

Pancreaticobiliary Cytology Review

Smears, ThinPrep, and Small Biopsy

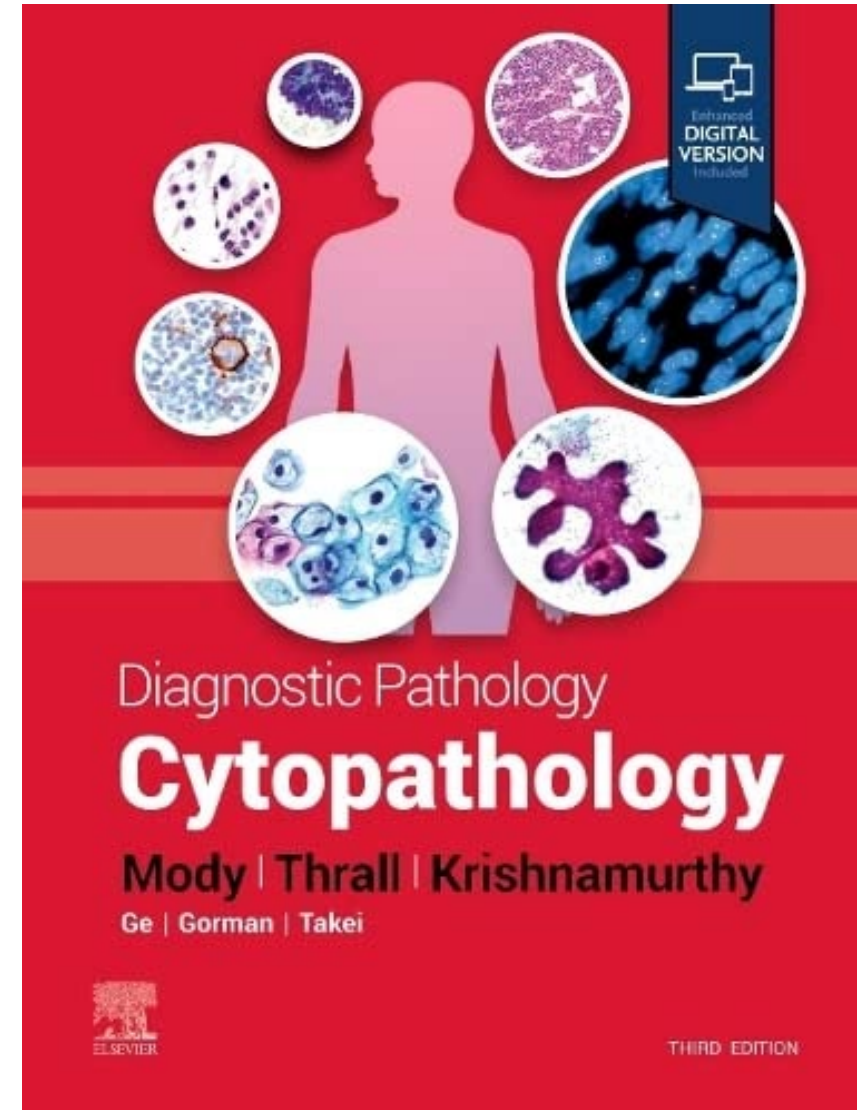
Michael Thrall MD

HOUSTON
Methodist[®]
LEADING MEDICINE

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- Director of Cytopathology Fellowship and Digital Pathology
 - Department of Pathology and Genomic Medicine
 - Houston Methodist Hospital in Houston, TX, USA
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- Co-editor of 2022 textbook
Diagnostic Pathology:
Cytopathology, 3rd Edition
Publisher: Amirsys/Elsevier



- Endoscopic ultrasound-guided (EUS) FNA and biopsies have become standard of care for the diagnosis of pancreatic tumors
- Rapid on-site evaluation (ROSE) is frequently offered in large US academic centers, but availability is limited in many settings
- However, EUS of pancreas has largely moved away from using ROSE in the era of flexible core needles

Endoscopic Ultrasound (EUS) Basics

Franseen and Fork-Tip Needles

- New core needles have changed EUS
- Many laboratories now rarely or never get called to perform ROSE

Review > [Minerva Med.](#) 2017 Dec;108(6):547-553. doi: 10.23736/S0026-4806.17.05327-7.

Epub 2017 Jul 27.

Endoscopic ultrasound core needle for diagnosing of solid pancreatic lesions: is rapid on-site evaluation really necessary?

Monica Arena ¹, Leonardo H Eusebi ², Rinaldo Pellicano ³, Maria A Palamara ¹, Giuseppe Iabichino ¹, Pierluigi Consolo ⁴, Sharmila Fagoonee ⁵, Enrico Opocher ⁶, Matteo Barabino ⁶, Carmelo Luigiano ⁷

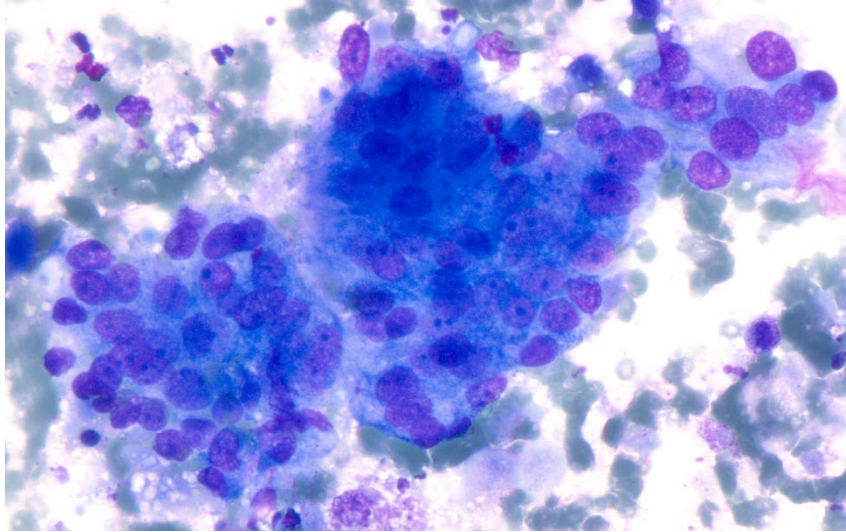


- We have gone from several EUS ROSE per day in the early 2010s to only one or two per month now, despite much higher volumes of EUS procedures in our hospital system
- We receive EUS samples in CytoLyt (ThinPrep) vials
- If large “forcepsable” pieces are present, these may be removed and processed as a biopsy
- Small fragments and the fluid are processed into a ThinPrep slide and a cell block

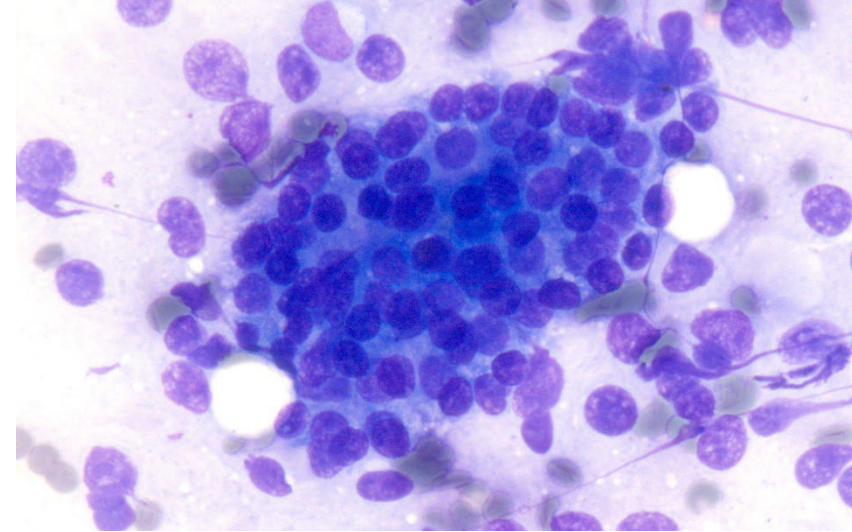
Solid Tumors of the Pancreas

The Big Four (Adult Solid Tumors)

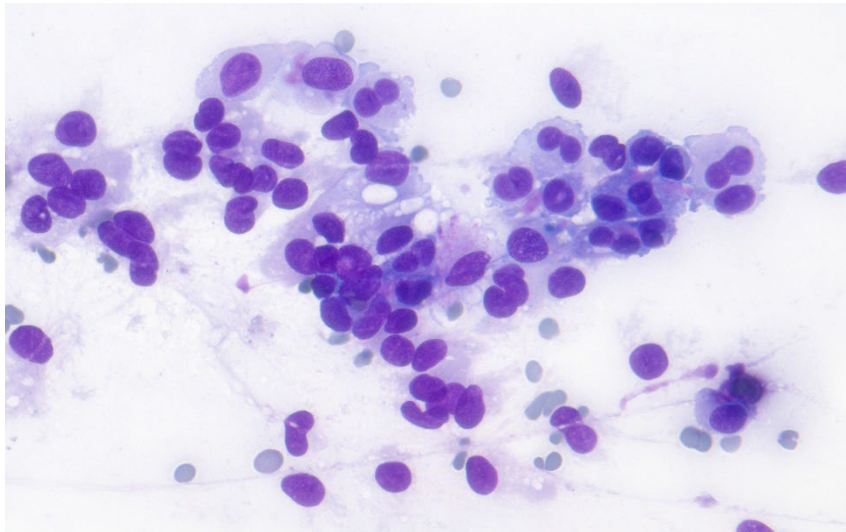
Ductal
adenocarcinoma



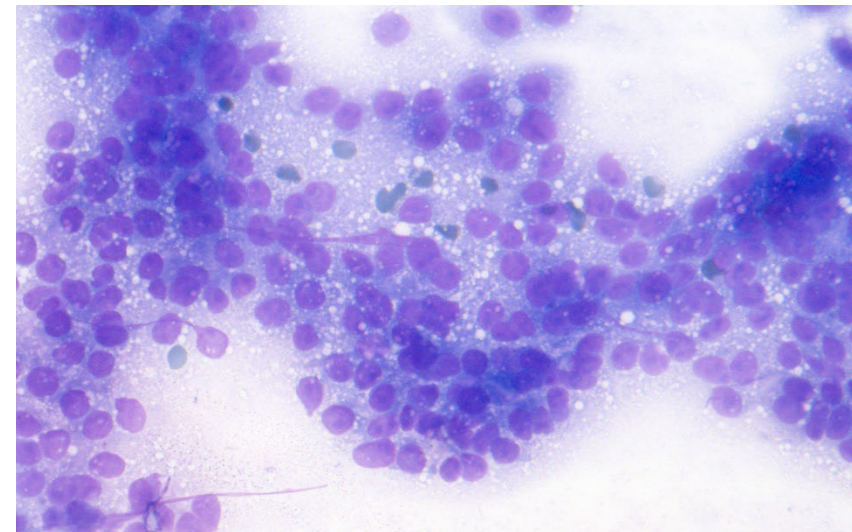
Well-
differentiated
neuroendocrine
tumor



Solid
pseudopapillary
neoplasm

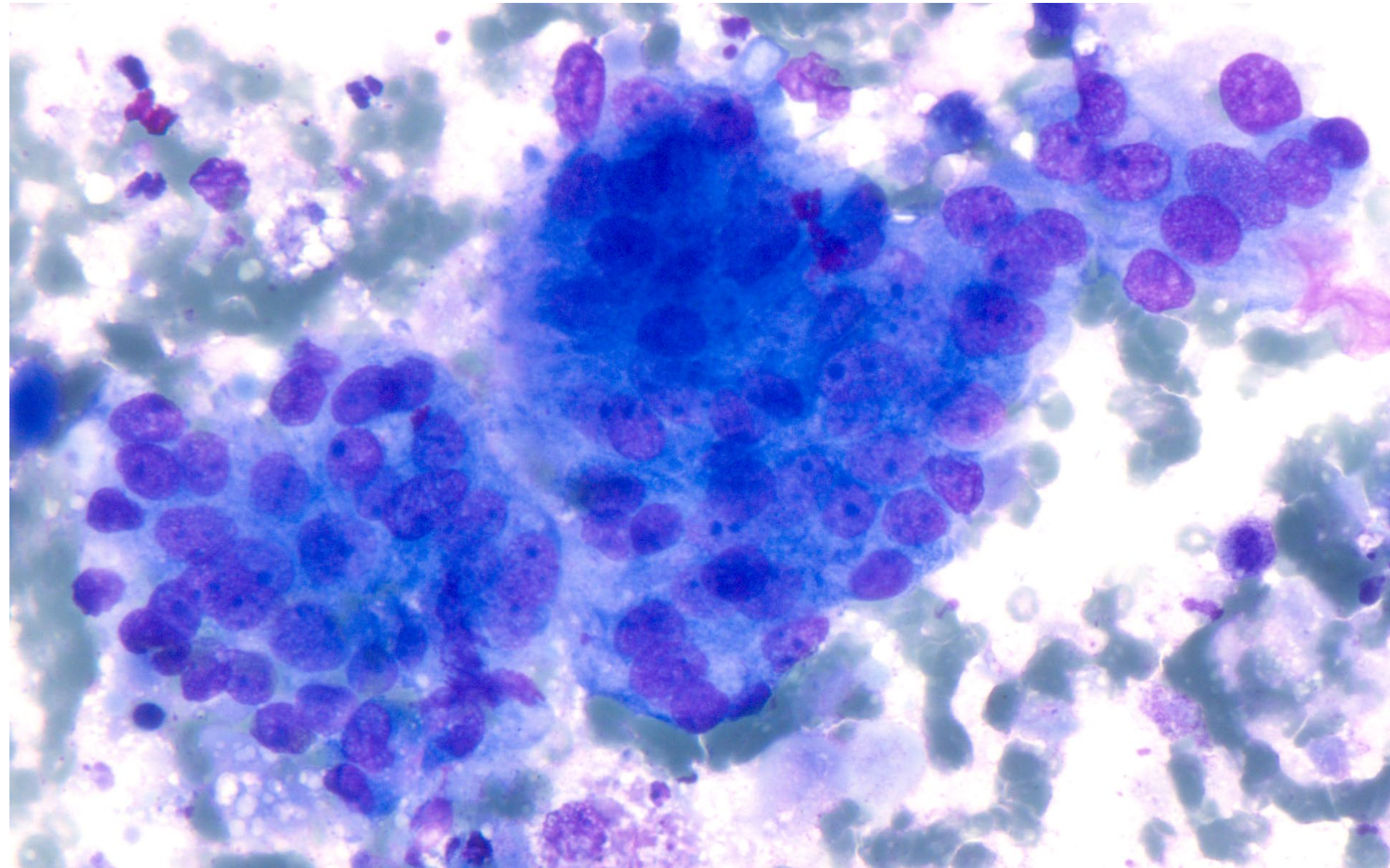


Acinar cell
carcinoma



- The most prominent “other” category to think about when getting a sample from a solid tumor:
peri-pancreatic mass lesions
 - Lymph nodes / lymphoma
 - Ectopic spleen / splenule
 - Gastric GIST or other stomach wall tumor

Diff-Quik of Ductal Adenocarcinoma



High
cellularity

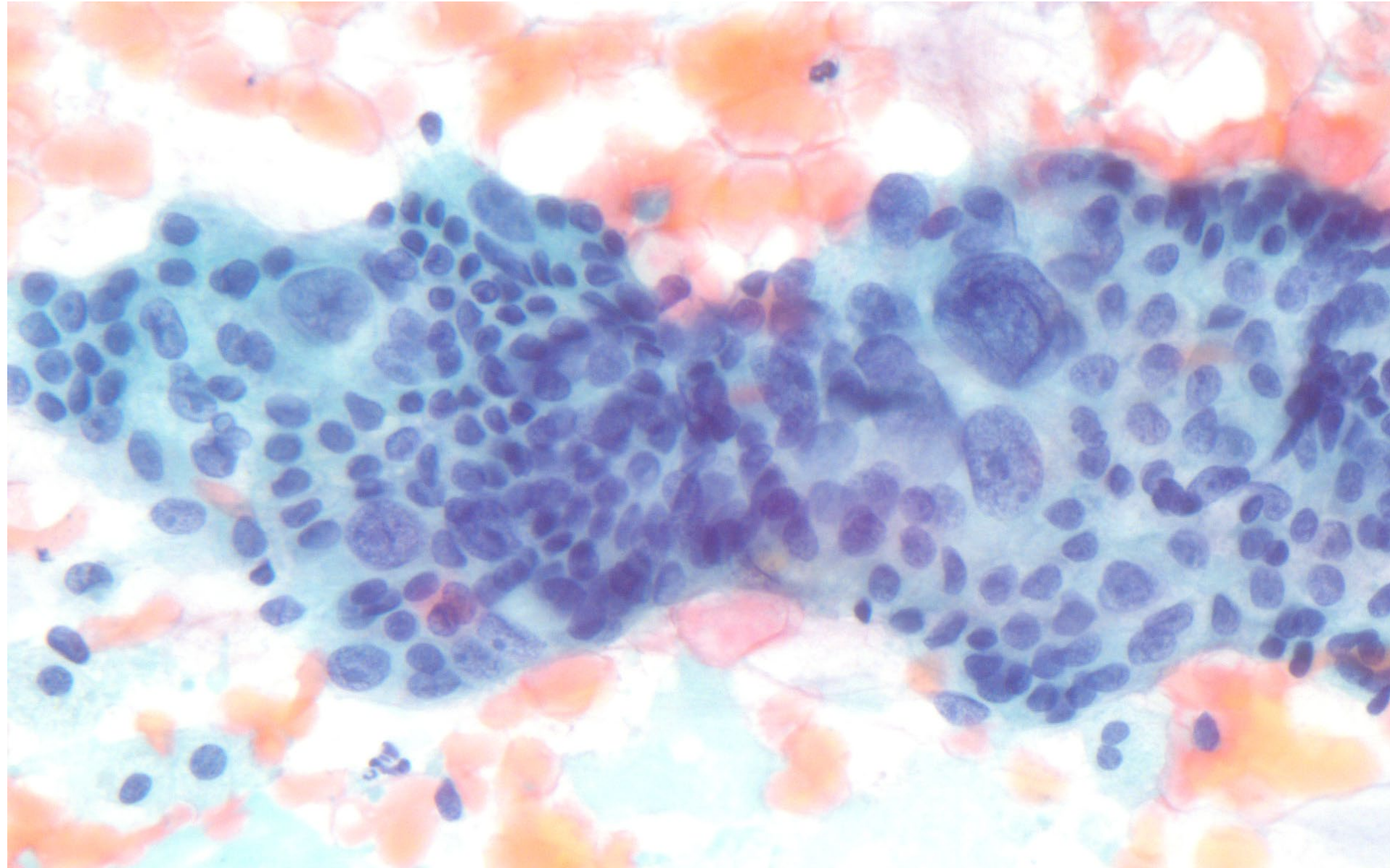
Overlapping,
large,
pleomorphic
nuclei with
prominent
nucleoli

