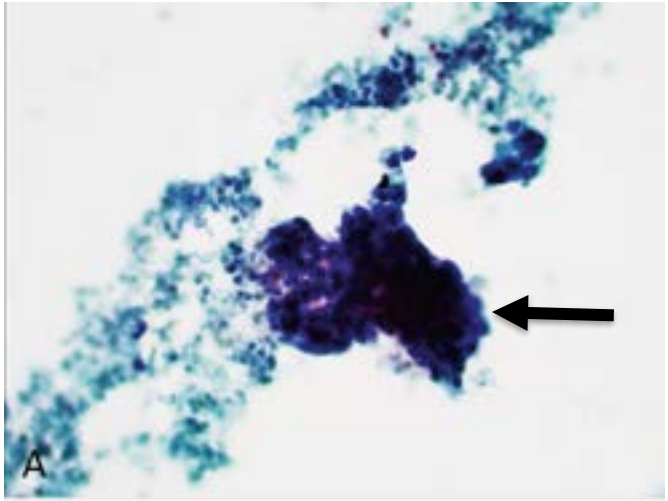
Serous Cystadenoma Cytology

EUS-FNA cytology is often paucicellular with blood and hemosiderin-laden macrophages

Fluid analysis: Low-viscosity clear fluid, low CEA levels

When cells are present, they should be non-mucinous, small, and lack nucleoli

Serous Cystadenoma Cytology



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Serous Cystadenoma Surgical Pathology

Gross exam: Fibrosis with central scar

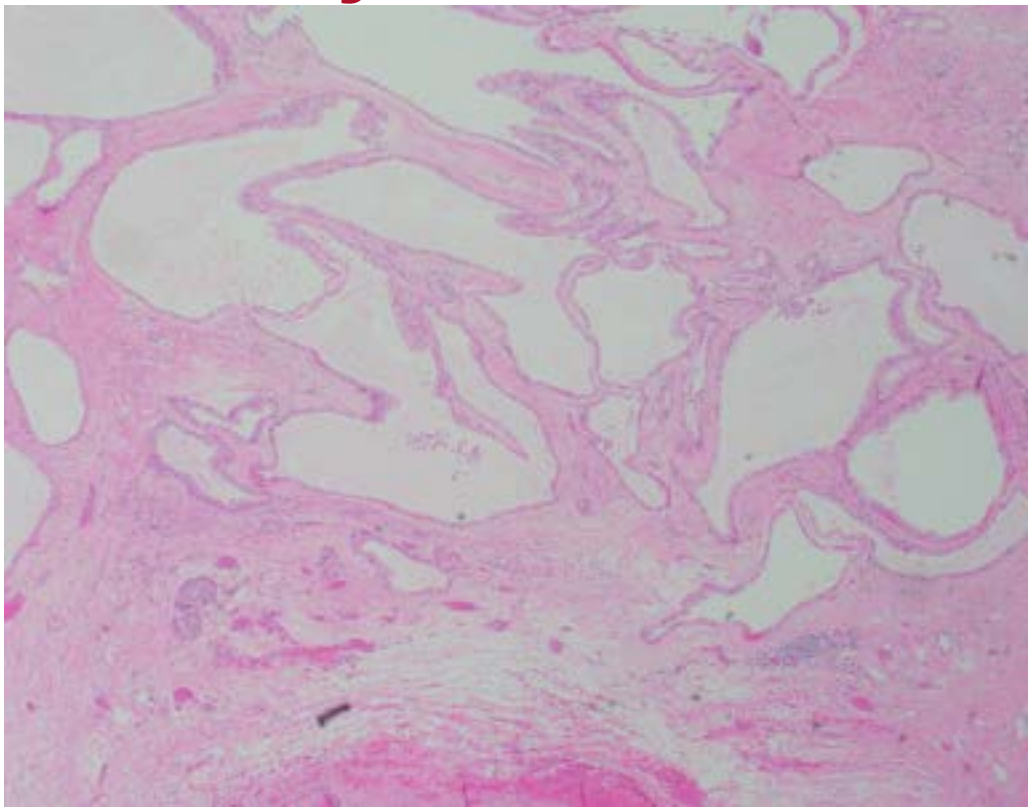
May be microcystic or oligocystic

Epithelium lined by bland cuboidal epithelium

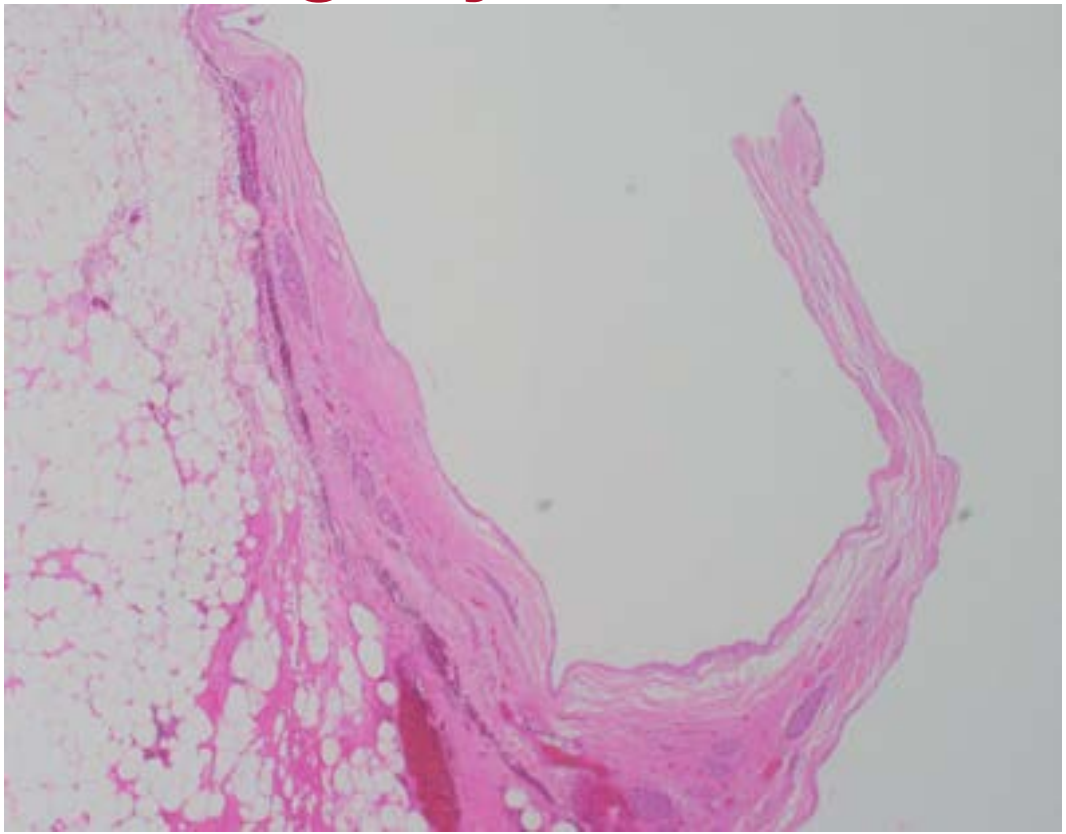
Cells contain glycogen: PAS positive, diastase sensitive

Molecular: Mutations in *VHL*

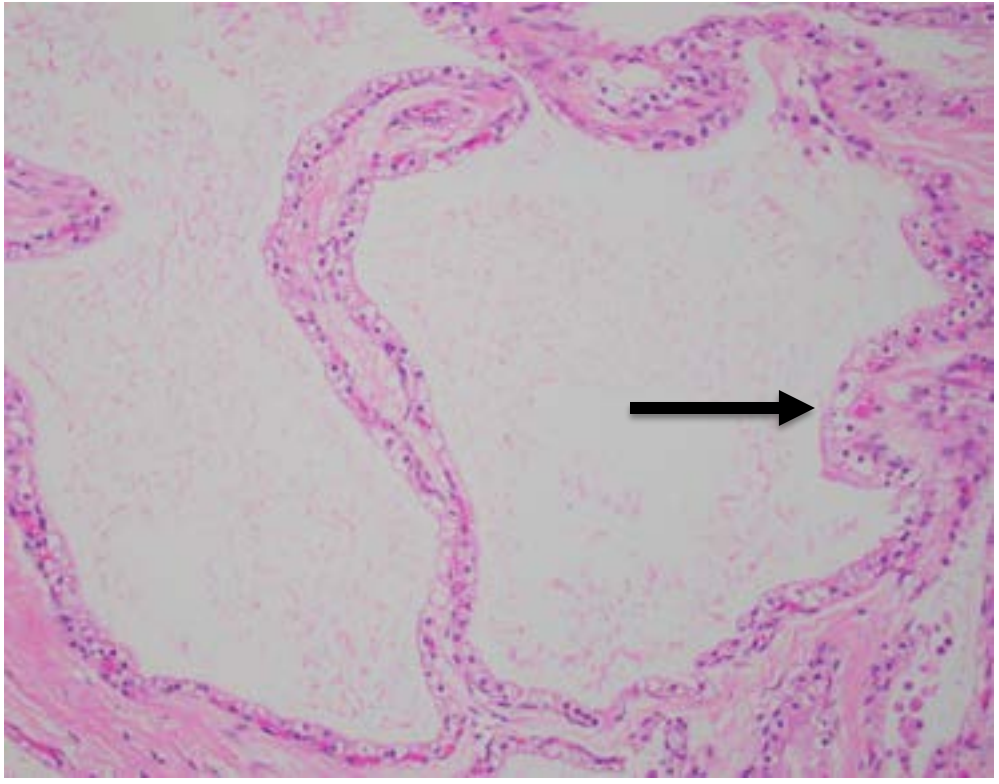
Serous Cystadenoma Microcystic



Serous Cystadenoma Oligocystic



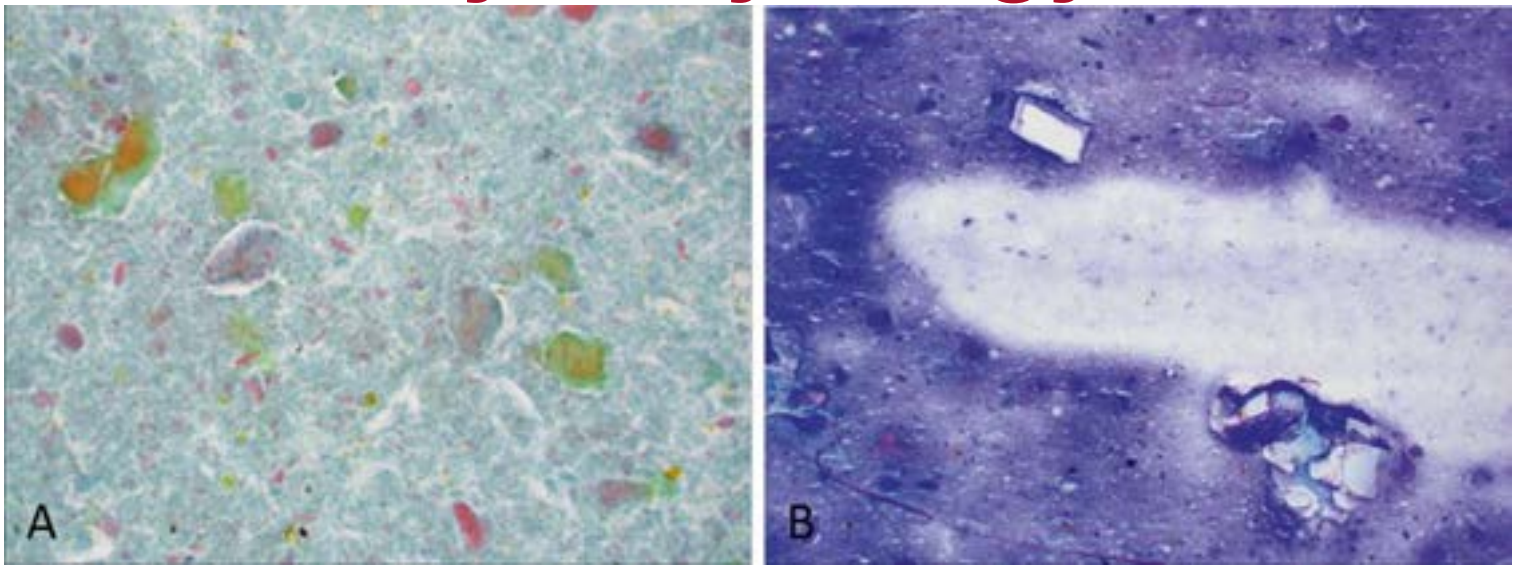
Serous Cystadenoma High power



Pseudocyst Cytology

- Cytologic exam (from EUS-FNA) will show blood, inflammatory cells, and debris
- Cytology may also show yellow pigment, crystals
- Have elevated levels of amylase (usually >1K units per liter) in cyst fluid
- Cyst fluid does not have elevated carcinoembryonic antigen (CEA) levels greater than 200 ng/mL

Pseudocyst Cytology



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Pseudocyst
Surgical Pathology

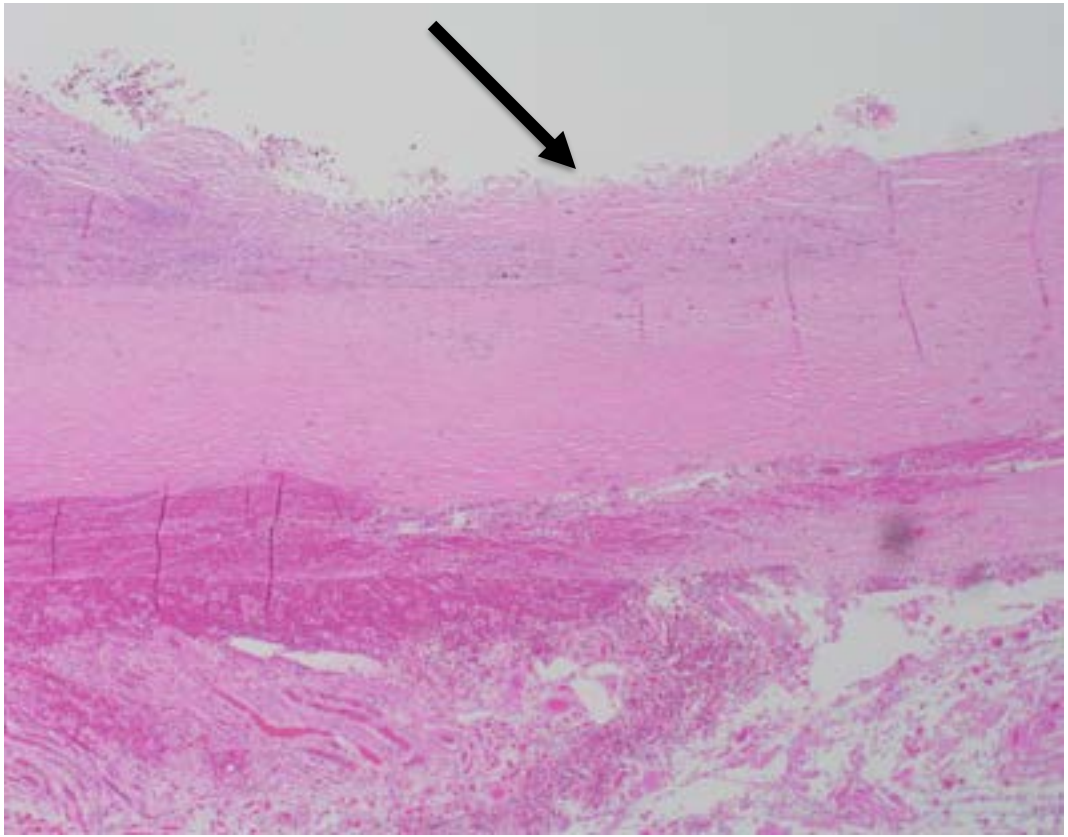
Grossly see a unilocular cyst with a thick fibrotic wall