Necrotic
debris in the
background

Pap-Stained Smear of Ductal Adenoca

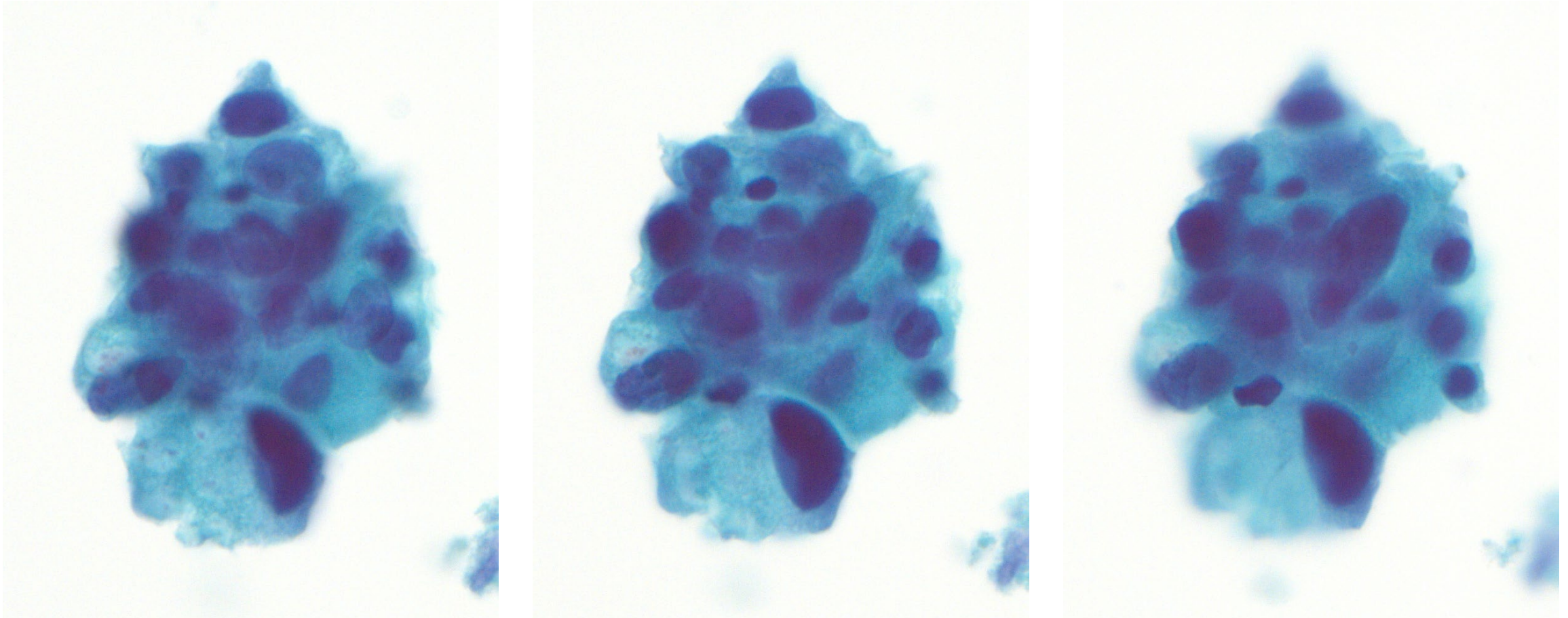
Prominent
anisonucleosis,
however,
beware
“endocrine
atypia”

Must also look
for other
features:
irregularities of
nuclear
chromatin and
contour



Ductal Adenocarcinoma - ThinPrep

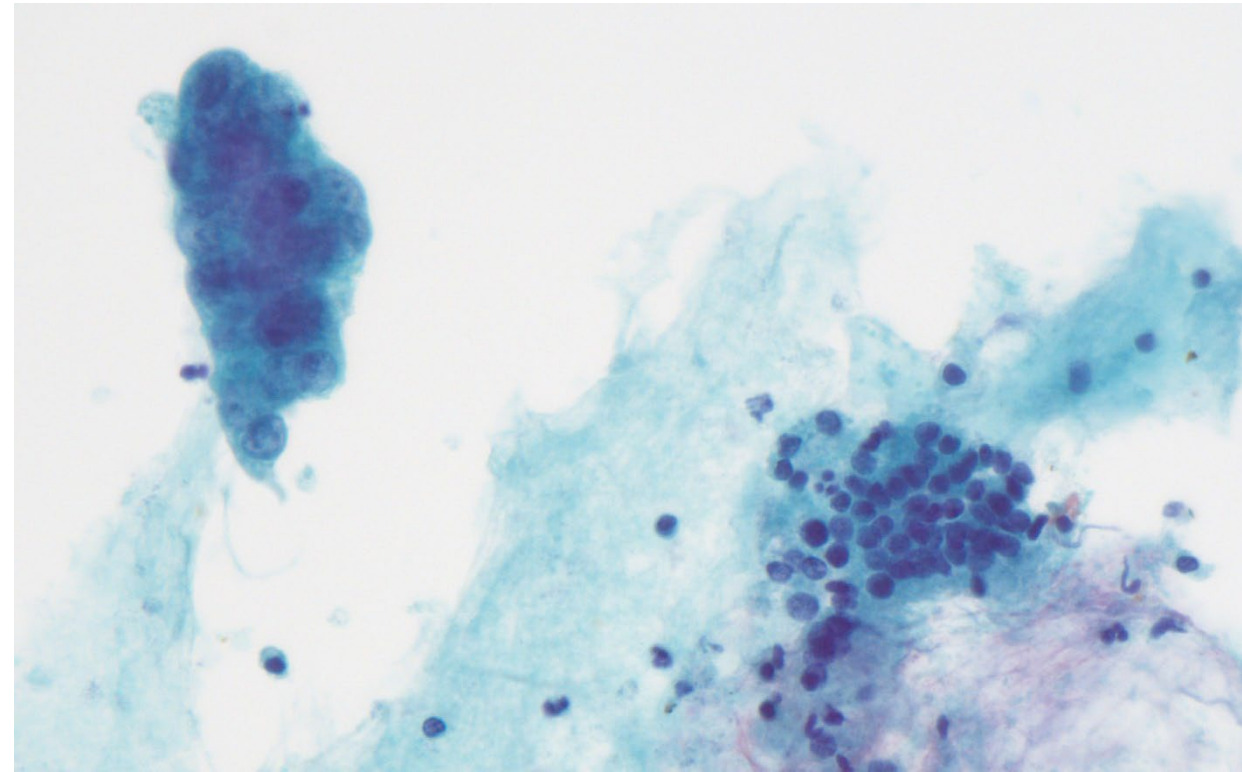
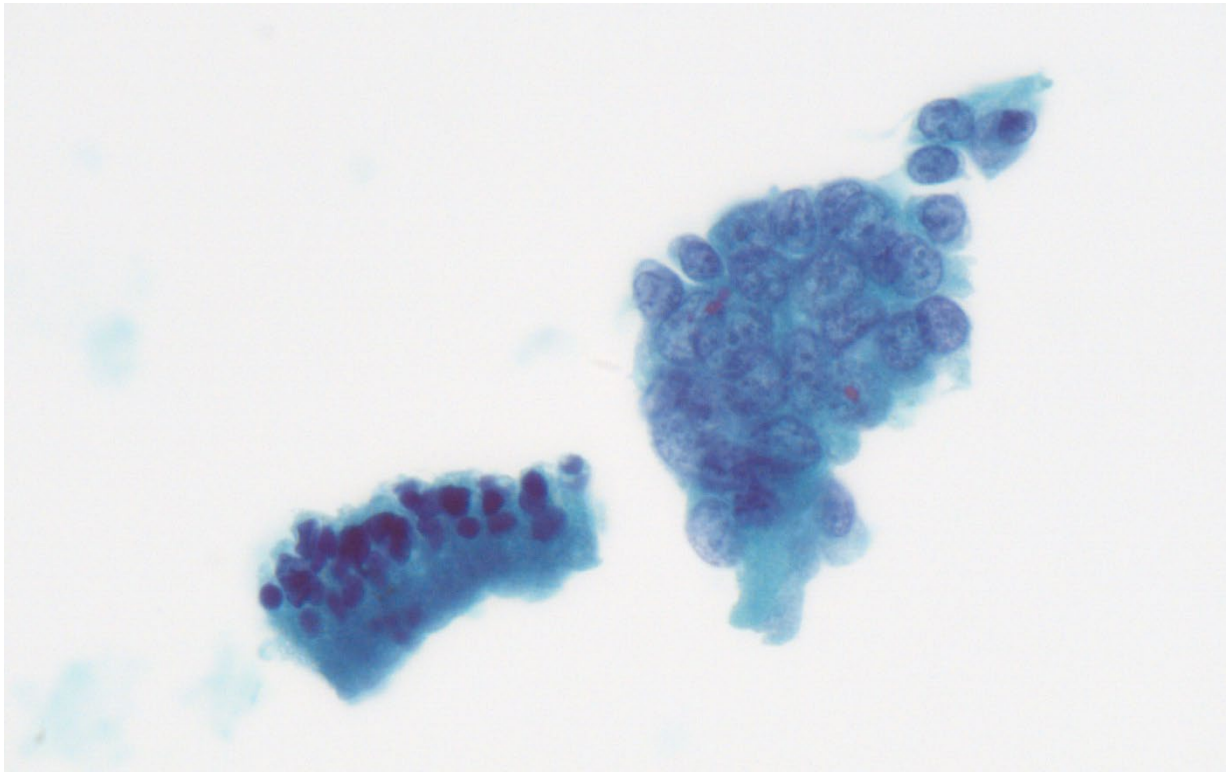
Marked anisonucleosis: ratio of smallest to largest nuclei in same cluster greater than 4:1



Disordered architecture with overlapping nuclei; Hyperchromasia and nuclear contour irregularities

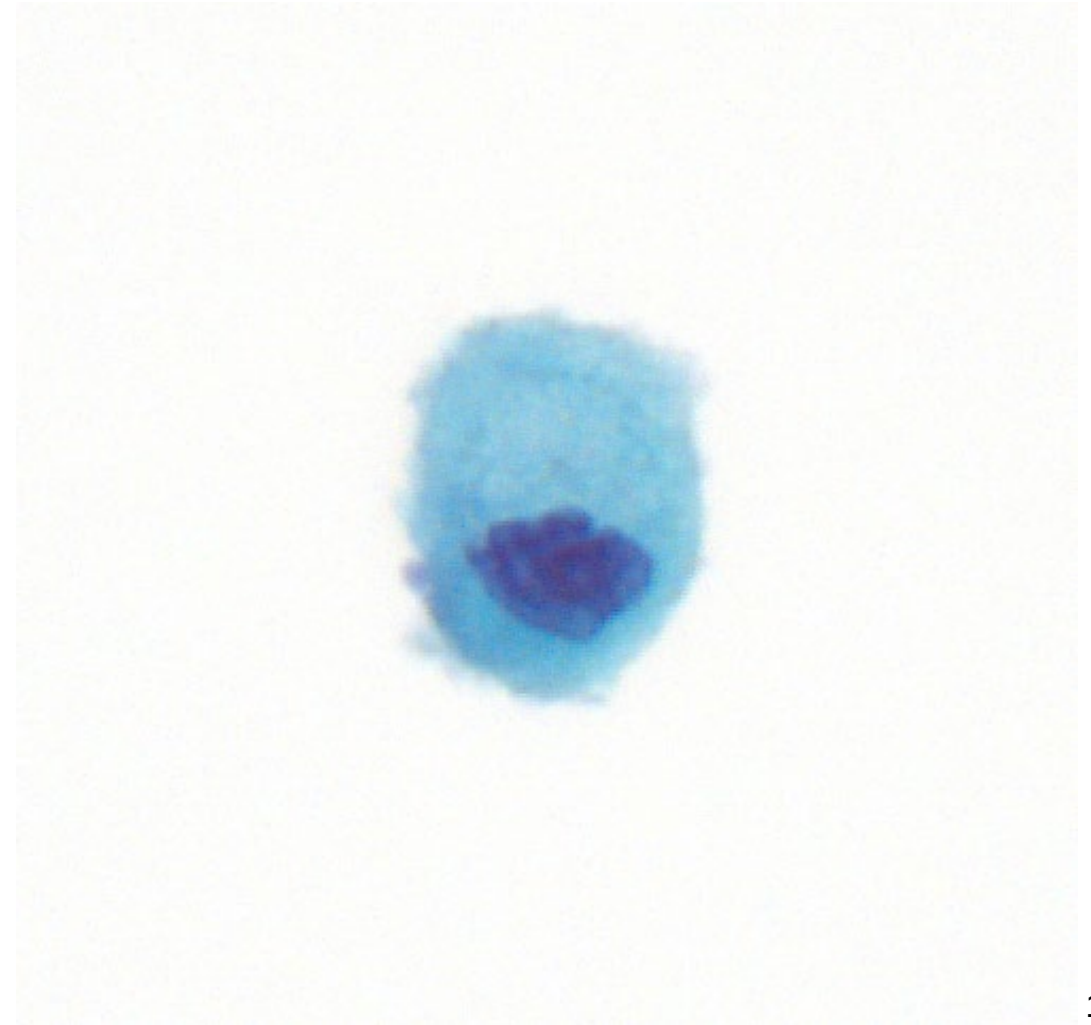
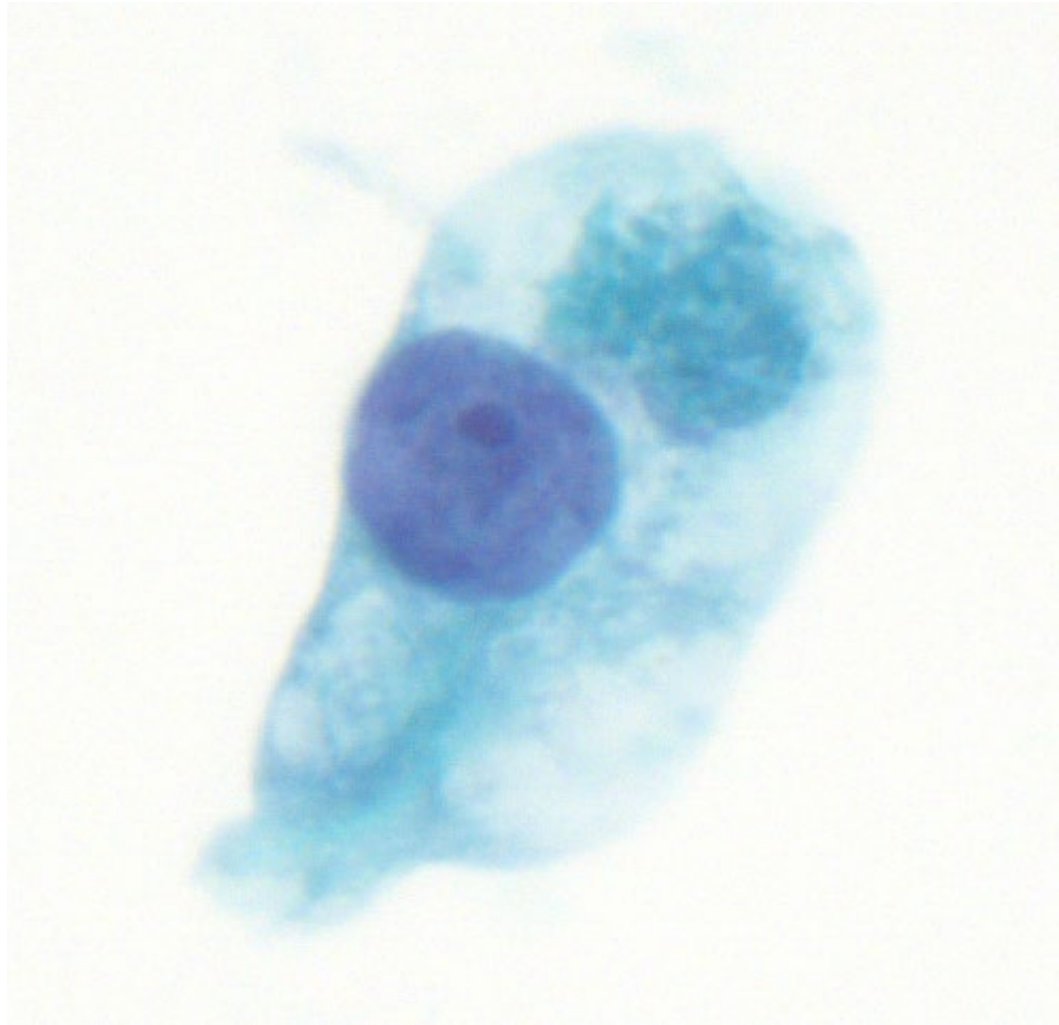
Contrast with Normal

Nuclear enlargement with high nucleus:cytoplasm ratio; Clumpy irregular chromatin



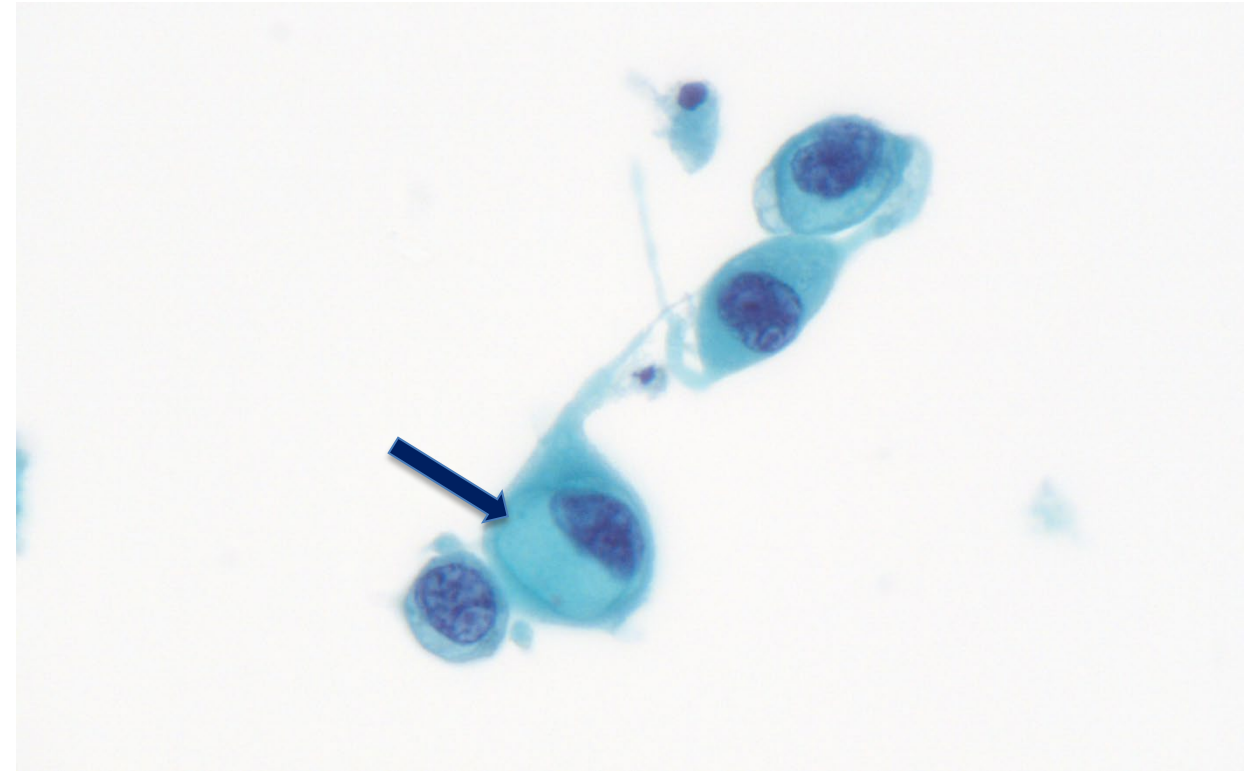
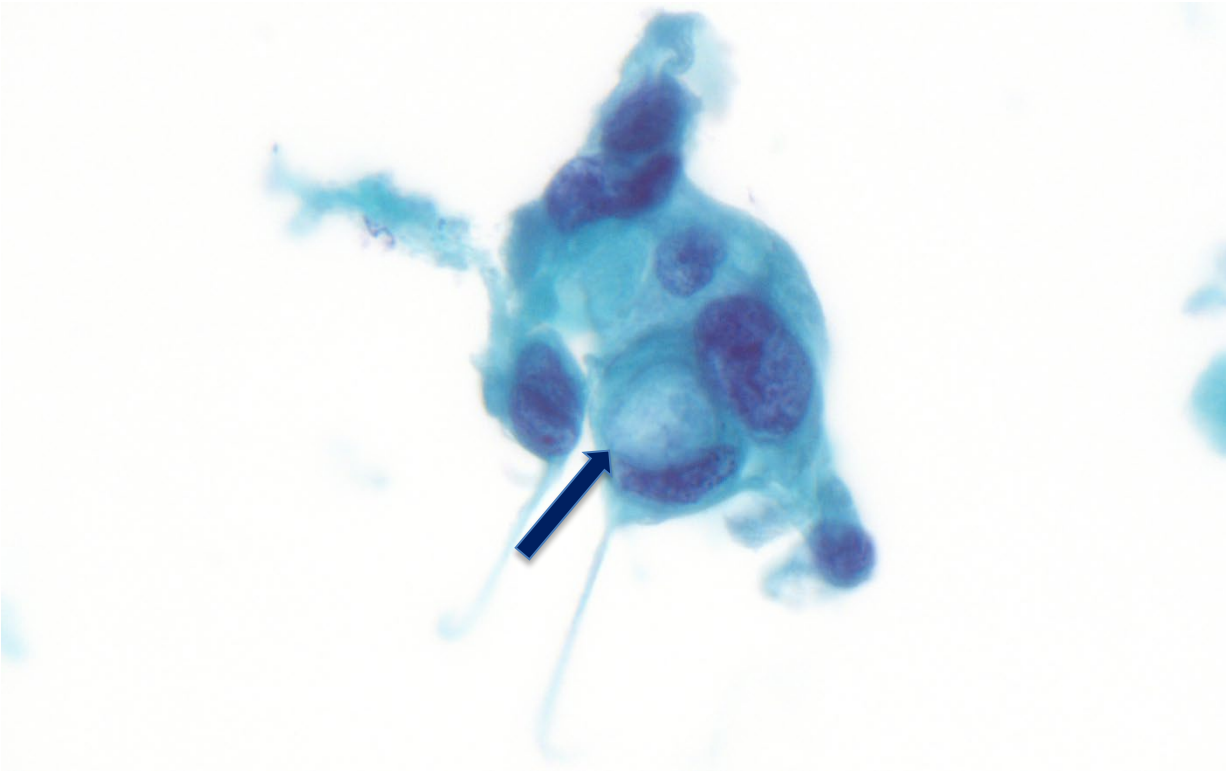
“Tombstone” Cells