Under the microscope --> lacks an epithelial lining, thus a pseudocyst

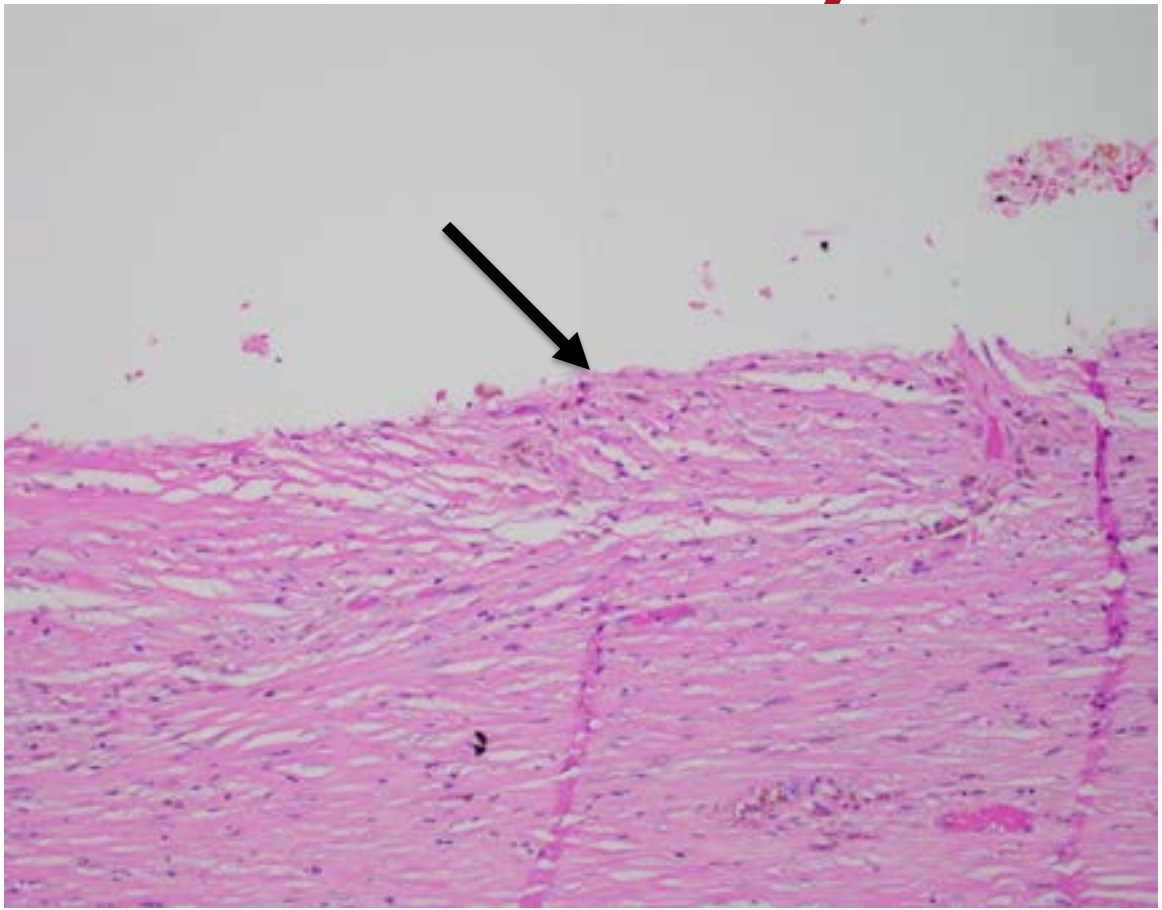
Cyst wall lined by granulation tissue

Background pancreas (if present): acute or chronic pancreatitis

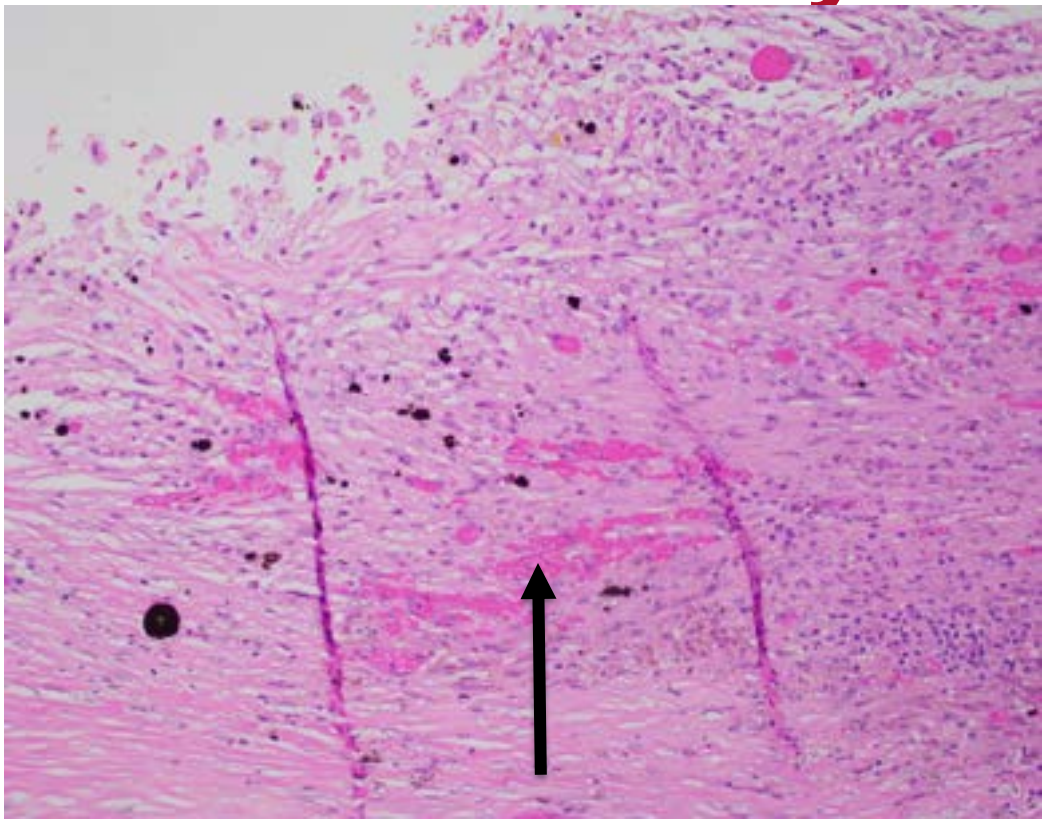
Pseudocyst



Pseudocyst



Pseudocyst



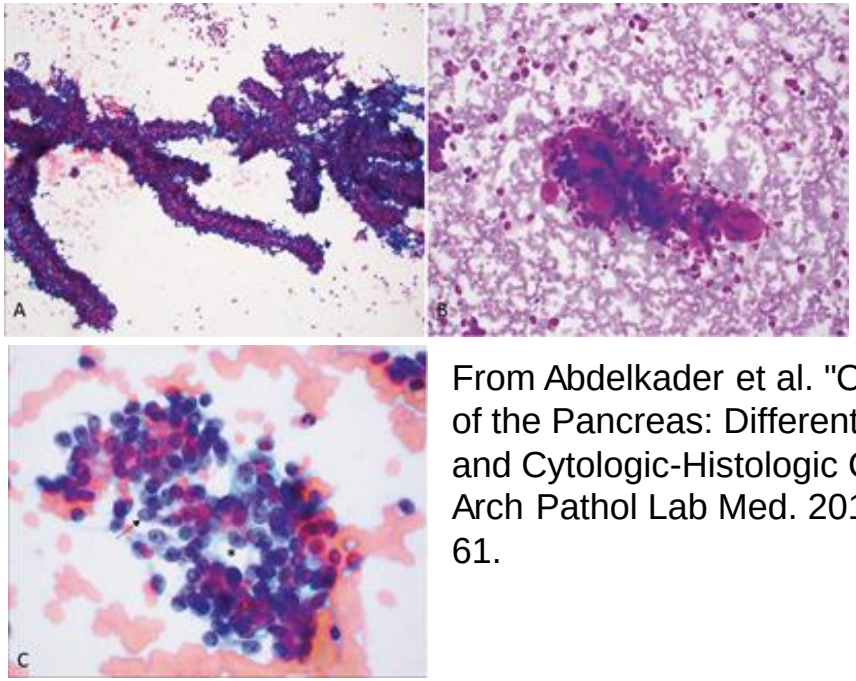
Solid Pseudopapillary Neoplasm Cytology

EUS-FNA cytology: Hypercellular smear

Papillary-like structures and a myxoid-like matrix

Monomorphic cuboidal cells, sometimes with grooves

Solid Pseudopapillary Neoplasm Cytology



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Solid Pseudopapillary Neoplasm Surgical Pathology