Large individual columnar cells with large nuclei



“Signet Ring” Cells

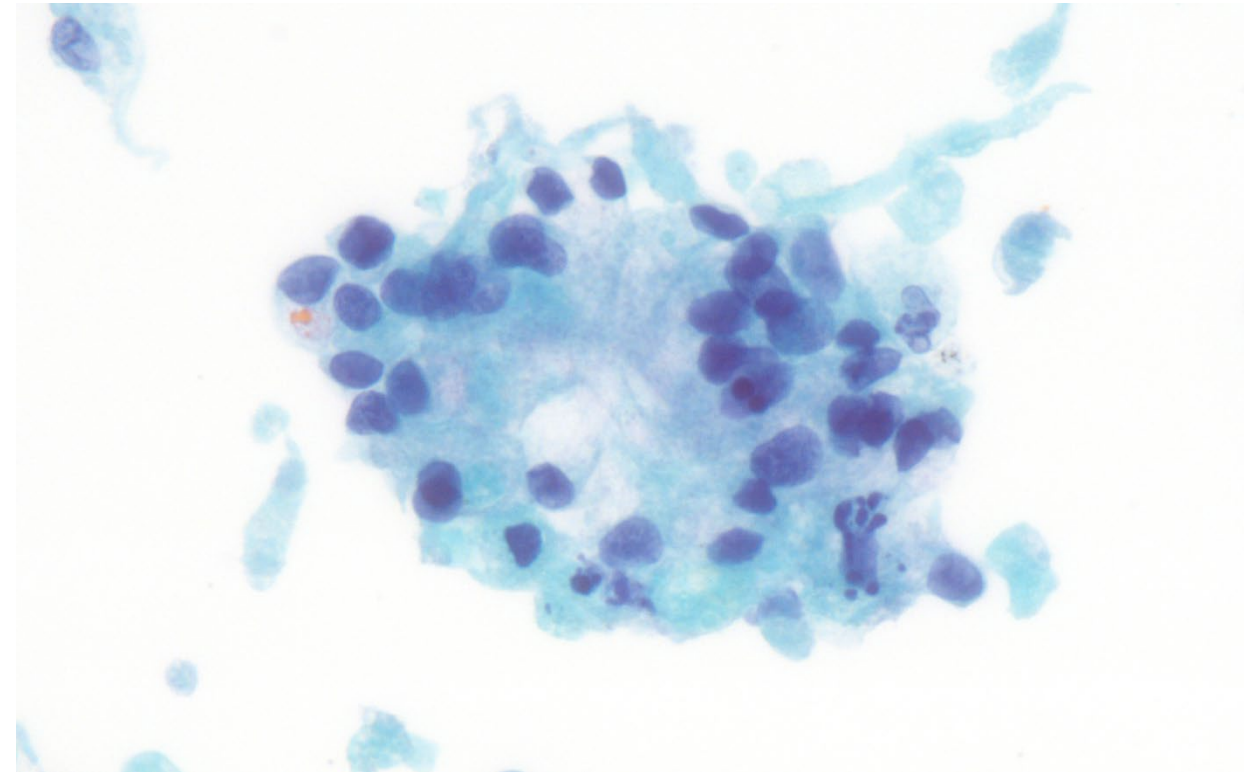
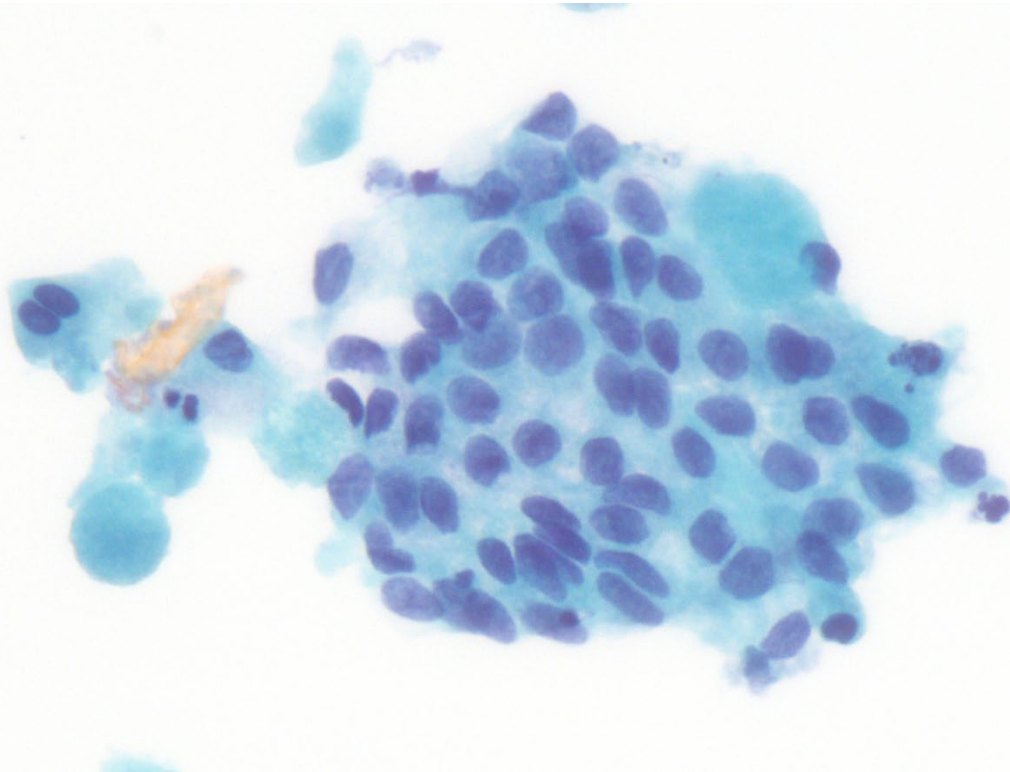
Prominent cytoplasmic mucin vacuoles and numerous individual malignant cells



Often associated with cystic mucinous neoplasms; may be a minor component in dysplastic background

“Drunken Honeycomb”

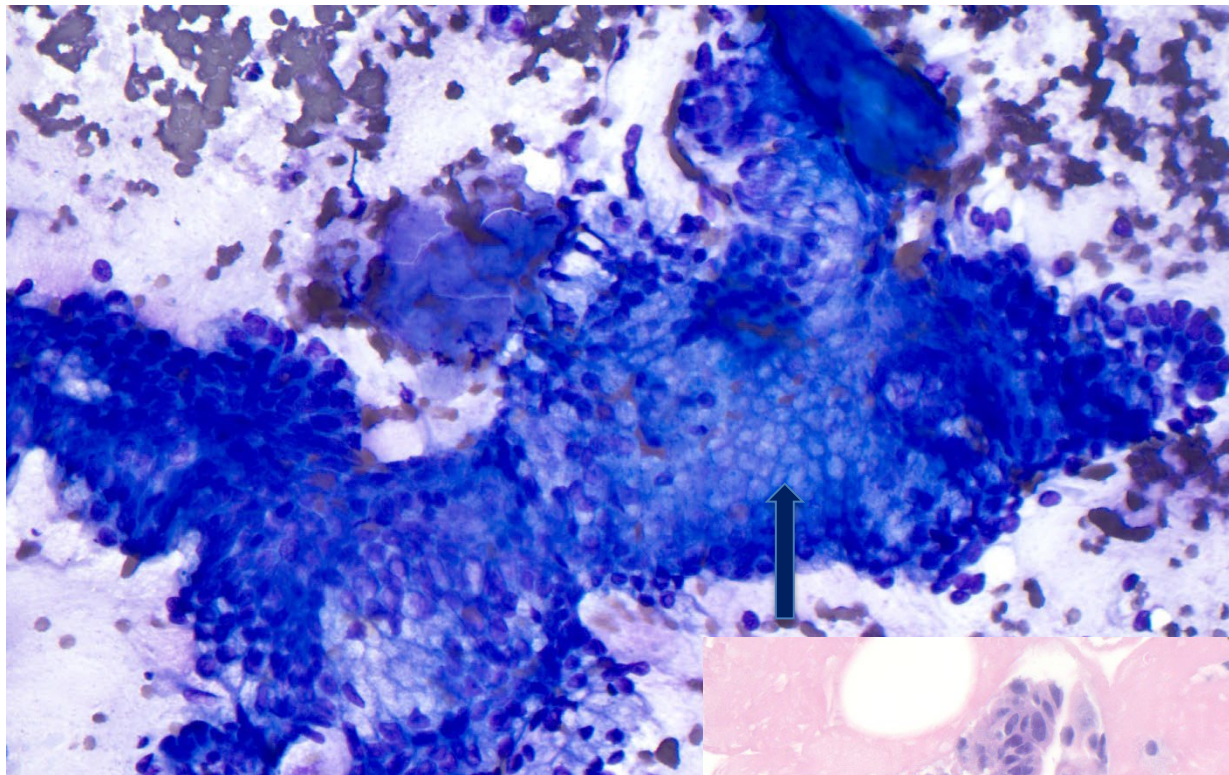
Well-differentiated: subtle architectural and nuclear irregularities, abundant cytoplasm



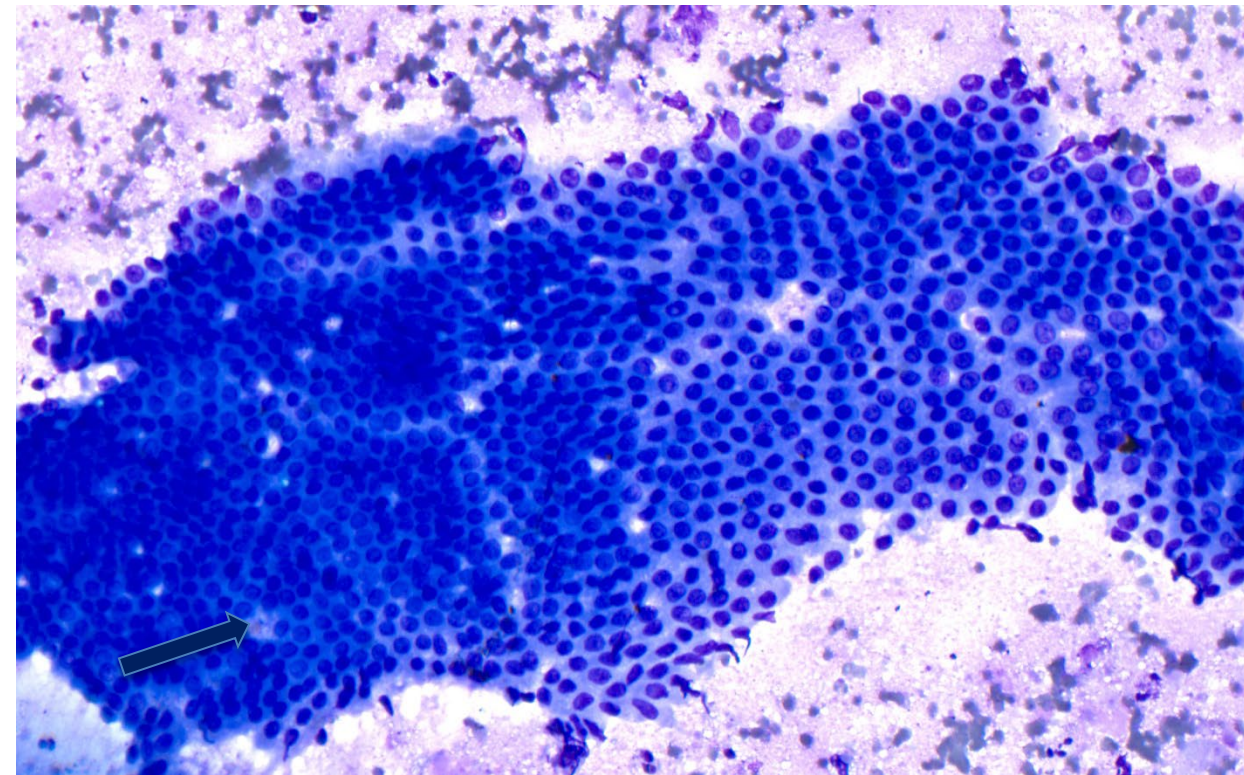
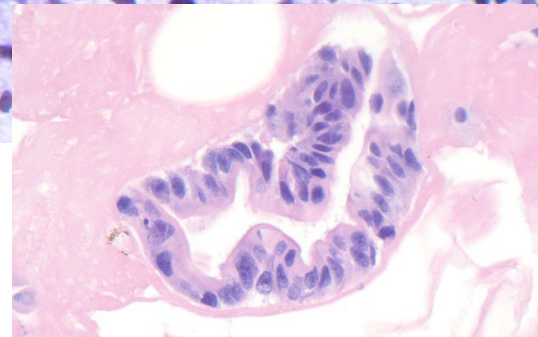
Look for more atypical groups or “tombstones”; Cell block of cores may be extremely helpful

WD Adenocarcinoma Vs Duodenum

Well-differentiated adenocarcinoma: prominent cytoplasmic borders, lack of goblet cells

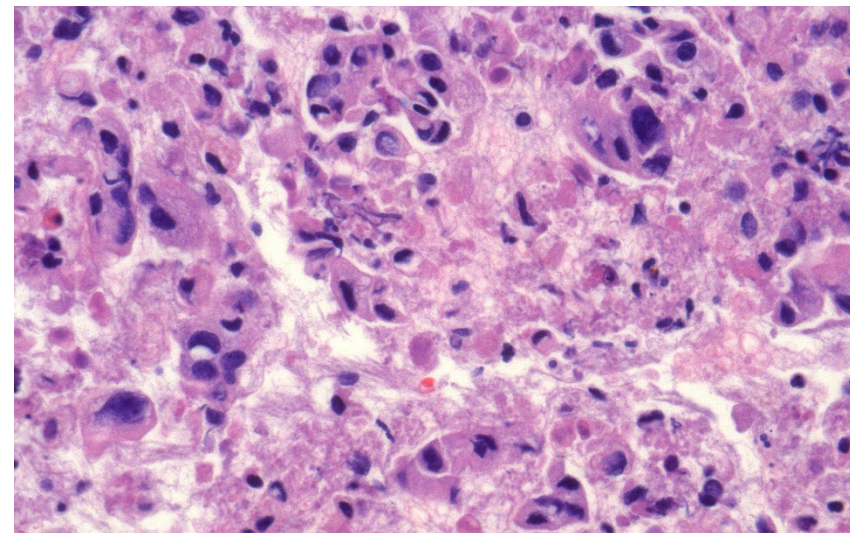
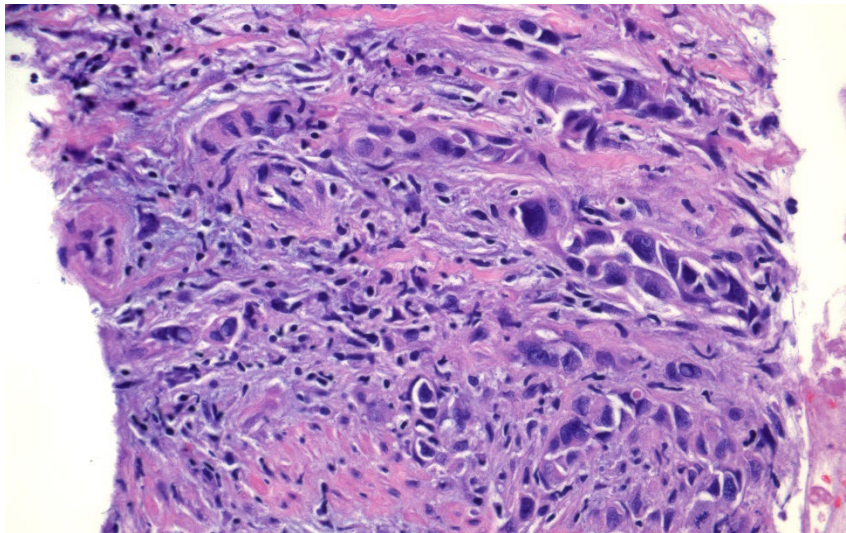
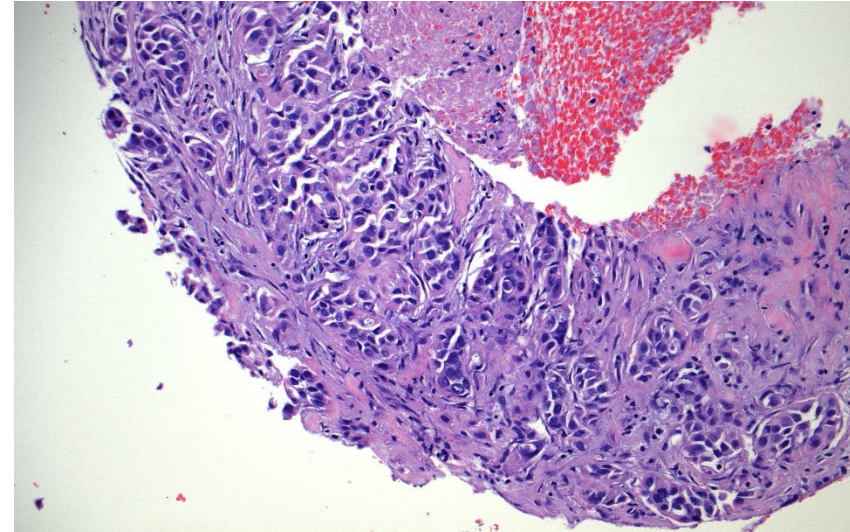
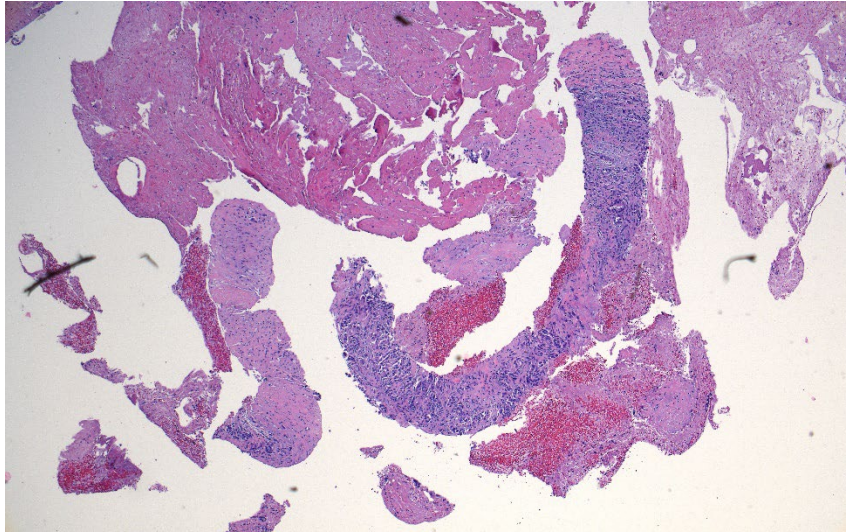