Solid neoplasm growth with cells surrounding blood vessels intact

Degeneration of cells farther away from blood vessels

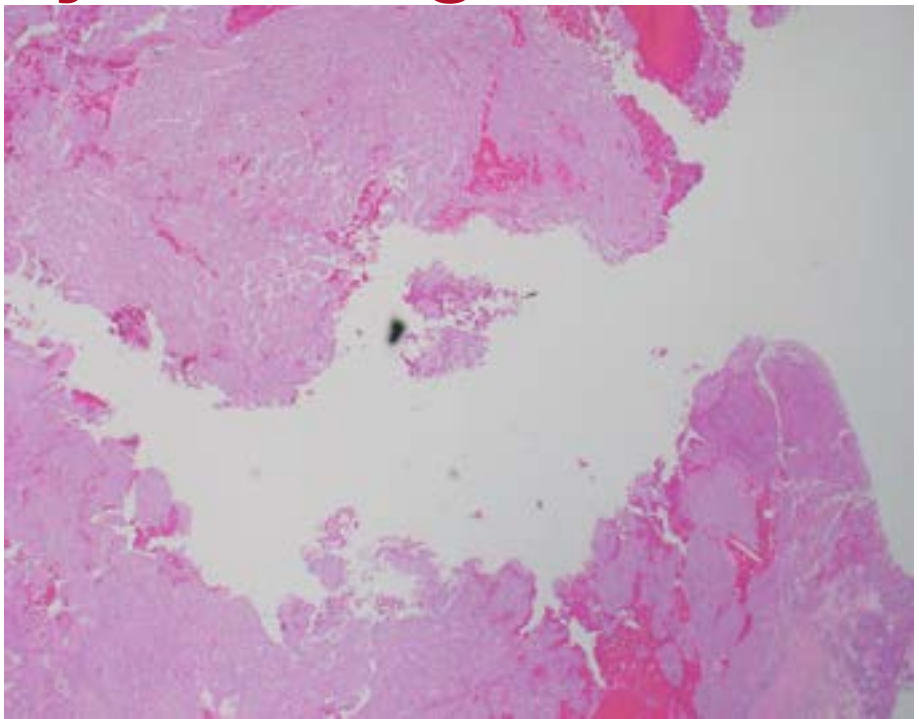
Bland/uniform round cells

Nuclear grooves

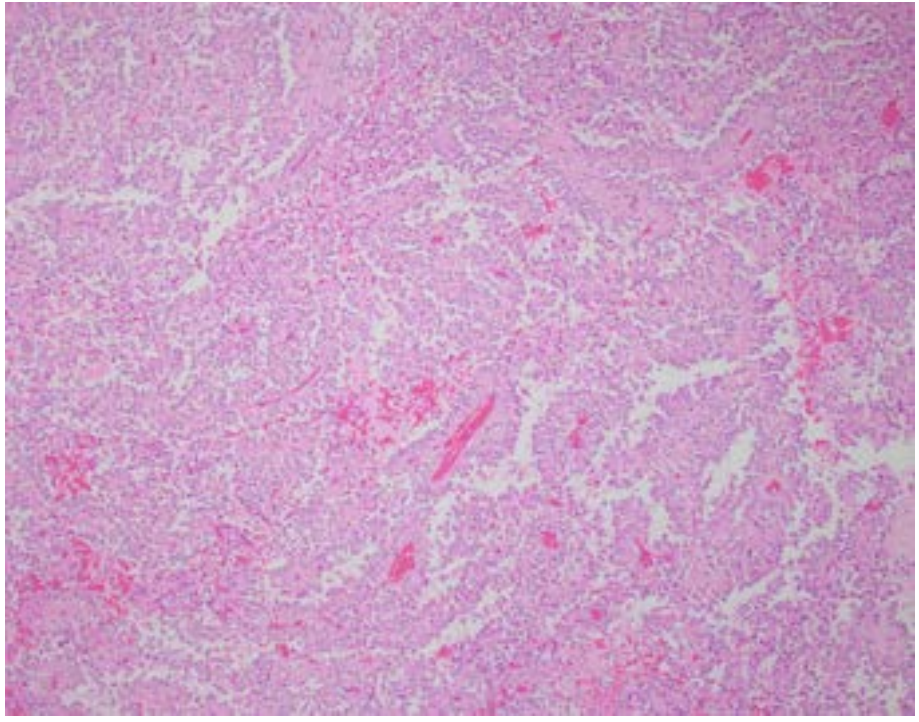
Positive nuclear staining for beta-catenin

Molecular: Mutations in *CTNNB1*

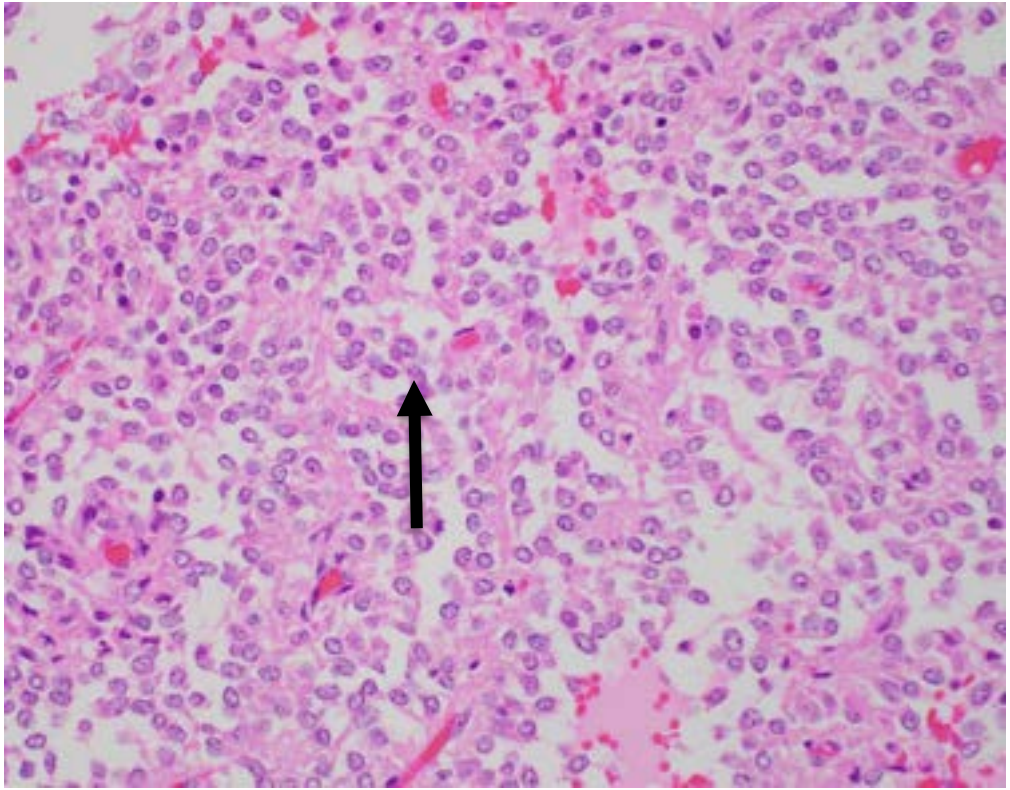
Solid Pseudopapillary Neoplasm Cystic Degeneration



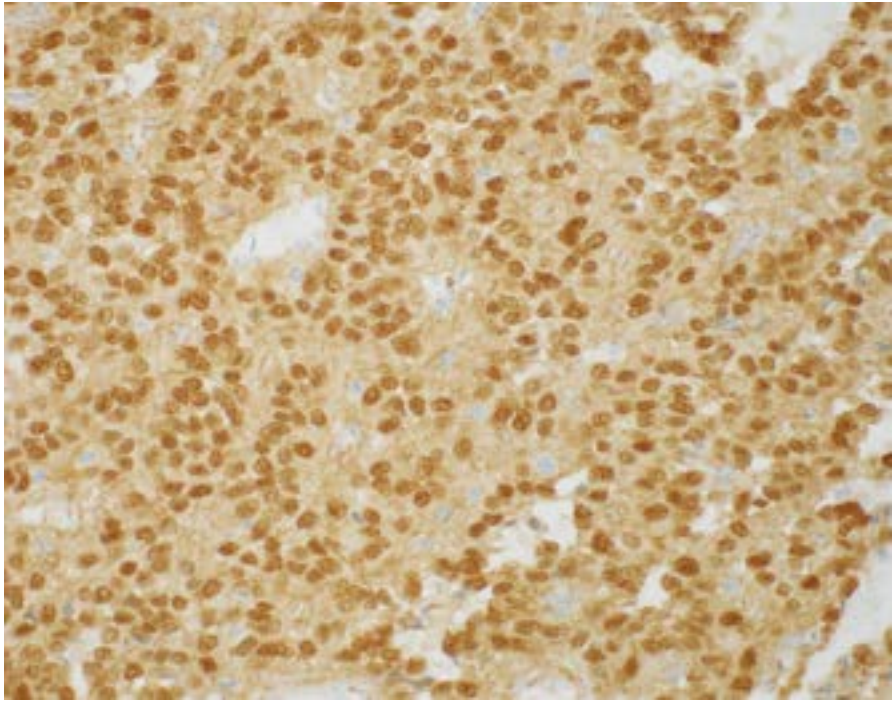
SPN
Pseudopapillary Growth



SPN-Bland cells with grooves



SPN
Nuclear beta-catenin staining



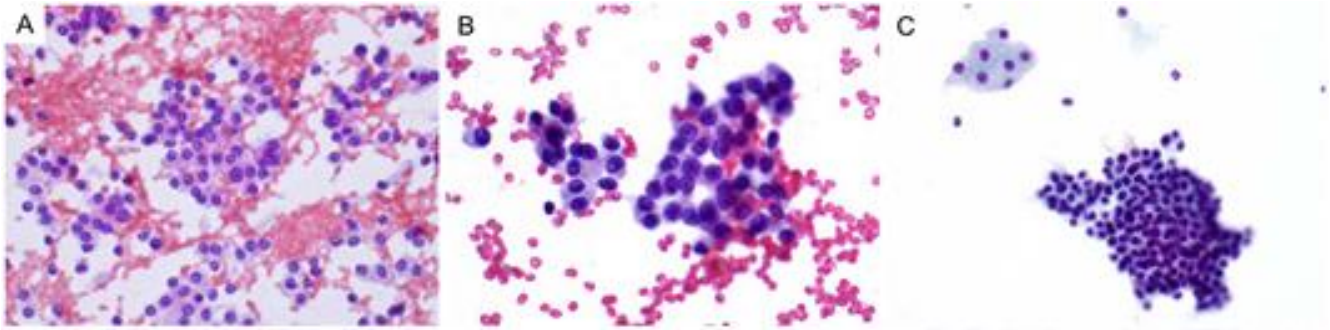
Neuroendocrine Tumors (NET or PanNET) Cytology

Low power microscopic examination shows loosely packed clusters of cells with plasmacytoid features

Cells are monotonous (uniform)

Fine (salt and pepper) chromatin

PanNET Cytology



Sigel CS. "Advances in the cytologic diagnosis of gastroenteropancreatic neuroendocrine neoplasms."Cancer Cytopathology, Volume: 126, Issue: 12, Pages: 980-991.

PanNET Surgical Pathology

Solid growth, sometimes with cystic degeneration

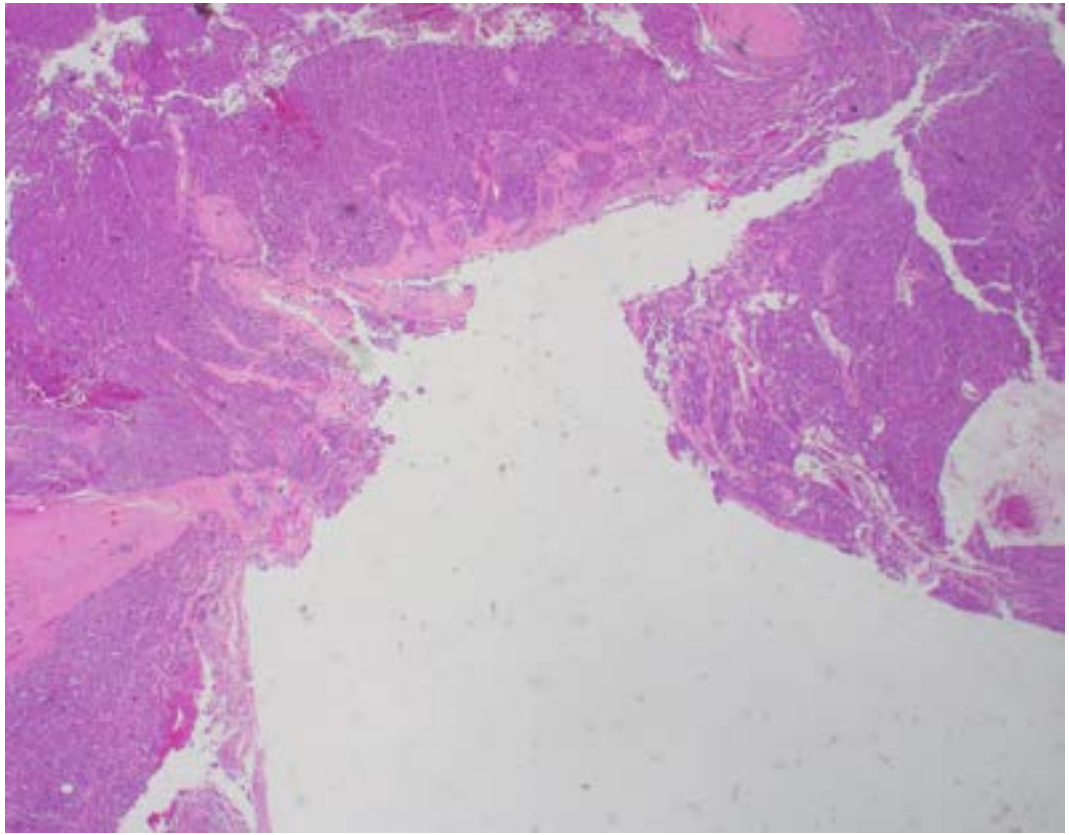
Cells are monotonous and grow in ribbons/nests

Fine (salt and pepper) chromatin

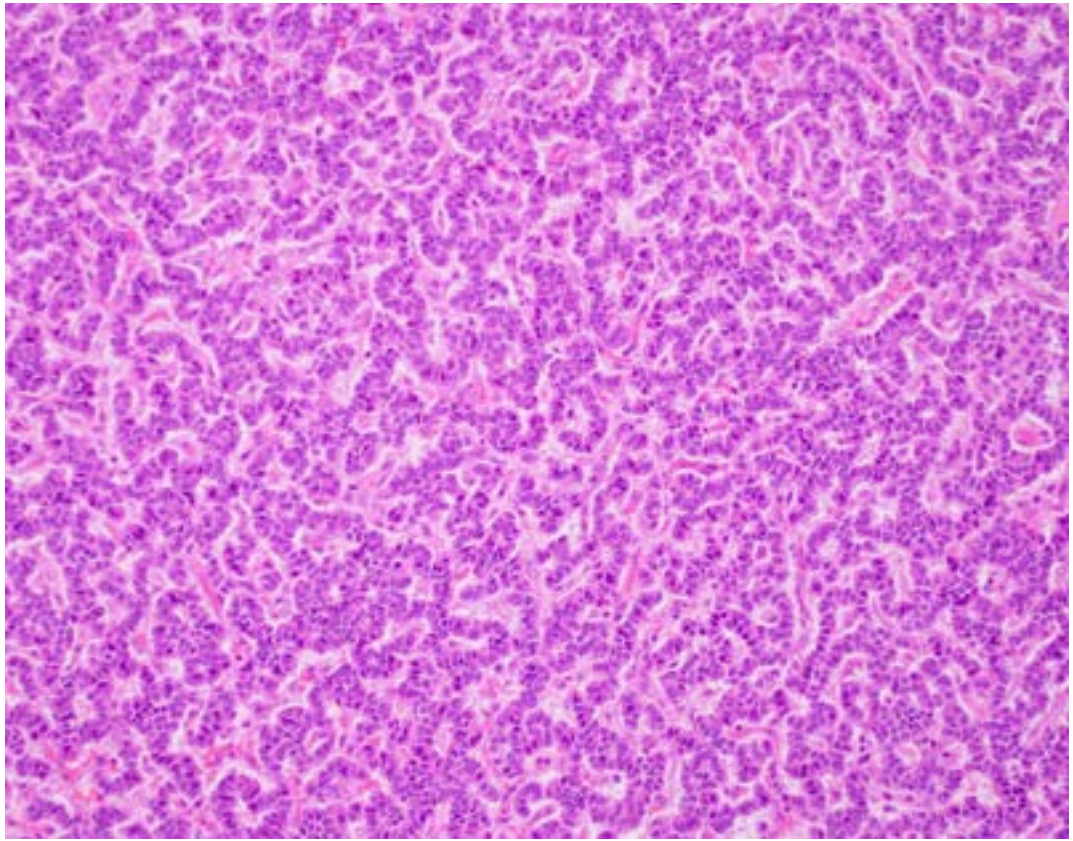
Tumors are graded by mitotic count, Ki-67