Well-diff adenocarcinoma



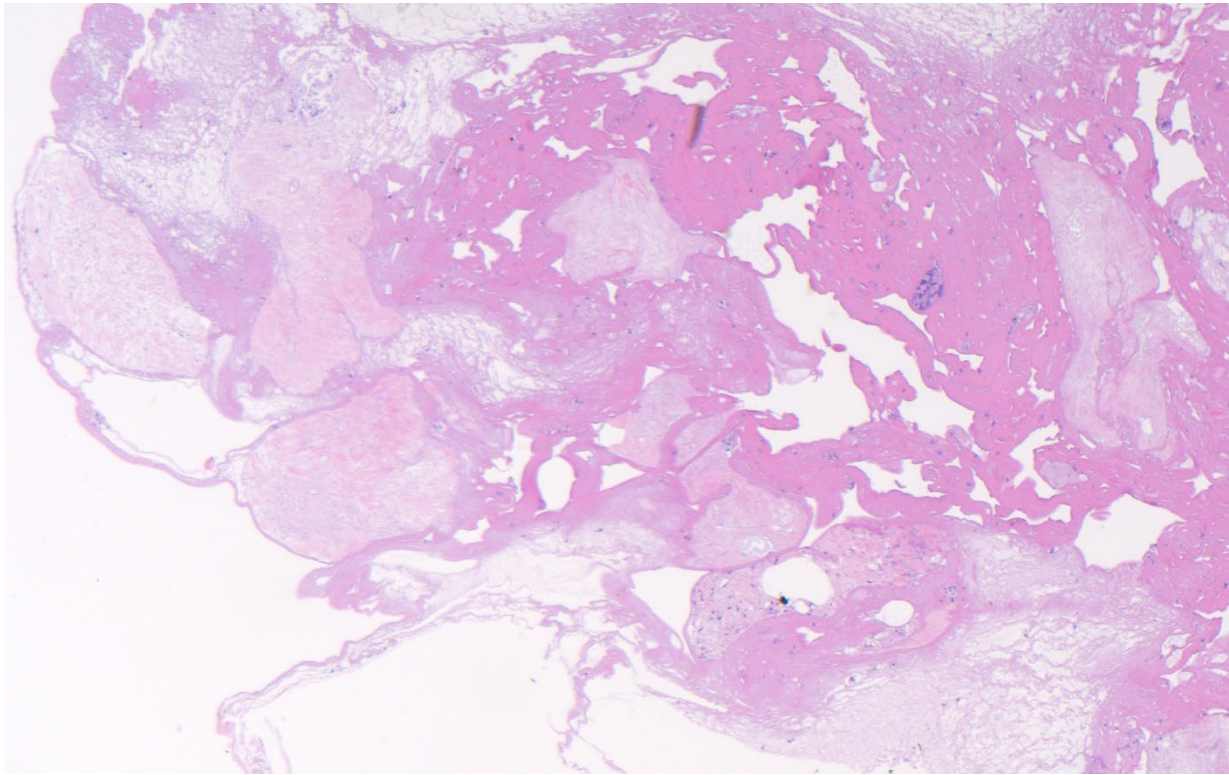
Normal duodenum (incidental sampling)

Core Biopsies in Cell Block

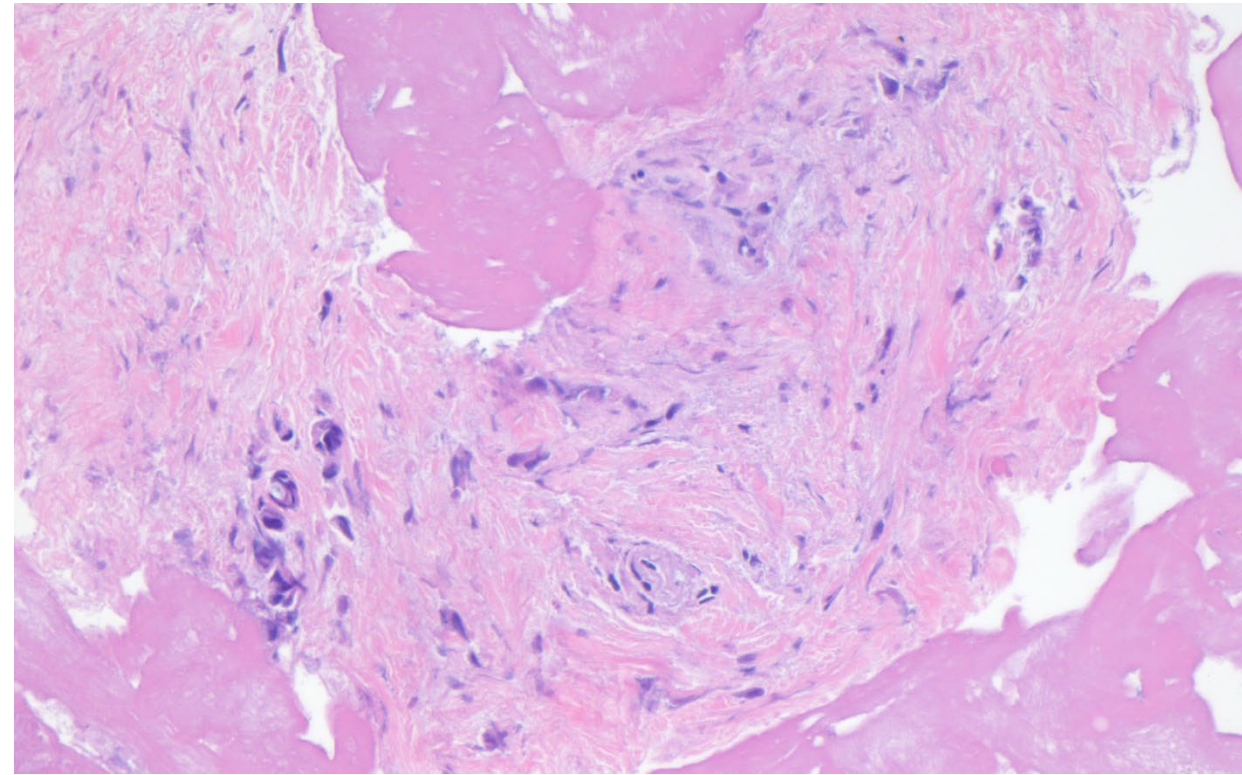


Paucicellular Core Fragments

Level 10



Level 45



Not uncommonly, cell blocks have desmoplastic core fragments with few cells; Deeper levels often help

What's old is new again...

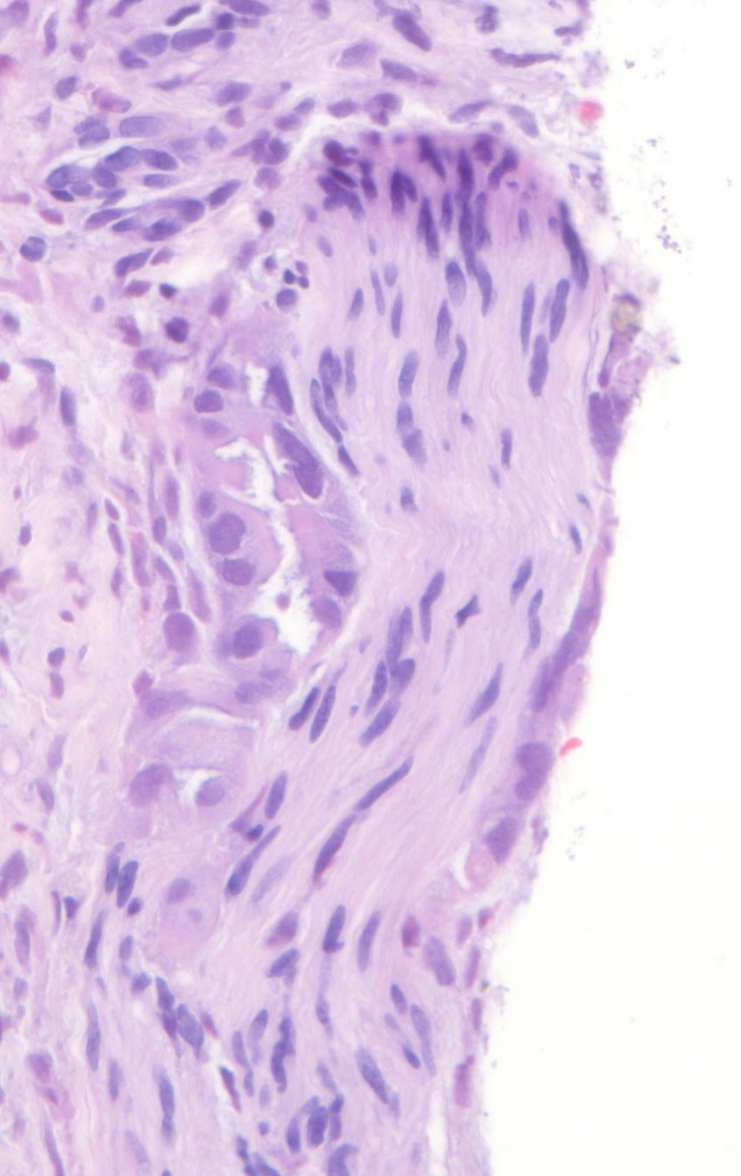
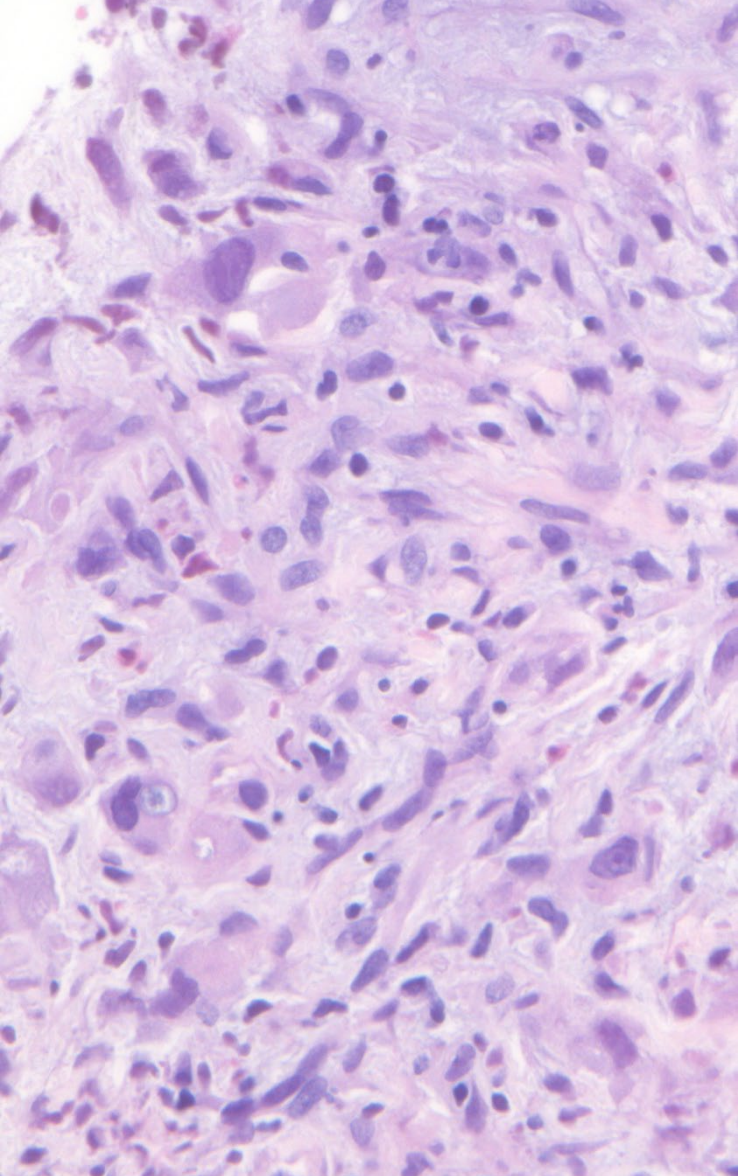
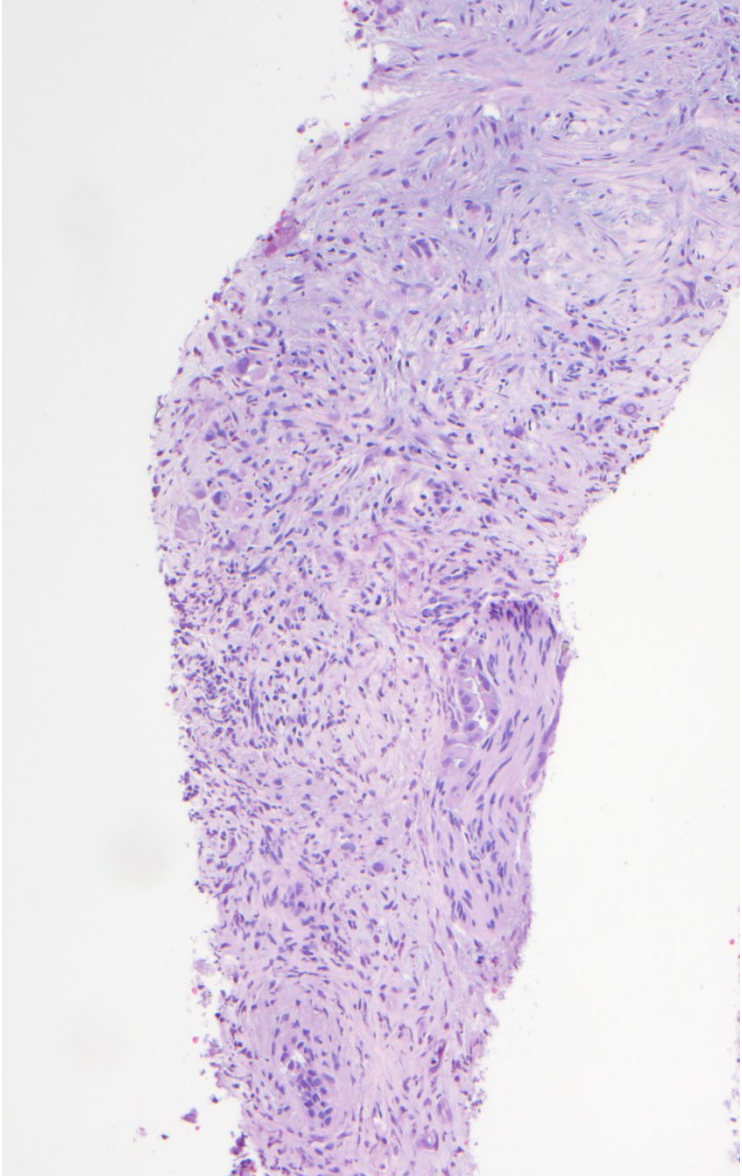
Frozen Section Diagnosis of Pancreatic Lesions

Adina M. Cioc, MD; E. Christopher Ellison, MD; Daniela M. Proca, MD; Joel G. Lucas, MD; Wendy L. Frankel, MD

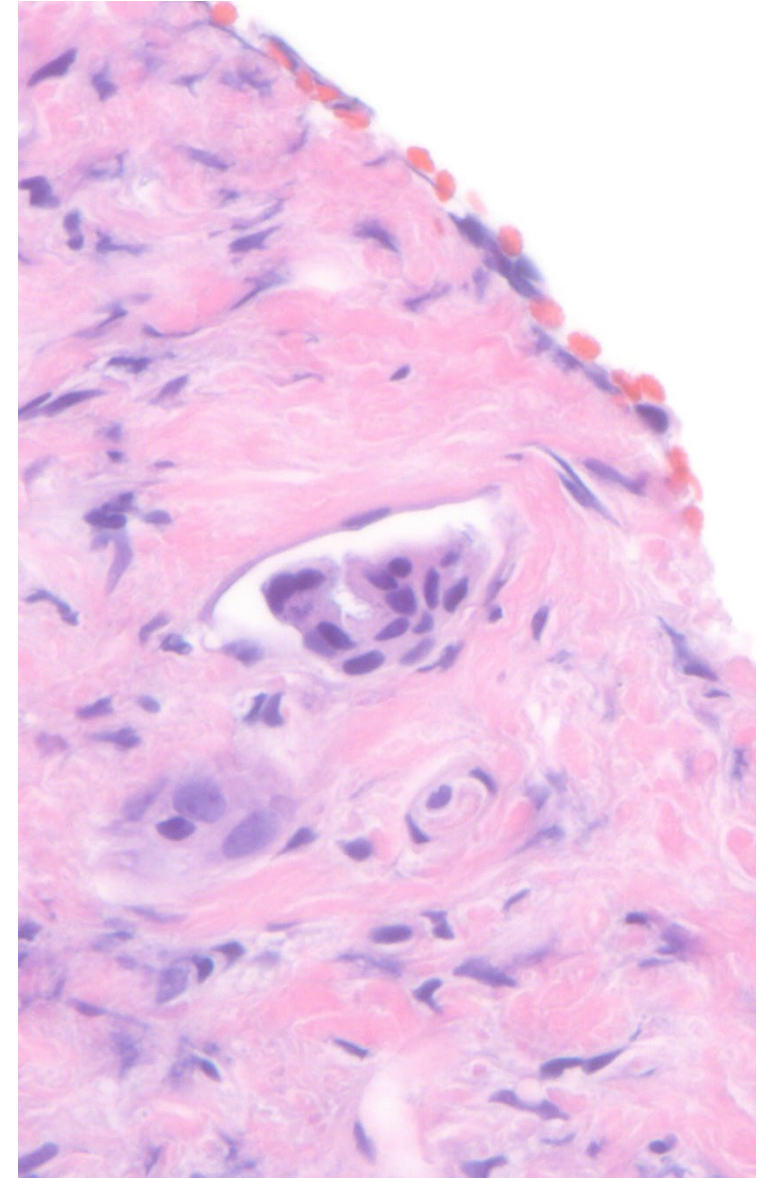
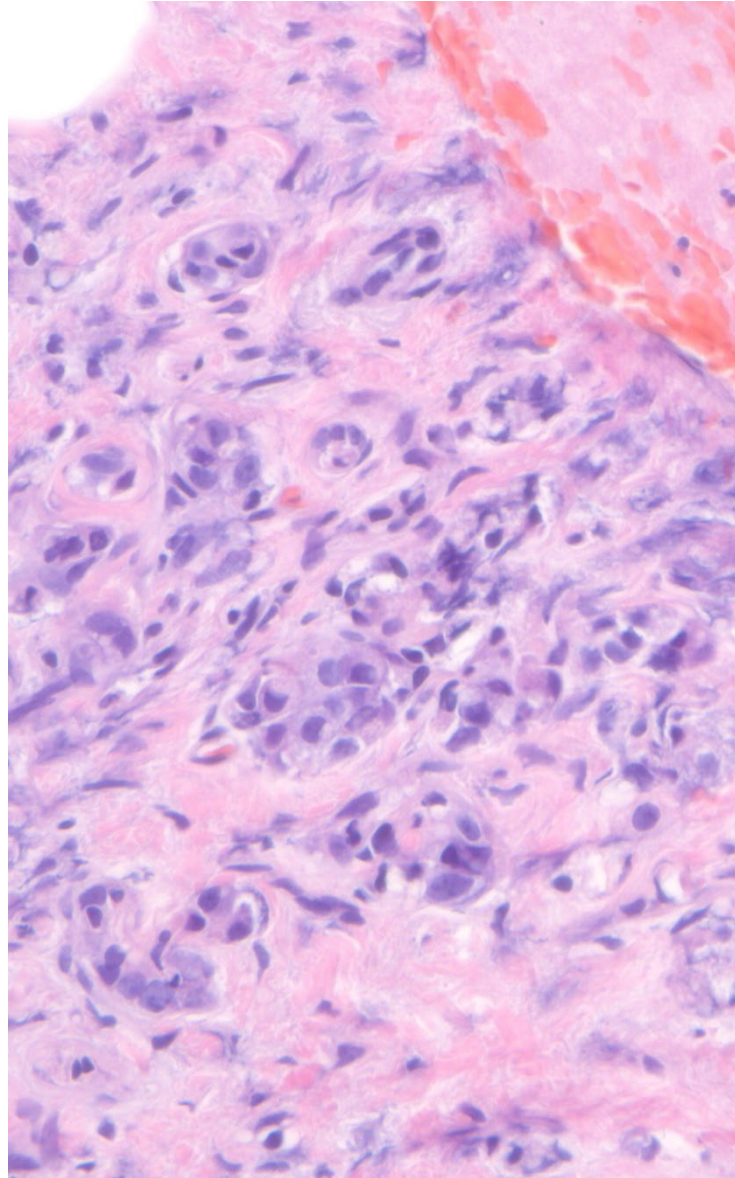
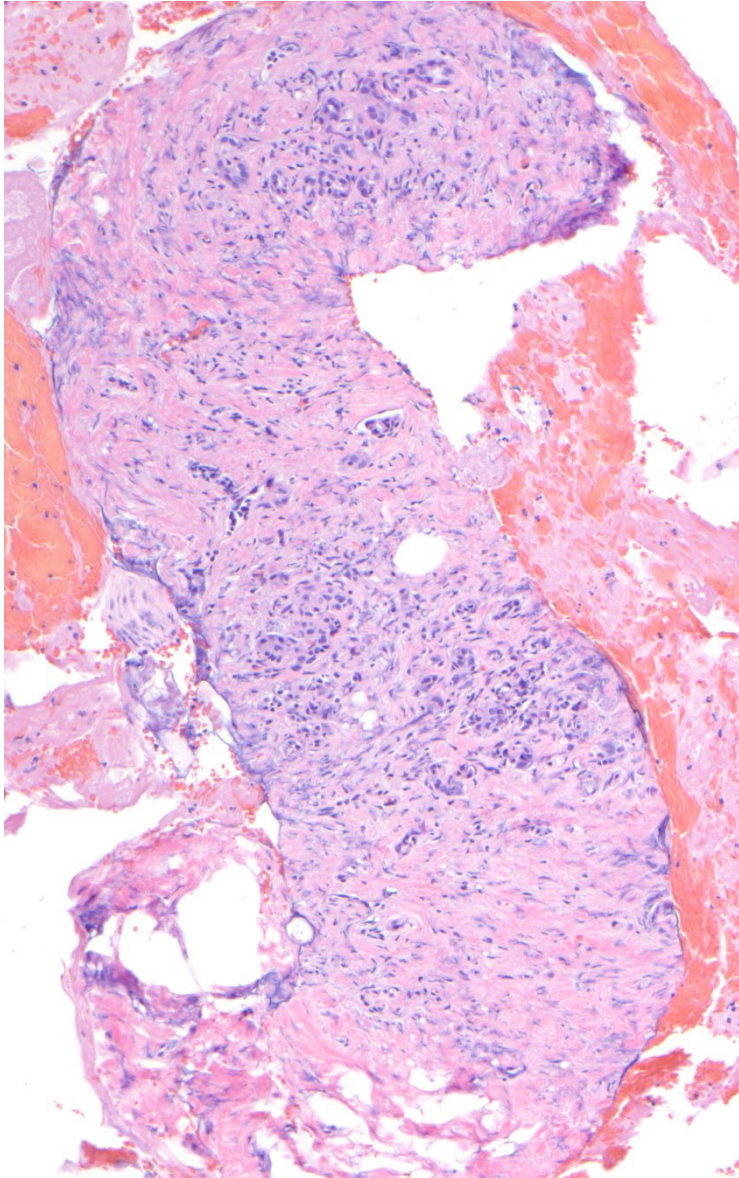
(Arch Pathol Lab Med. 2002;126:1169–1173)