Molecular: Mutations in MEN1, LOH

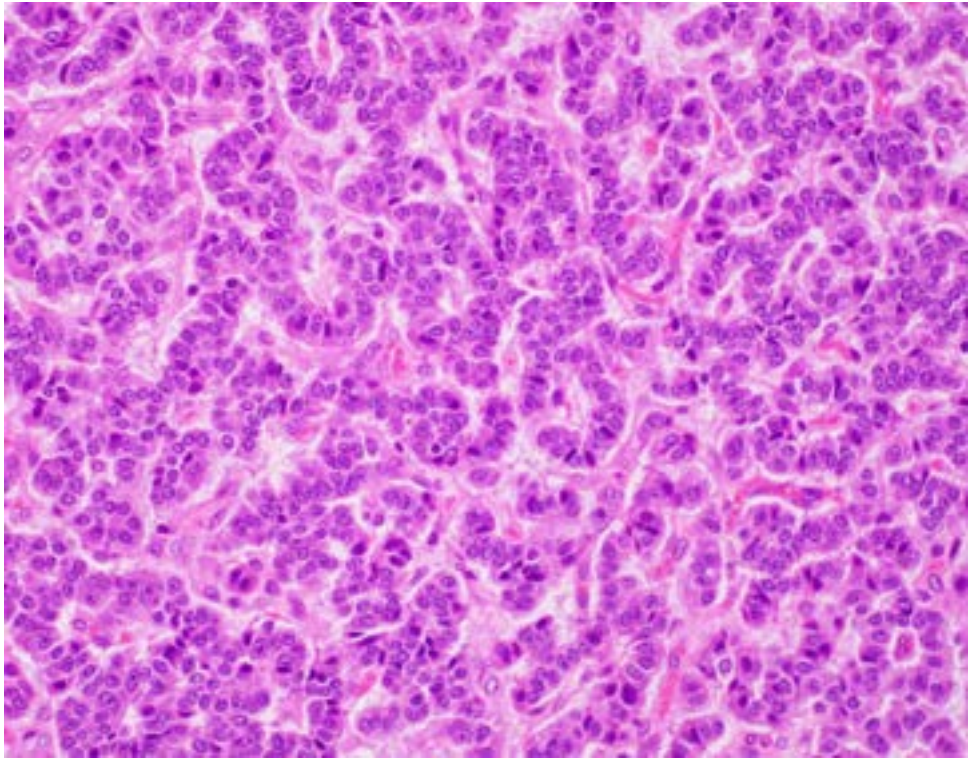
Cystic PanNET



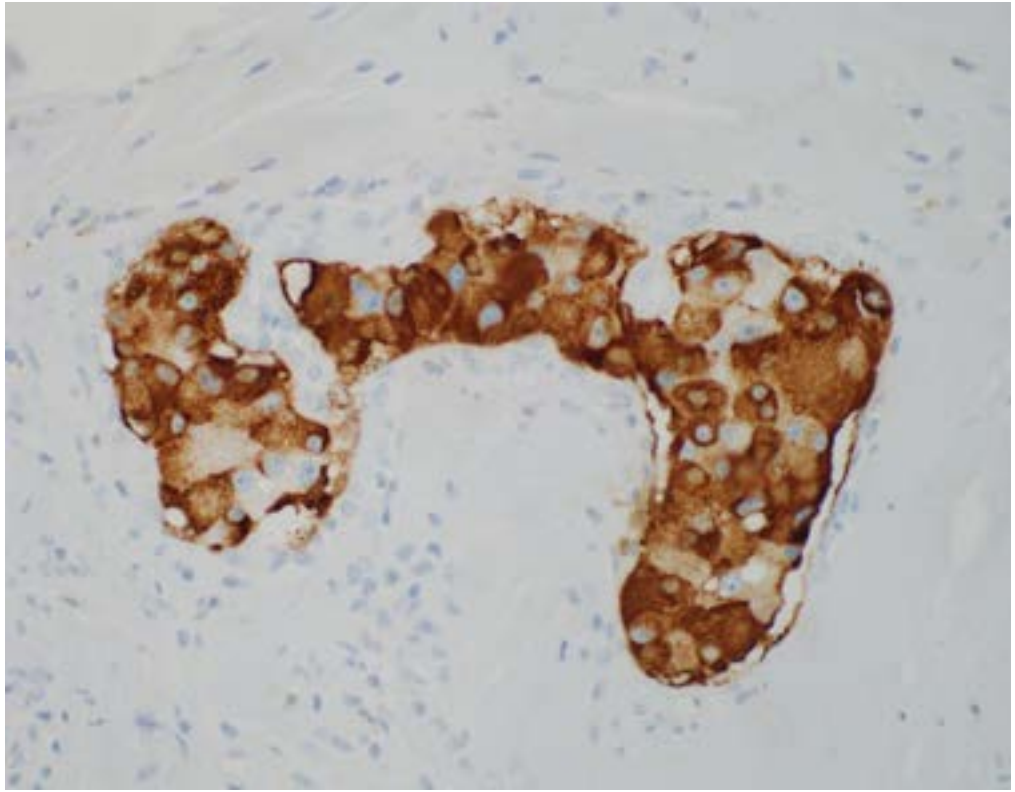
Cystic PanNET-Ribbon-Like Growth



Cystic PanNET
Monomorphic cells



Cystic PanNET-Chromogranin A

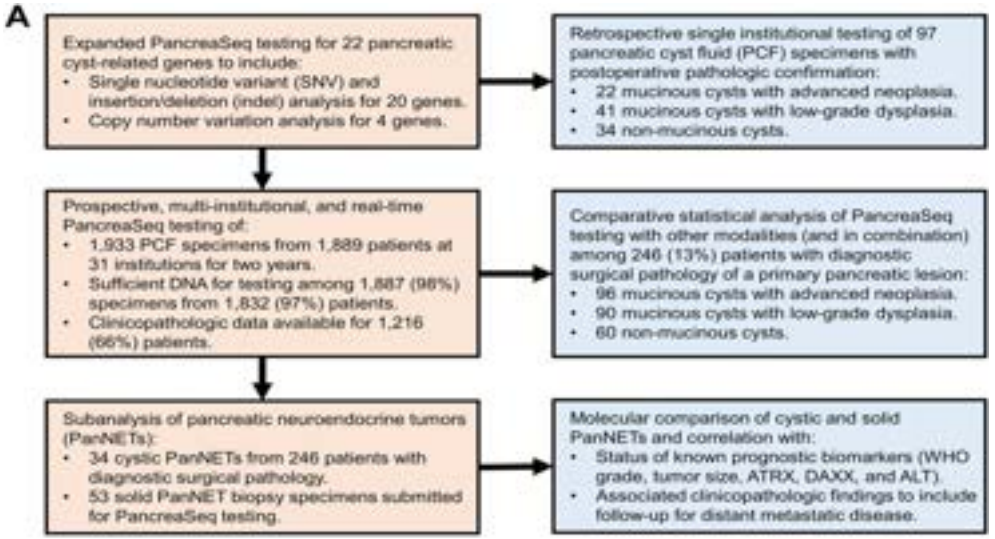


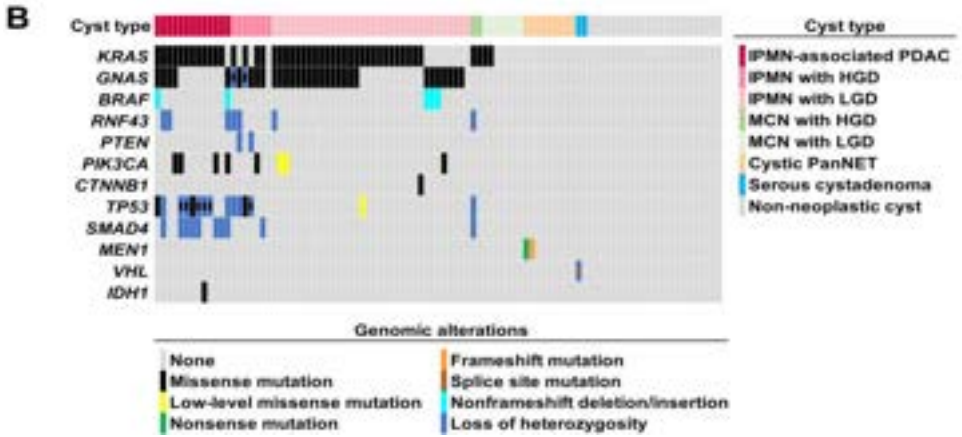
Prospective, Multi-Institutional, Real-Time Next-Generation Sequencing of Pancreatic Cyst Fluid Reveals Diverse Genomic Alterations That Improve the Clinical Management of Pancreatic Cysts

Alessandro Paniccia, Patricio M. Polanco, Brian A. Boone, Abigail I. Wald, Kevin McGrath, Randall E. Brand, Asif Khalid, Nisa Kubiliun, Anne Marie O'Broin-Lennon, Walter G. Park, Jason Klapman, Benjamin Tharian, Sumant Inamdar, Kenneth Fasanella, John Nasr, Jennifer Chennat, Rohit Das, John DeWitt, Jeffrey J. Easler, Benjamin Bick, Harkirat Singh, Kimberly J. Fairley, Savreet Sarkaria, Tarek Sawas, Wasseem Skef, Adam Slivka, Anna Tavakkoli, Shyam Thakkar, Victoria Kim, Hendrikus Dutch Vanderveldt, Allyson Richardson, Michael B. Wallace, Bhaumik Brahmbhatt, Megan Engels, Charles Gabbert, Mohannad Dugum, Samer El-Dika, Yasser Bhat, Sanjay Ramrakhiani, Gennadiy Bakis, Daniil Rolshud, Gordon Millspaugh, Thomas Tielleman, Carl Schmidt, John Mansour, Wallis Marsh, Melanie Ongchin, Barbara Centeno, Sara E. Monaco, N. Paul Ohori, Sigfred Lajara, Elizabeth D. Thompson, Ralph H. Hruban, Phoenix D. Bell, Katelyn Smith, Jennifer B. Permuth, Christopher Vandenbussche, Wayne Ernst, Maria Grupillo, Cihan Kaya, Melissa Hogg, Jin He, Christopher L. Wolfgang, Kenneth K. Lee, Herbert Zeh, Amer Zureikat, Marina N. Nikiforova, Aatur D. Singhi

Gastroenterology

DOI: 10.1053/j.gastro.2022.09.028





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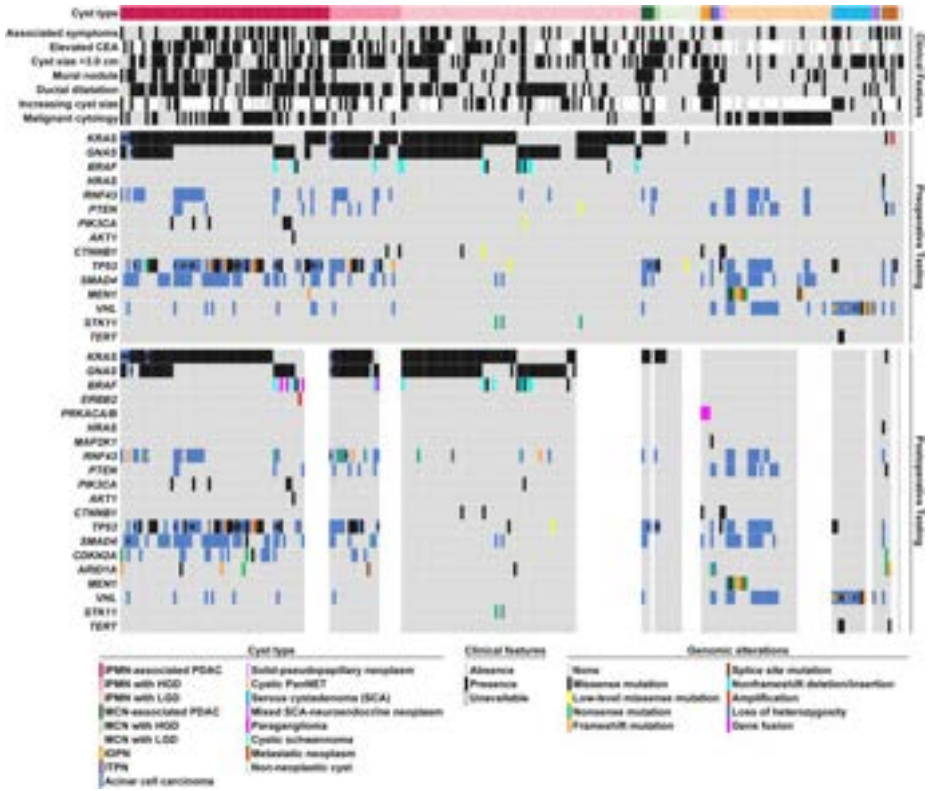