Histologic Feature	Pancreatic Adenocarcinoma, No. (%) (n = 38)	Chronic Pancreatitis, No. (%) (n = 14)
Disorganized duct distribution	37 (97)	0
Variation in nuclear size of 4:1 or more	36 (94)	1 (7)
Incomplete duct lumen	34 (89)	2 (14)
Disorganized stroma	37 (97)	3 (21)
Single-cell infiltration	27 (71)	0
Cribriform glands	16 (42)	0
Epithelial mitoses	16 (42)	0
Necrotic glandular debris	11 (29)	0
Large nucleoli	11 (29)	0
Perineural invasion	11 (29)	0

Single Cell Invasion and Perineural Invasion

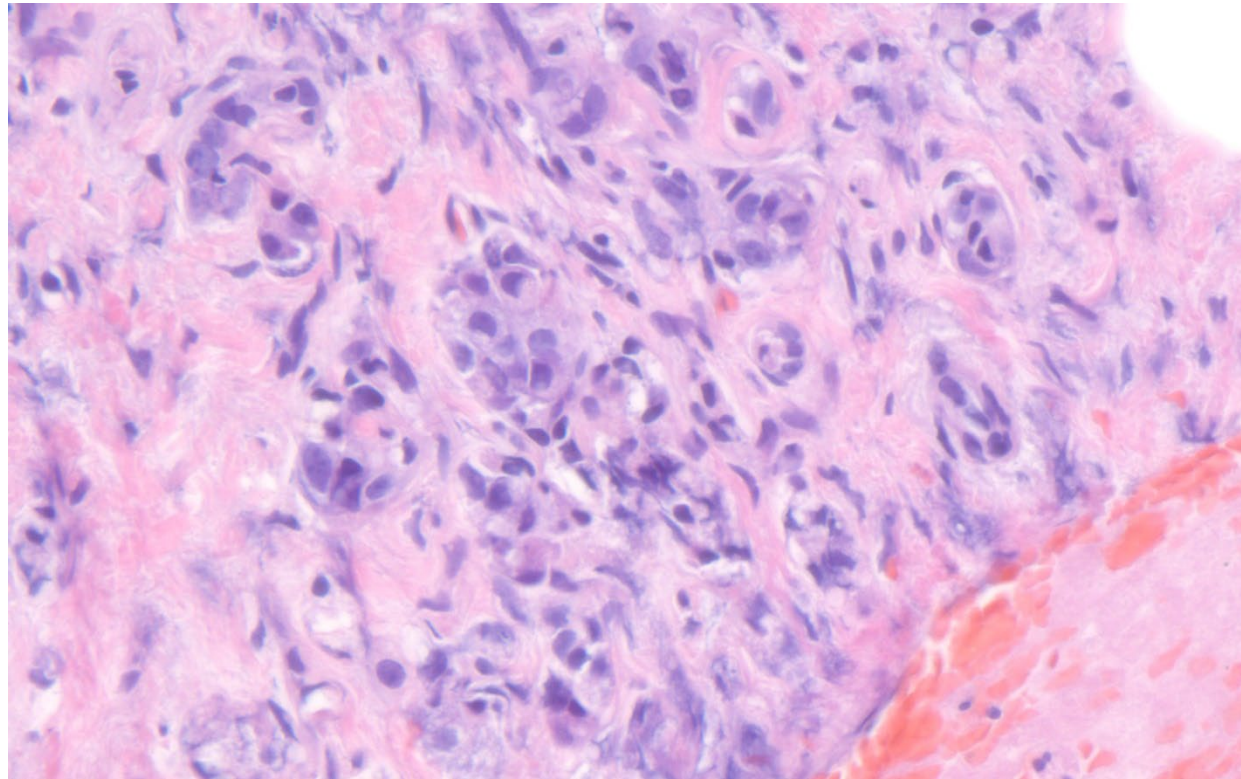


Incomplete Glands and Lymphatic Invasion

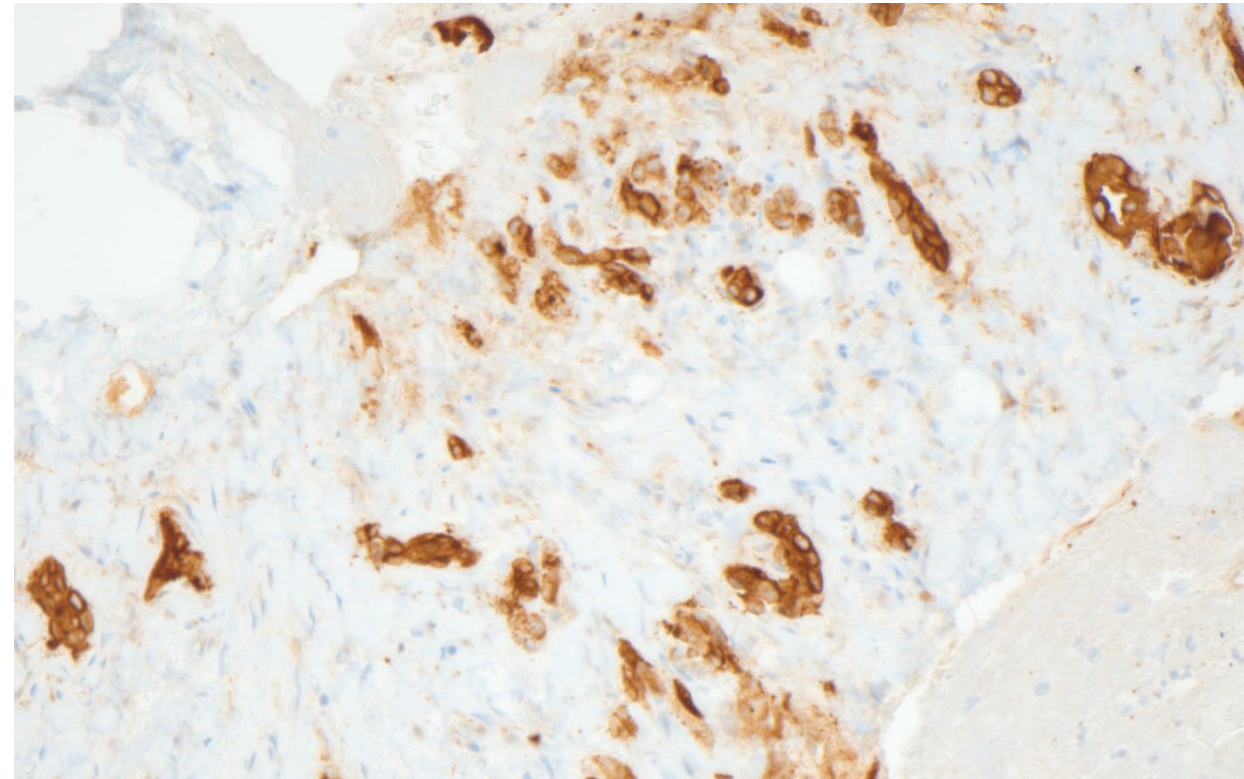


Utility of Pancytokeratin Stain?

H&E

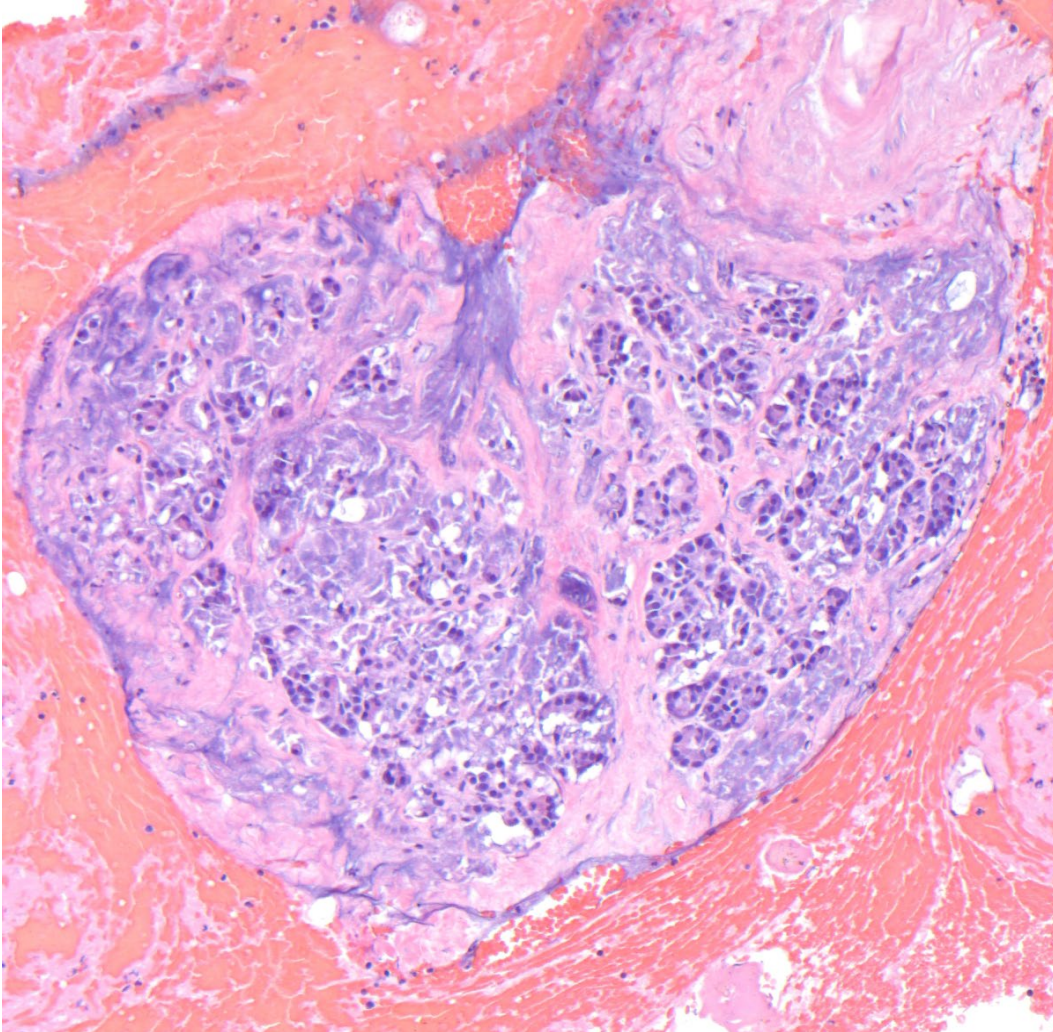


Pancytokeratin



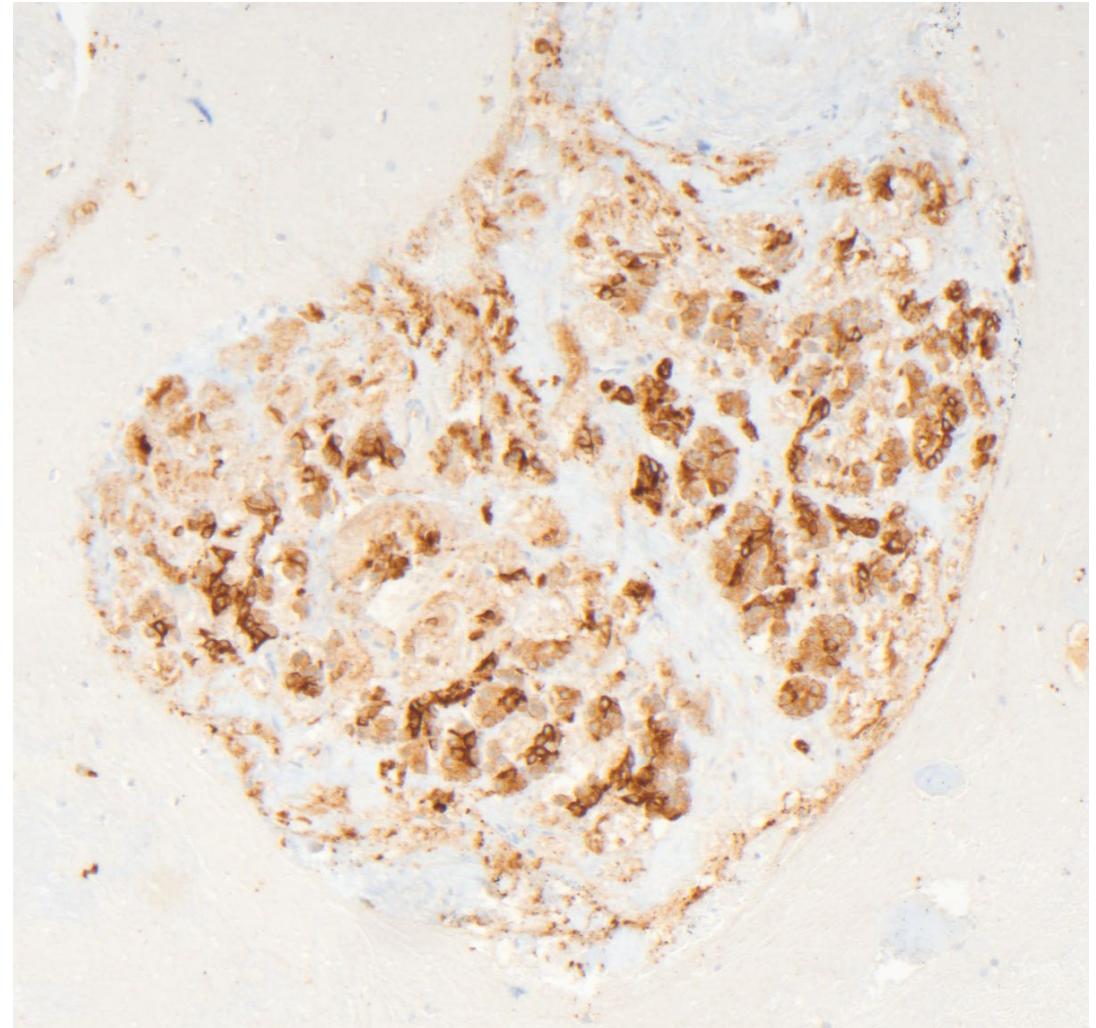
Utility of Pancytokeratin Stain?