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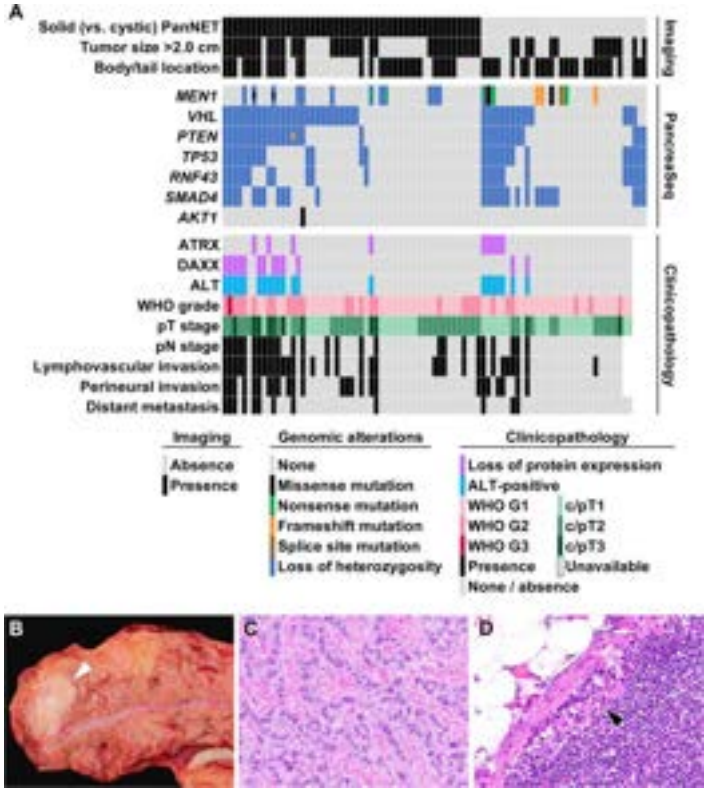
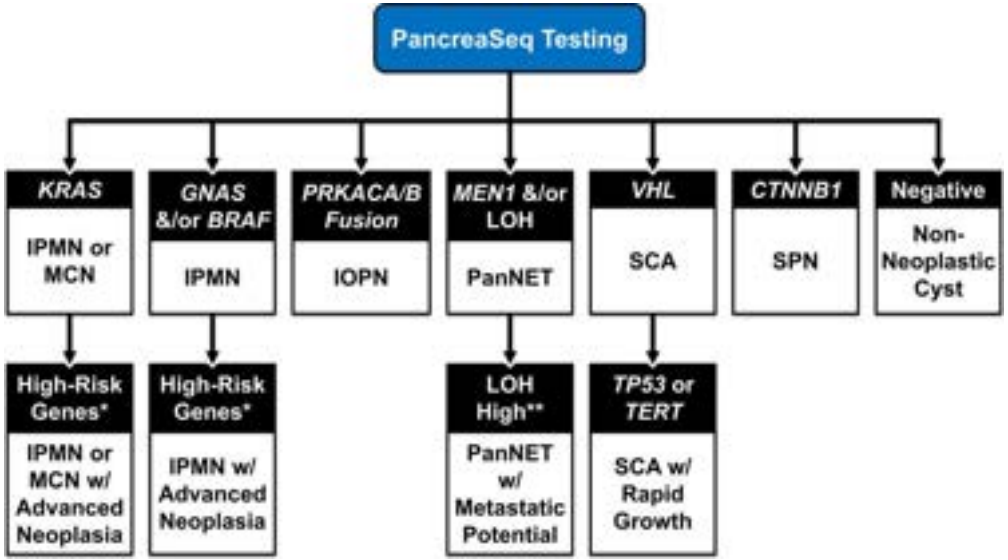


Gastroenterology 2023 Jan;164(1):117-133.

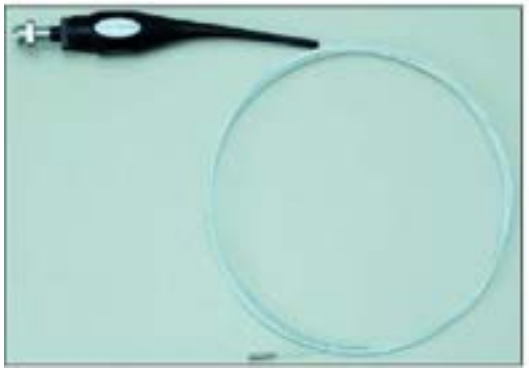


Findings of *Paniccia et al.* paper

Prospective (over 1900 samples), multi-institutional study with 14% of cases having surgical pathology correlation (with repeat molecular), 50% of cases had clinical follow-up

NGS (PancreaSeq) with cytopathologic evaluation had improved diagnostic performance (sensitivity, specificity, and PPV, NPV) compared to AGA or IAP/Fukuoka guidelines for the detection of mucinous cysts (IPMN and MCN) as well as the detection of advanced neoplasia

Confocal Laser Endomicroscopy in Gastrointestinal and Pancreatobiliary diseases



Digestive Endoscopy
pages86-94, 28 AUG 2013 DOI: 10.1111/den.12152
http://onlinelibrary.wiley.com/doi/10.1111/den.12152/full#den12152-fig-0002

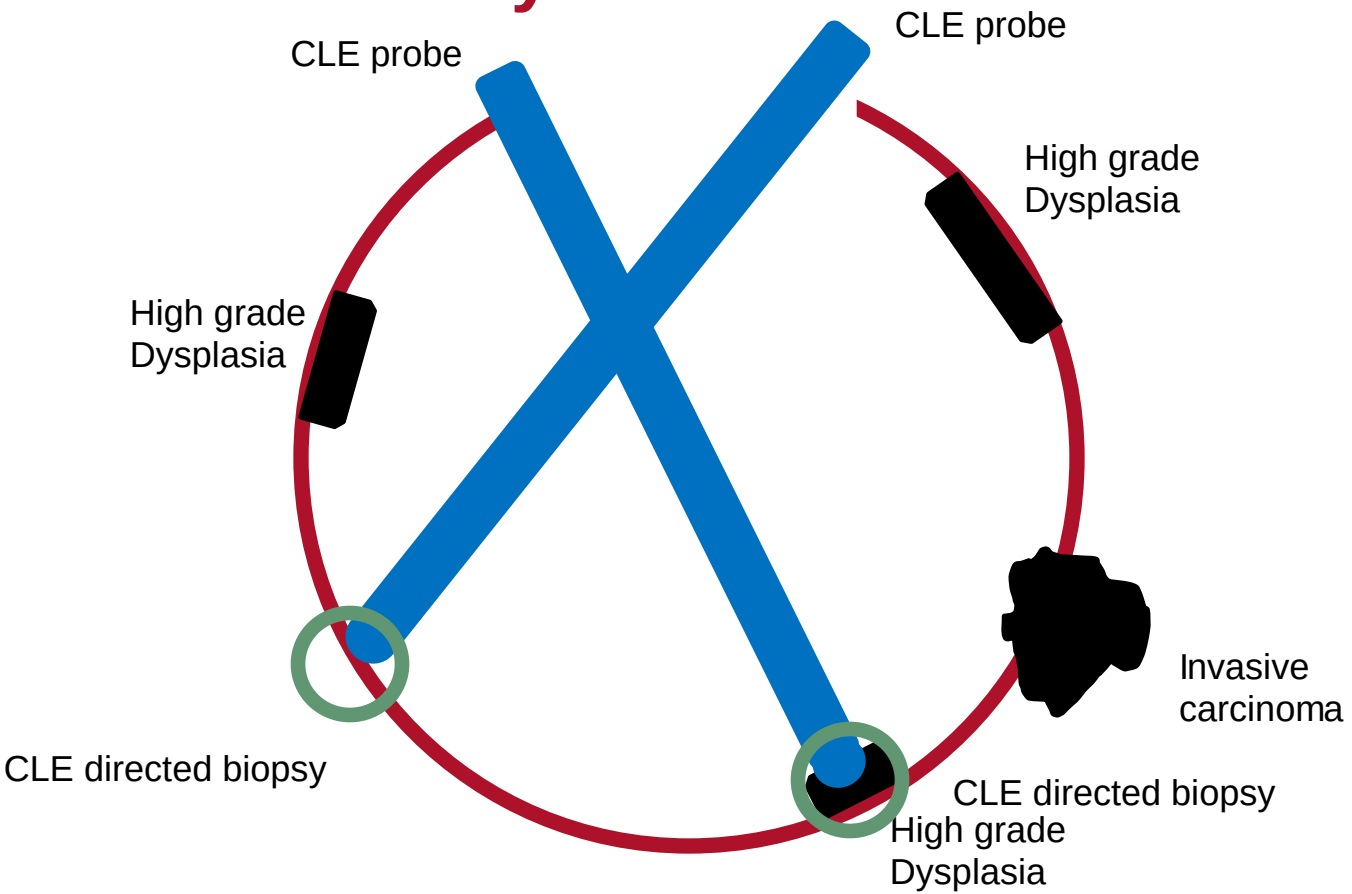
Procedure

1. Patient with PCL → EUS-FNA
2. In-vivo nCLE images are obtained/recorded
3. Tumor board→ Surgery
4. IV fluorescein (2.5-5 mL 10% fluorescein sodium) injected prior to ligation of the pancreatic blood vessels
5. Pancreas (whipple or distal pancreatectomy) removed by surgeon→ gross room
6. Pancreas and cyst characteristics recorded

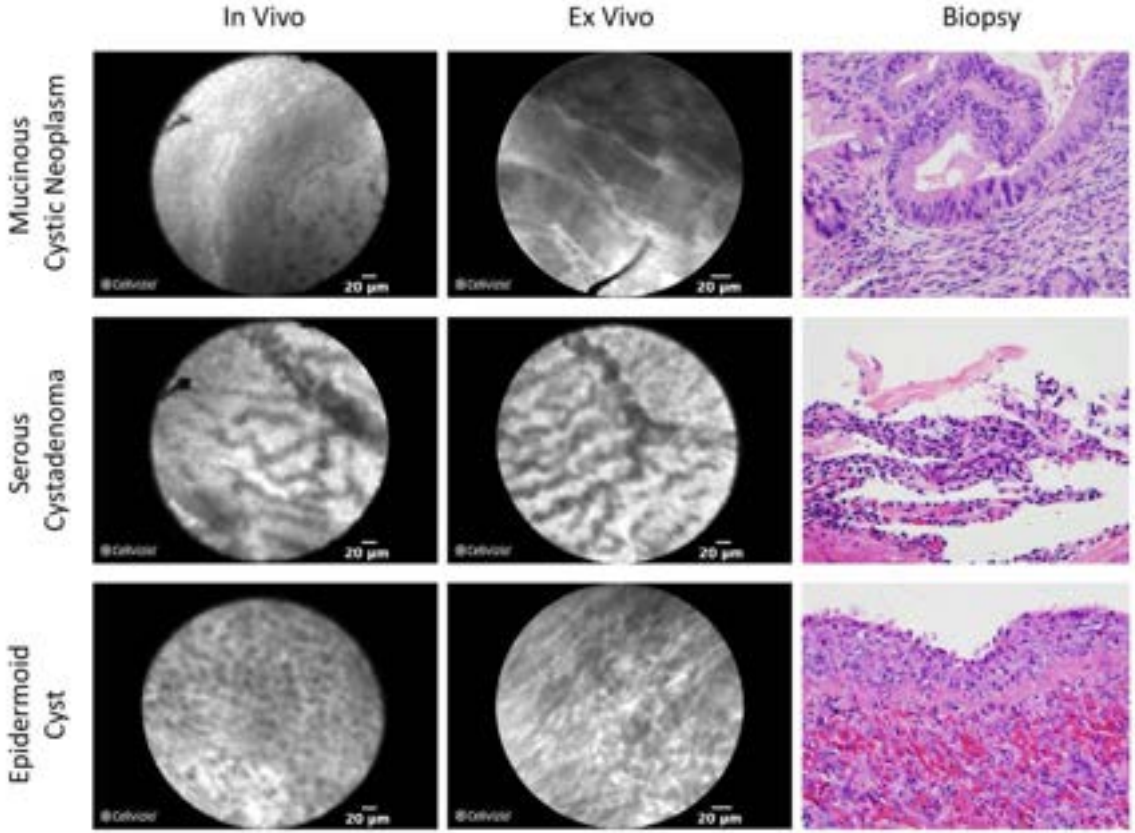
Procedure Continued

7. Ex-vivo nCLE with AQ-Flex 19 probe
   - Cyst wall geographically scanned
   - Areas of interest identified
8. Real-time images reviewed by Dr. Swanson and Dr. Krishna
   - Area of interest recorded as a unique video file for 1-5 minutes.
9. Area of interest biopsied → becomes part of surgical pathology specimen
11. Correlation between paired biopsy specimens and ex-vivo nCLE images as well as in-vivo nCLE

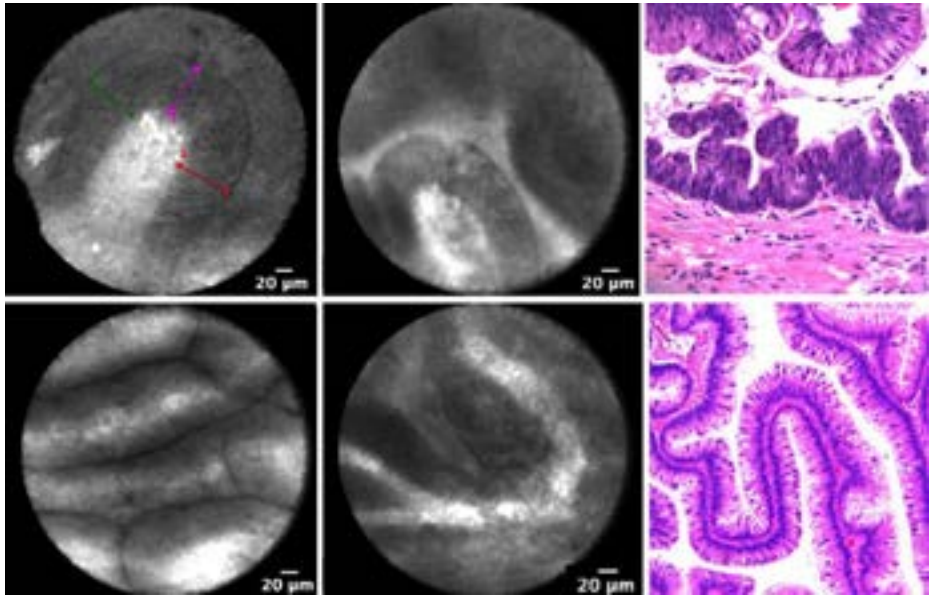
Ex Vivo Confocal Laser Endomicroscopy in Pancreatic Cystic Lesions



Case number	Final Diagnosis	Biopsy no.	Ex vivo CLE directed biopsy
1	MCN	1	MCN with LGD
		2	MCN with LGD
		3	MCN with LGD
2	Epidermoid cyst	1	Epidermoid cyst
		2	Epidermoid cyst
		3	Epidermoid cyst
		4	Epidermoid cyst
3	IPMN with low grade dysplasia	1	IPMN with low-grade dysplasia (gastric foveolar type)
		2	IPMN with low-grade dysplasia (gastric foveolar type)
		3	IPMN with low-grade dysplasia (gastric foveolar type)
		4	IPMN with low-grade dysplasia (gastric foveolar type)
		5	IPMN with low-grade dysplasia (gastric foveolar type)
4	MCN with moderate dysplasia	1	MCN with moderate dysplasia
		2	No epithelium present; only ovarian stroma
		3	MCN with moderate dysplasia
5	Serous Cystadenoma	13 biopsies	All serous cystadenoma
6	IPMN with focal high grade dysplasia	1	IPMN with low-grade dysplasia (intestinal type)
		2	IPMN with high-grade dysplasia (intestinal type)
		3	IPMN with low-grade dysplasia (intestinal type)
7	Cystic NET	1	Cystic NET
		2	Cystic NET
		3	Cystic NET
		4	Cystic NET
		5	Cystic NET


In Vivo
Ex Vivo
Biopsy
Mucinous Cystic Neoplasm
Serous Cystadenoma
Epidermoid Cyst
20 µm

IPMN



Confocal Endomicroscopy Results

IPMN: cases showed characteristic villous structures

MCN: band of epithelium and ovarian stroma was observed by ex vivo

SCA: characteristic vascular network observed with ex vivo

Summary

PCLs are common and are a clinical conundrum

Common cysts: Intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasm (MCN), serous cystadenoma (SCA), pseudocyst

Uncommon cysts: Cystic pancreatic adenocarcinoma, Solid pseudopapillary neoplasm (SPN), cystic Neuroendocrine tumors (NET)

Radiology (CT, MRI, MRCP) findings, Established guidelines (AGA IAP/Fukuoka), cyst fluid analysis, and cytology all currently play important roles in current management decision making

- Growing role for NGS (molecular testing) to help inform decision making

Thank you!

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