H&E



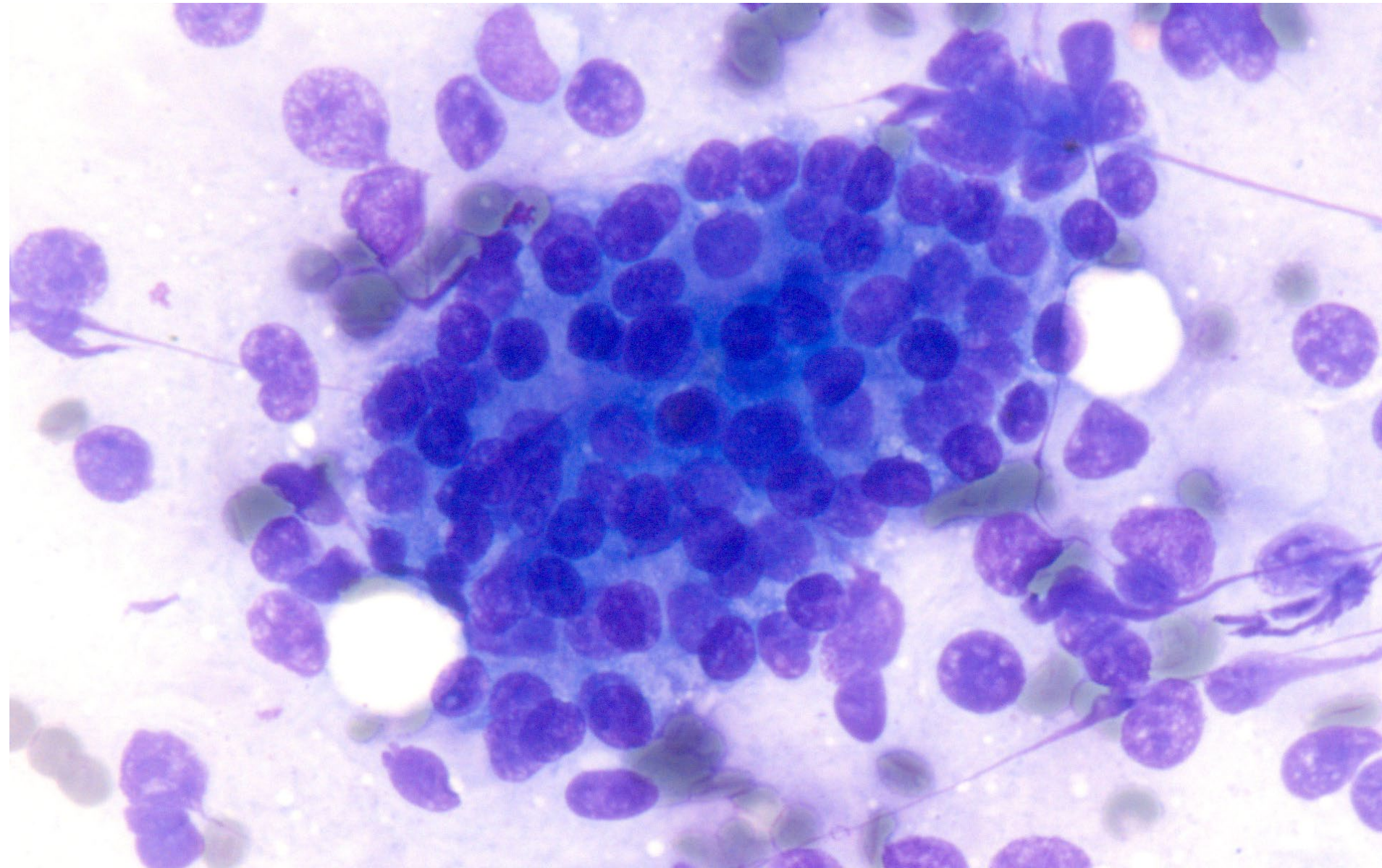
Autolytic normal in the same case

Pancytokeratin



Similar patchy staining

Diff-Quik of Well-Differentiated Neuroendocrine Tumor



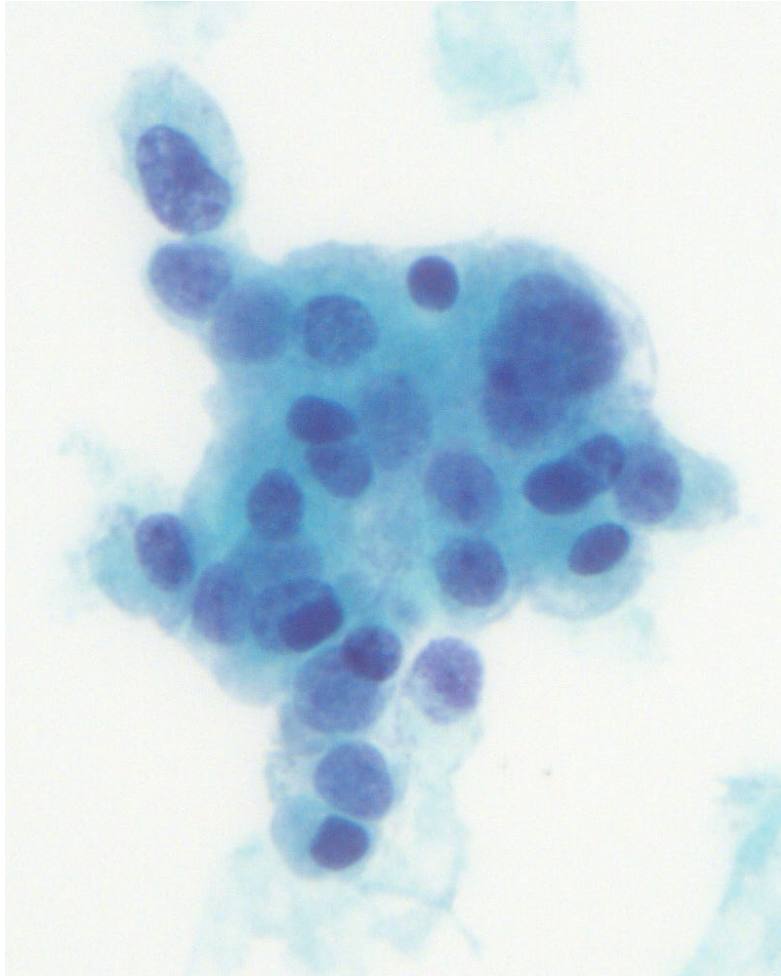
High
cellularity

Oval-round
nuclei with
granular
chromatin

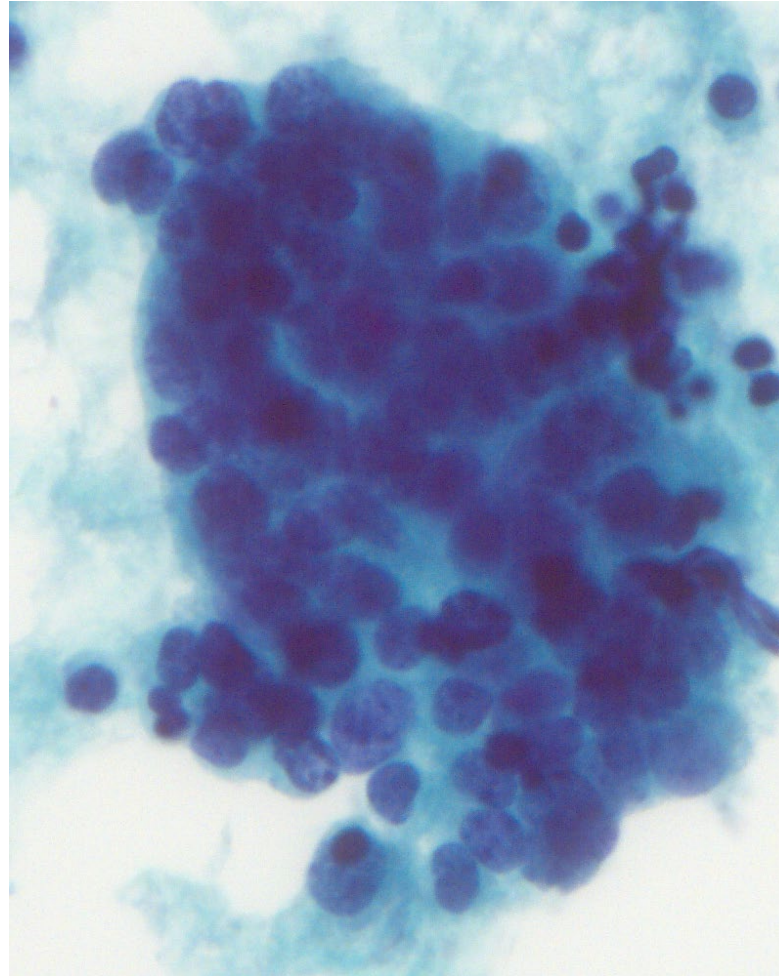
Rosette
architecture

Low mitotic
rate, no
apoptosis

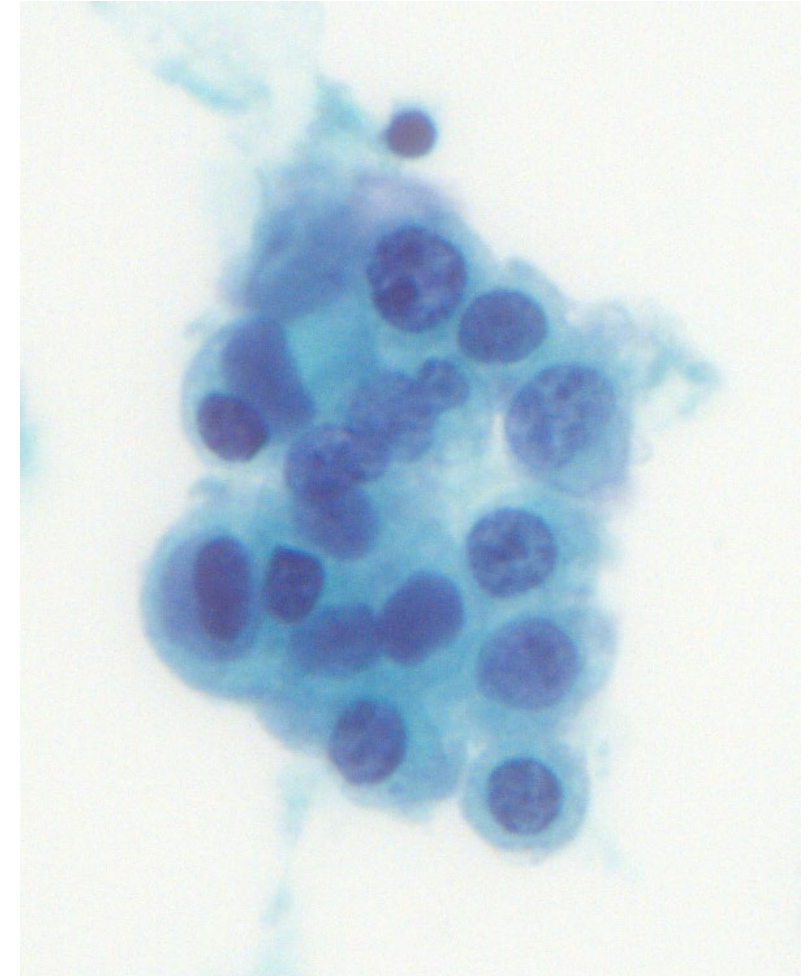
Well-Differentiated Neuroendocrine Tumor ThinPrep



Anisonucleosis,
but round and smooth

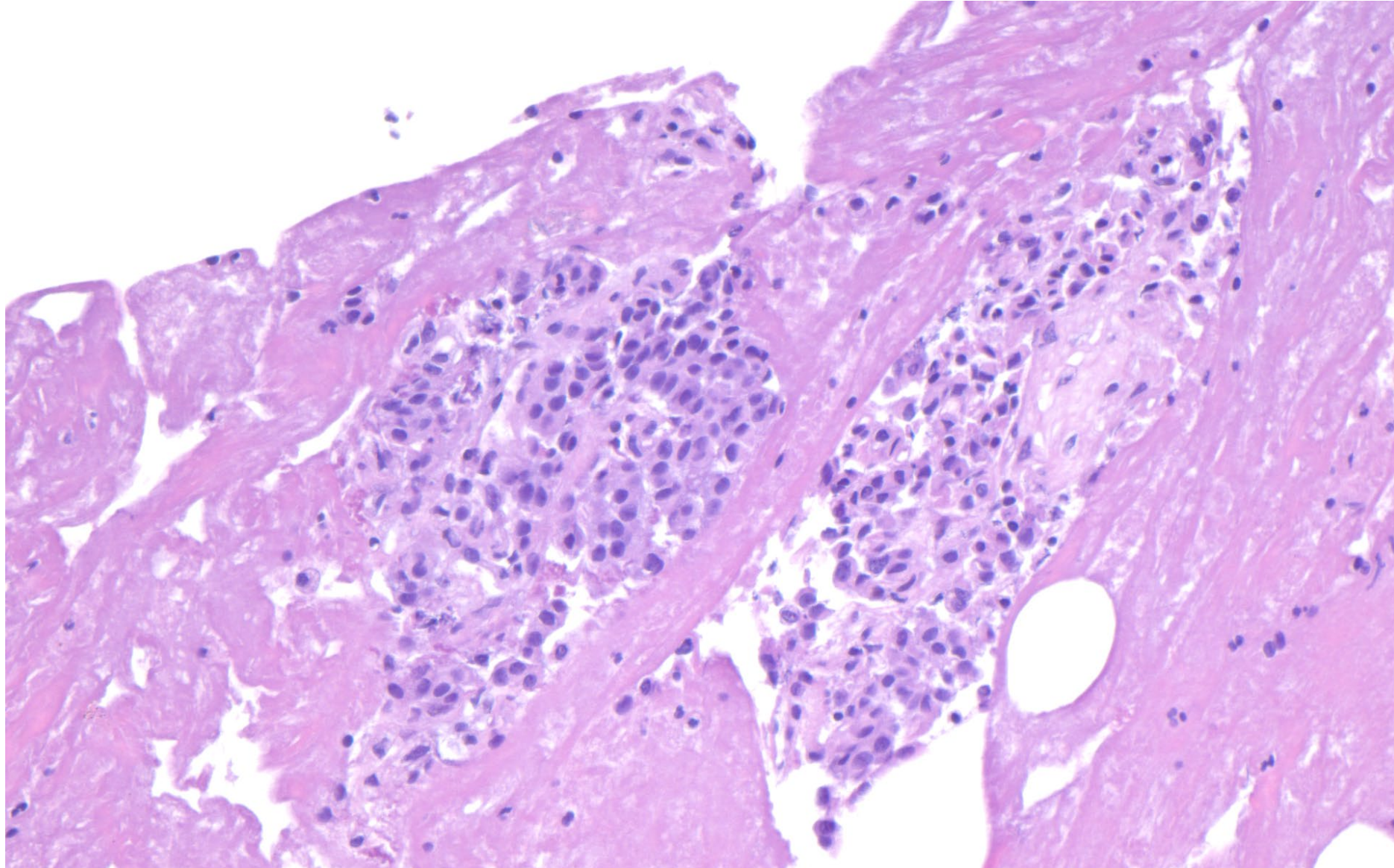


Complex 3D architecture



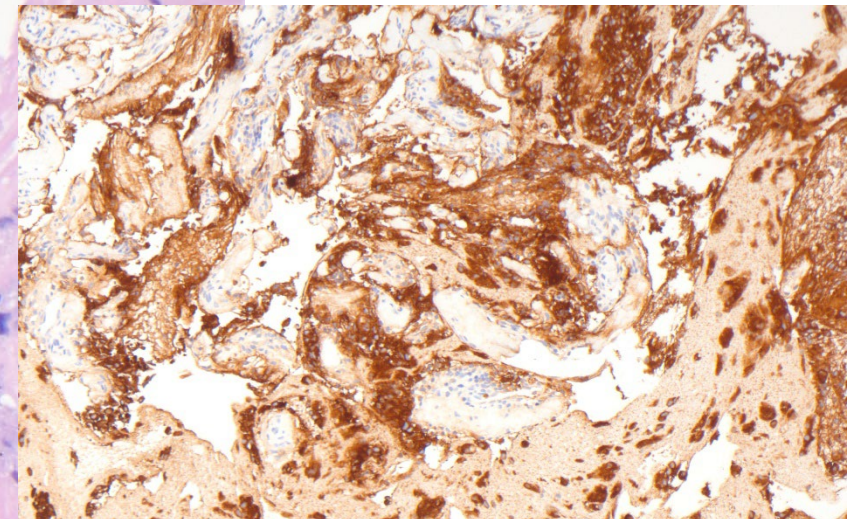
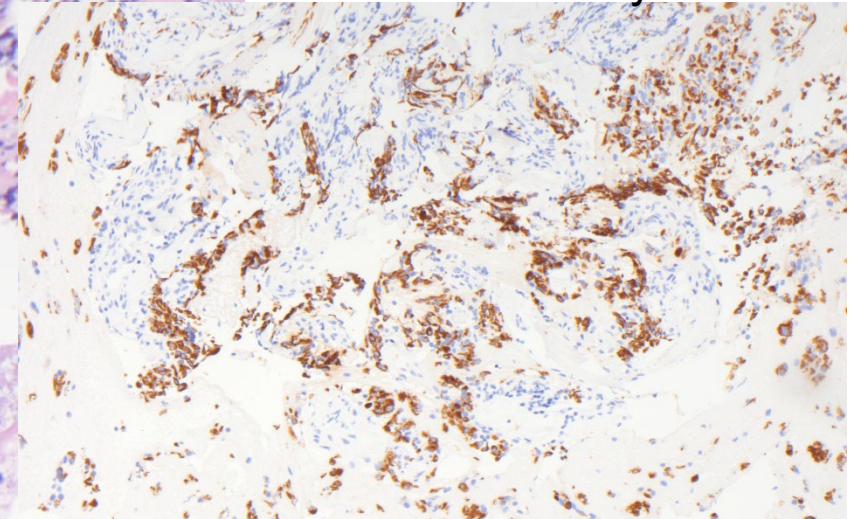
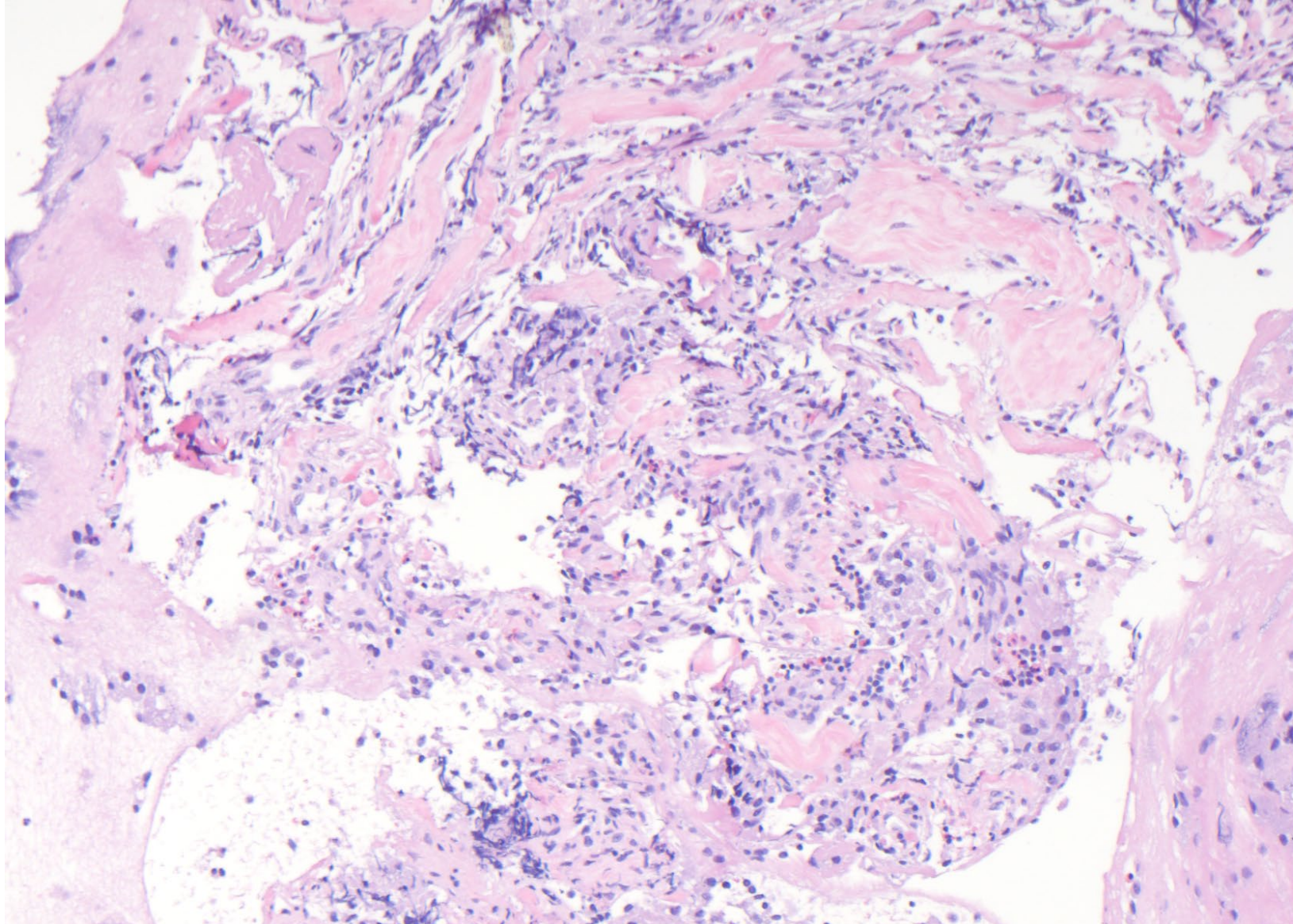
Coarse "neuroendocrine"
chromatin

Well-Differentiated Neuroendocrine Tumor Cell Block



Well-Differentiated Neuroendocrine Tumor Cell Block

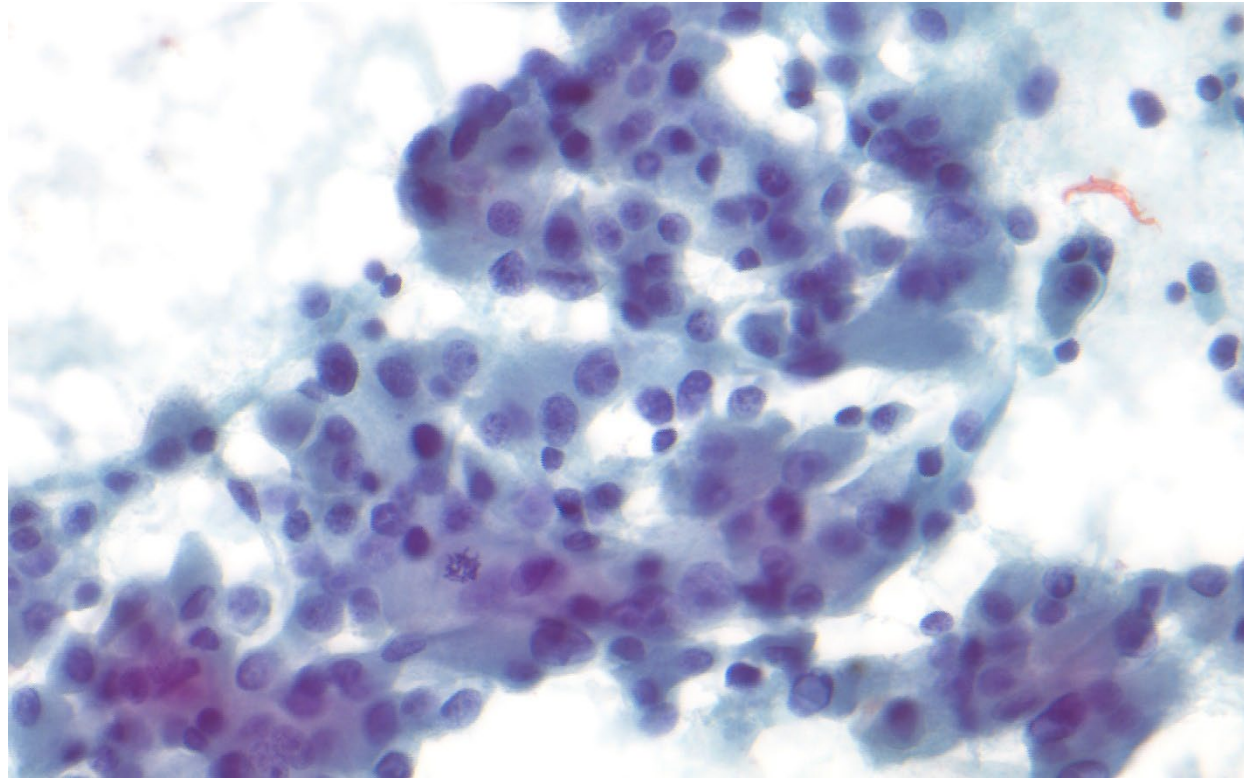
Pancytokeratin



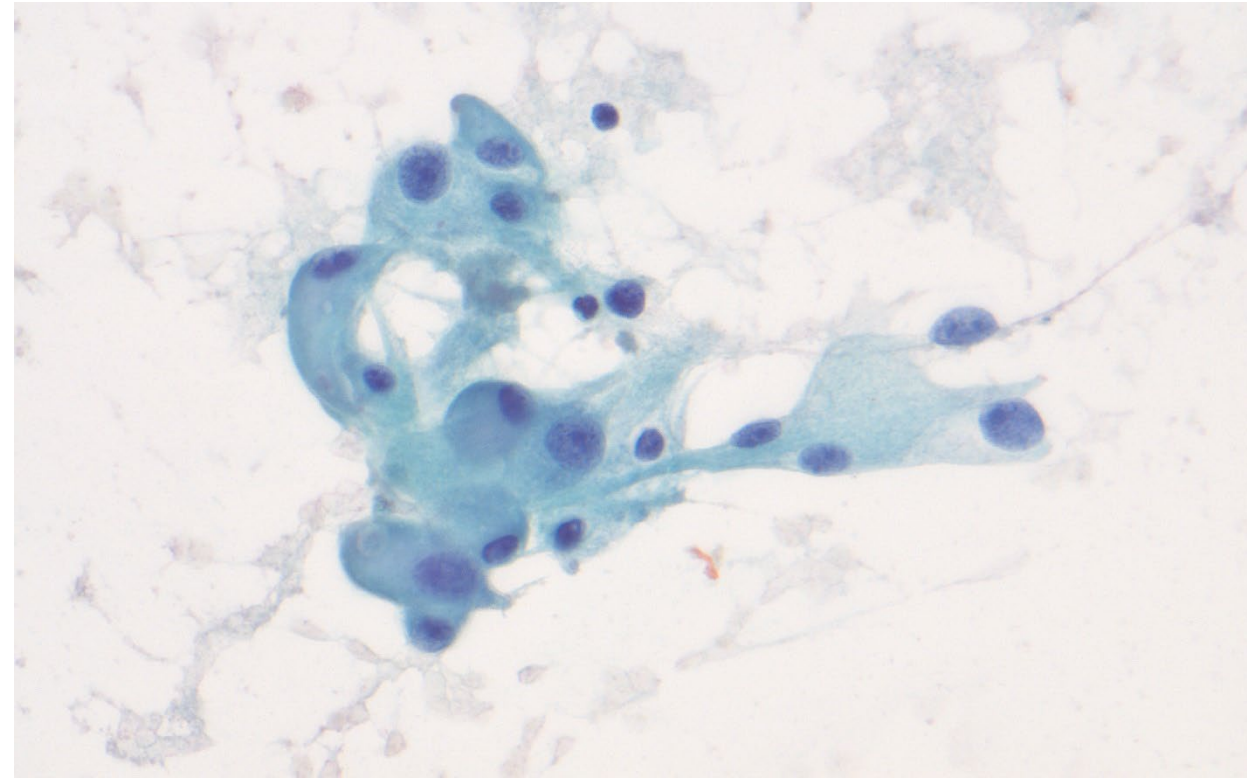
Synaptophysin

Oncocytic Variant of WDNET

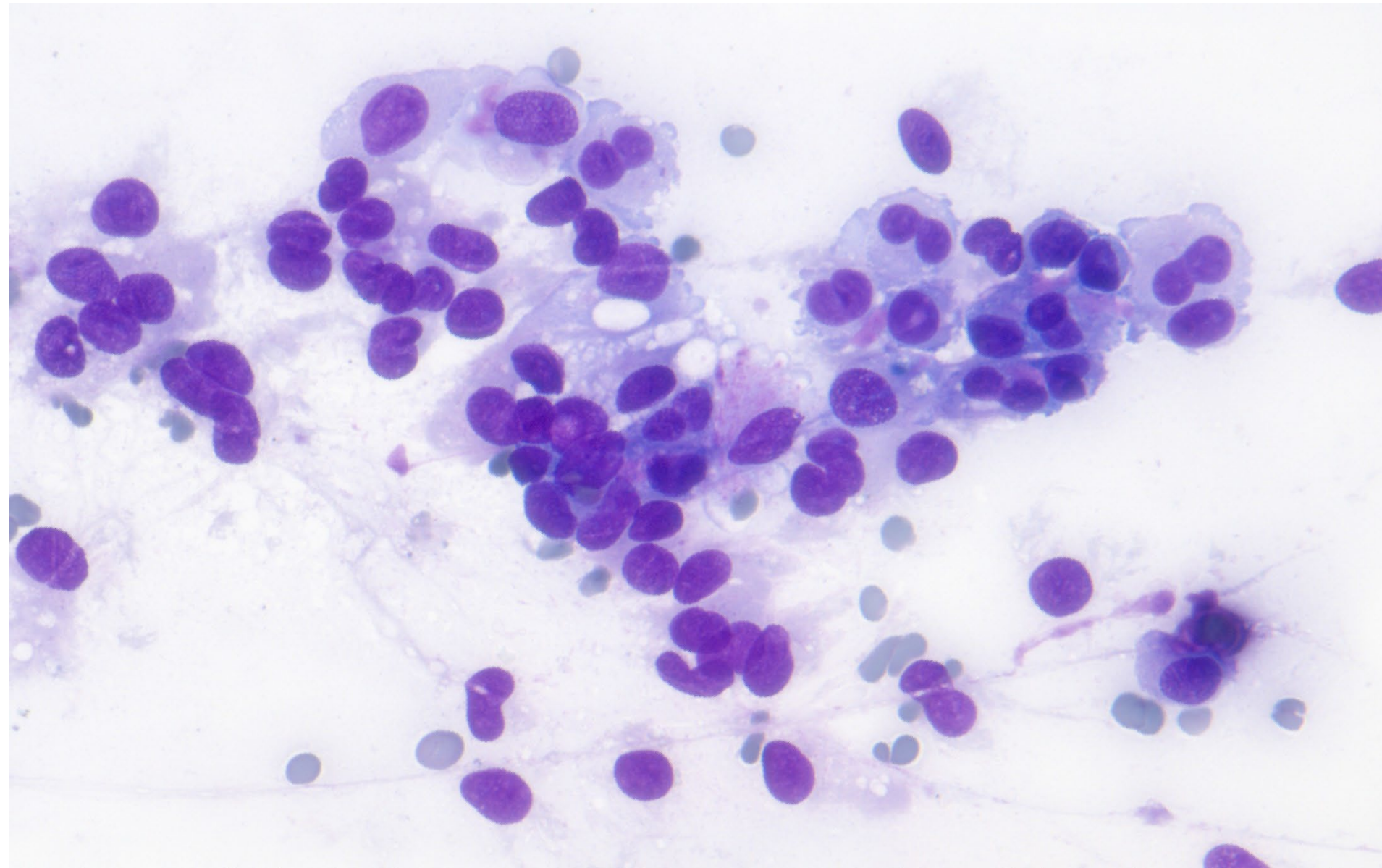
Abundant cytoplasm



Prominent nucleoli



Solid Pseudopapillary Neoplasm



High
cellularity

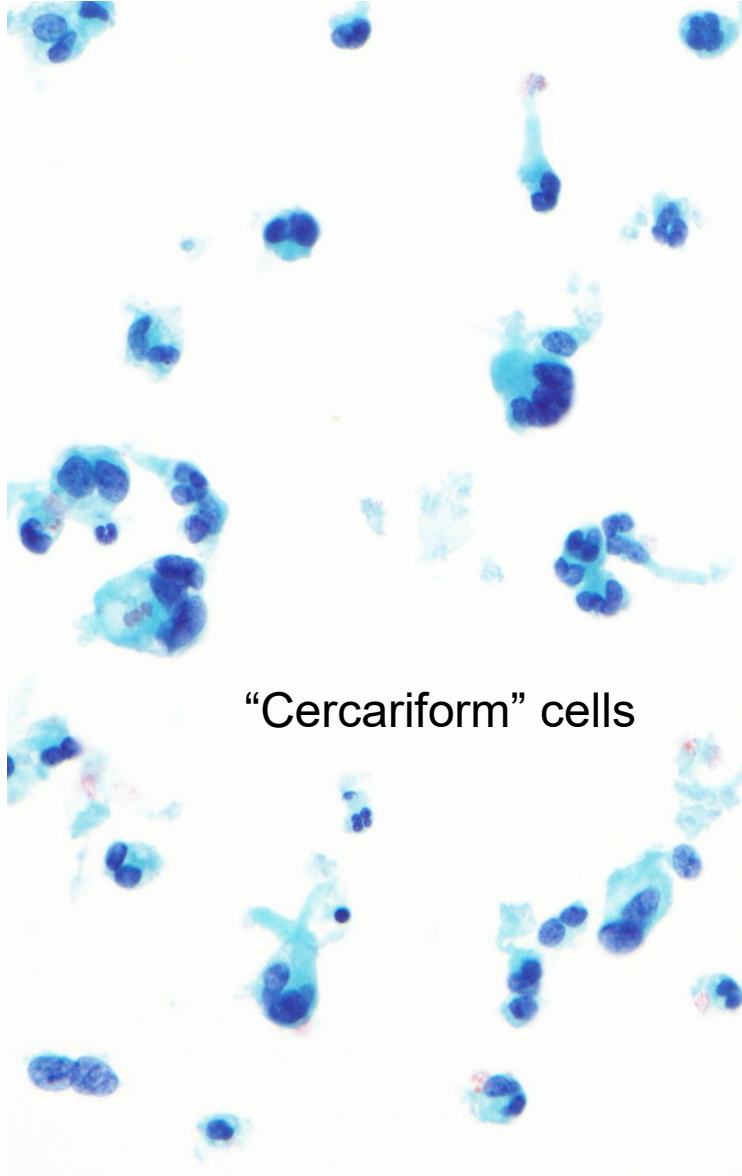
Oval-round
nuclei, finely
granular
chromatin

Moderate
cytoplasm

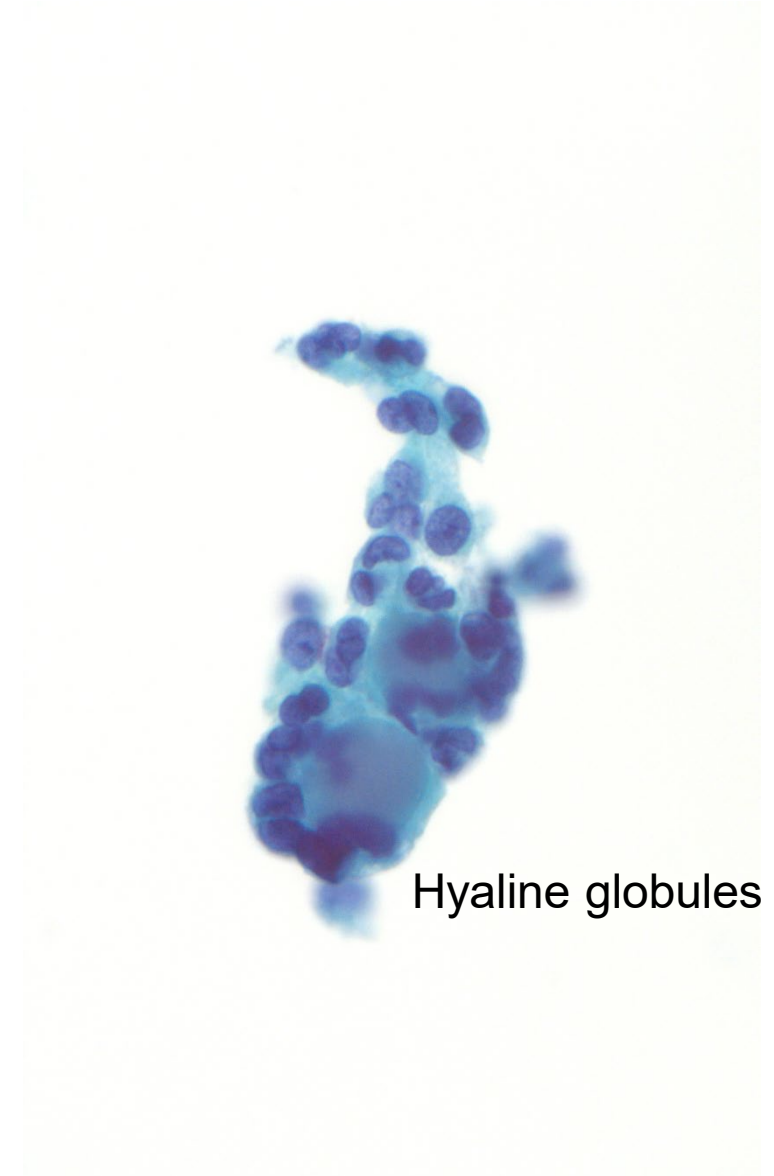
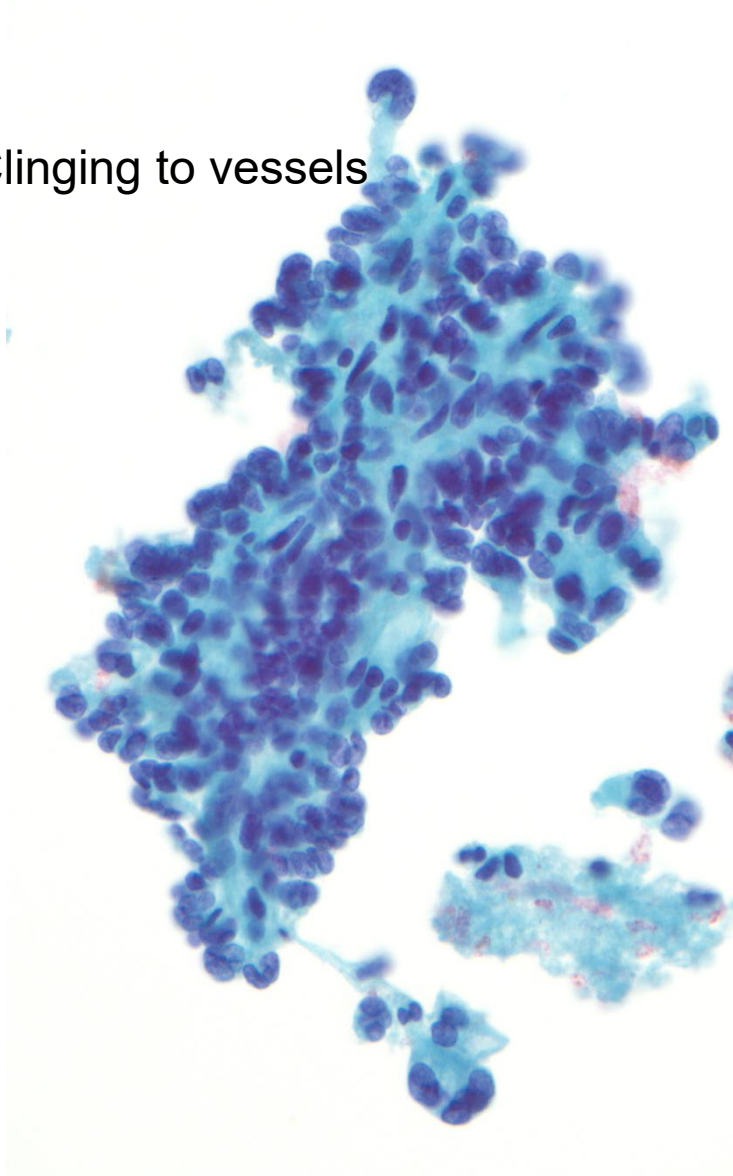
Discohesive

Low mitotic
rate

Solid Pseudopapillary Neoplasm ThinPrep Features

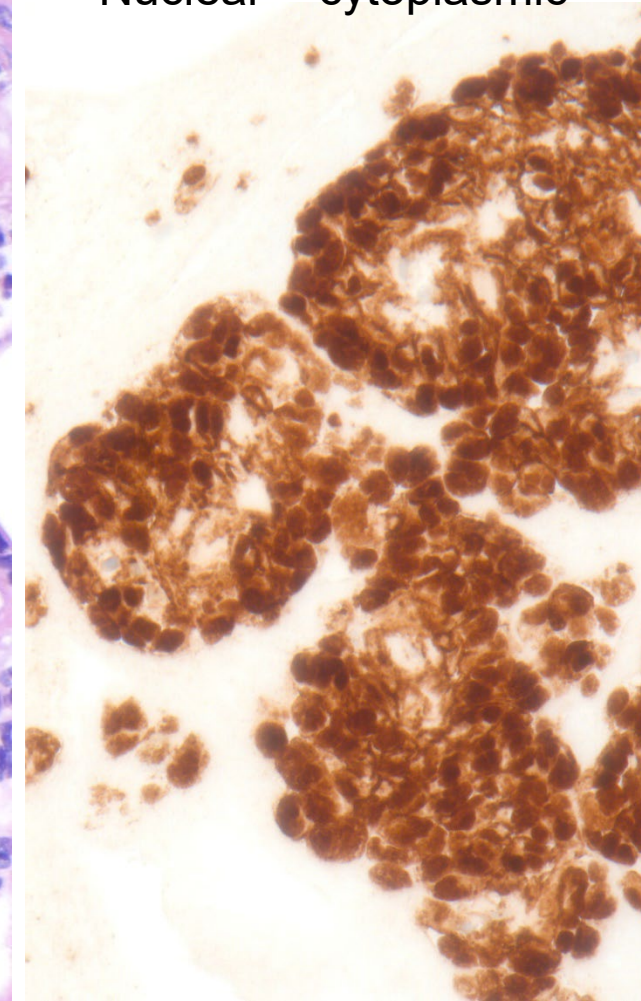
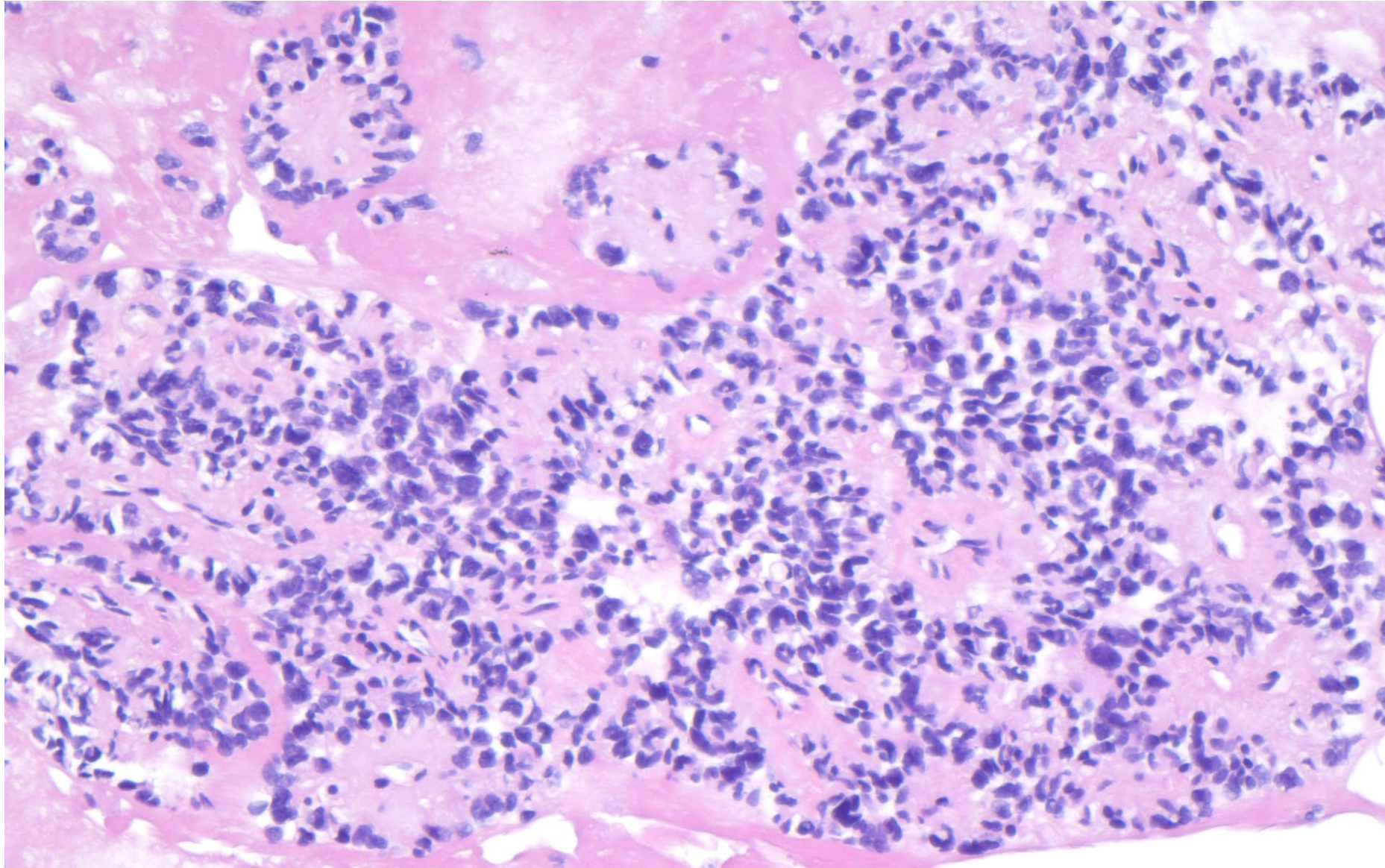


Clinging to vessels

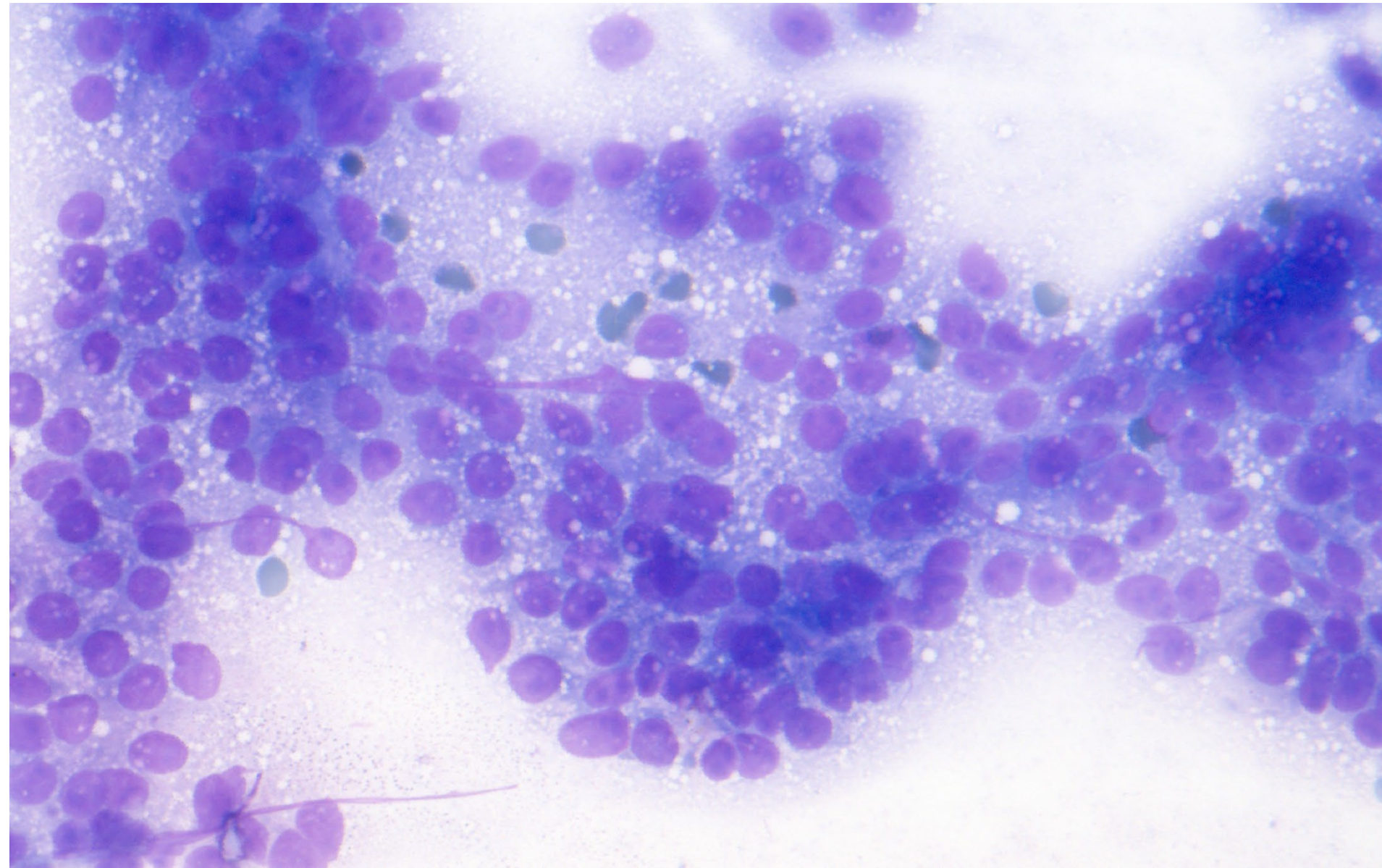


Solid Pseudopapillary Neoplasm Cell Block

Beta-catenin
Nuclear + cytoplasmic



Acinar Cell Carcinoma



High
cellularity

Round nuclei,
prominent
nucleoli

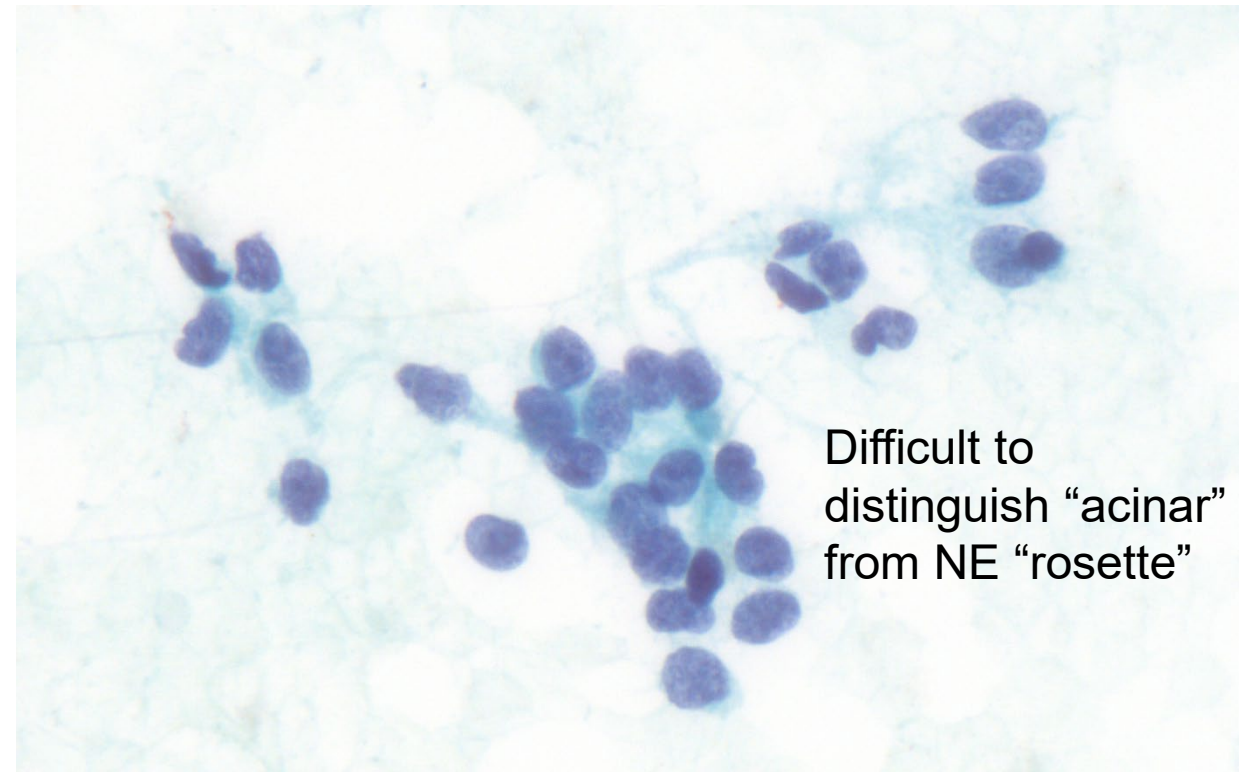
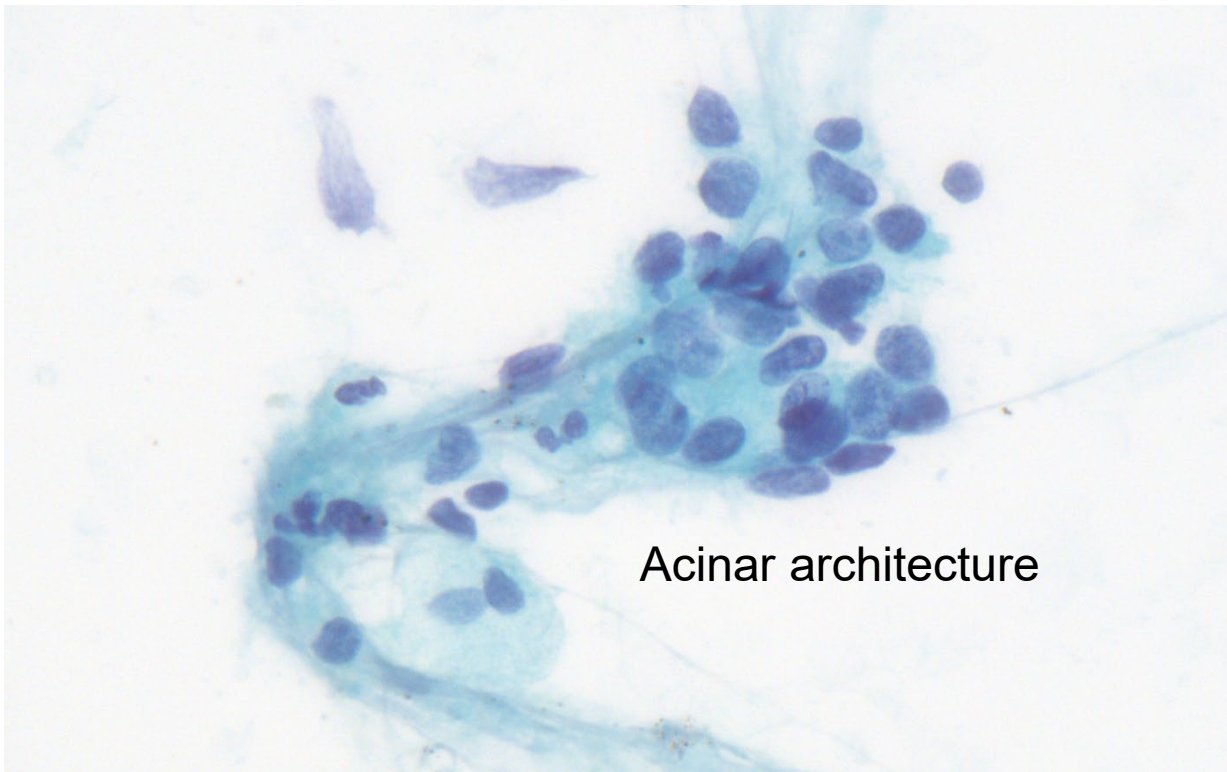
Moderate
cytoplasm

Loosely
cohesive

Mitosis and
necrosis

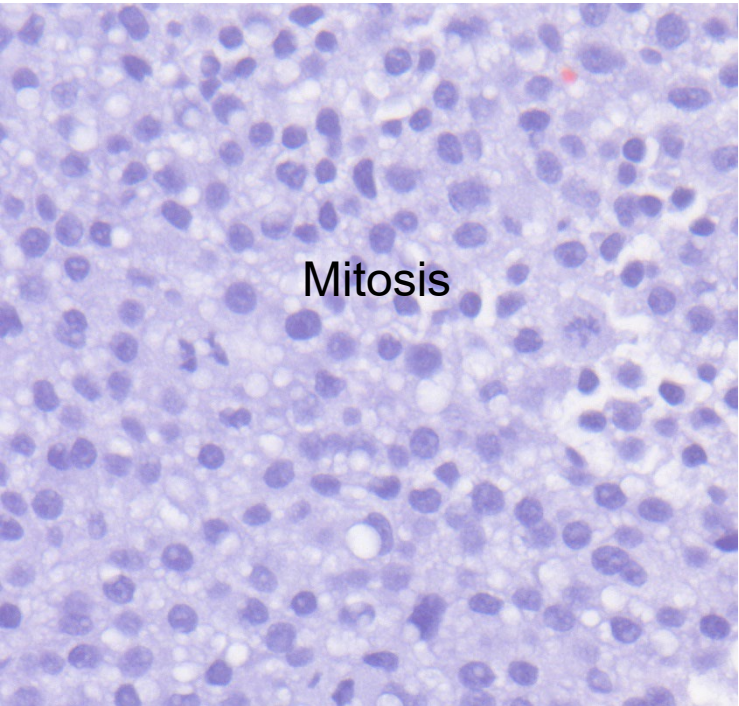
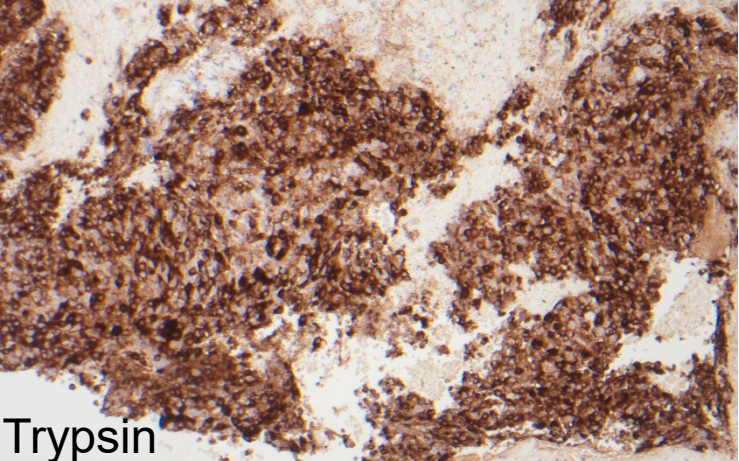
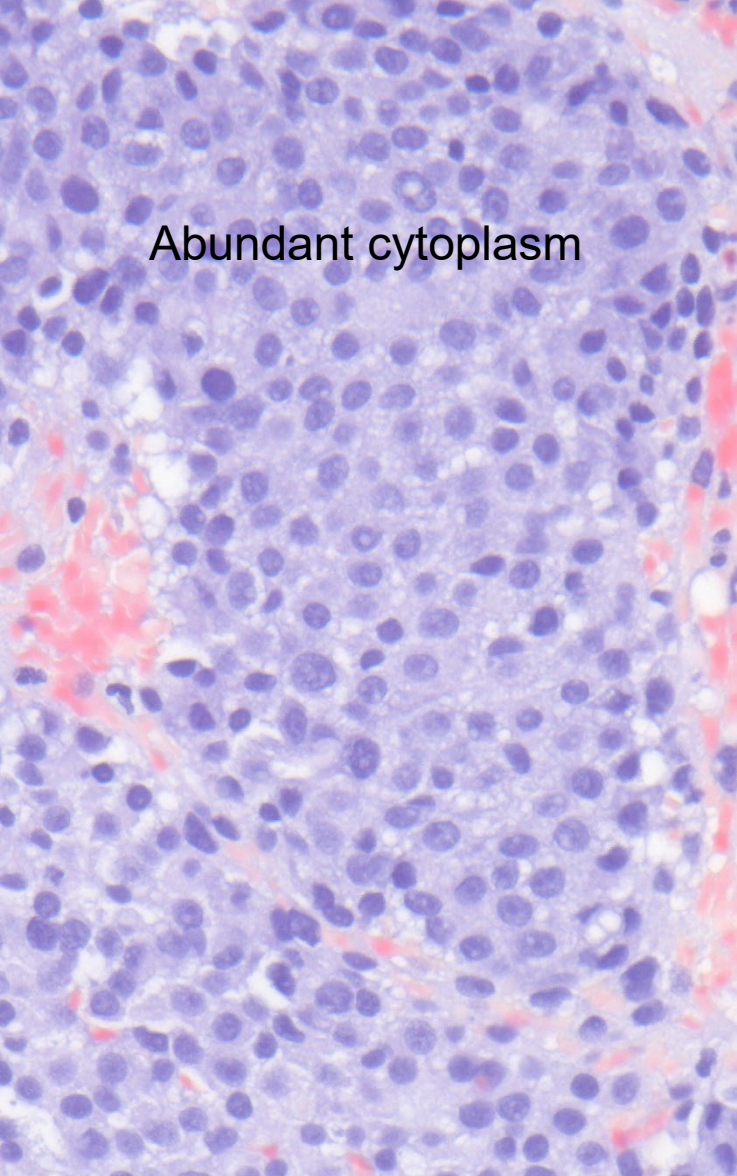
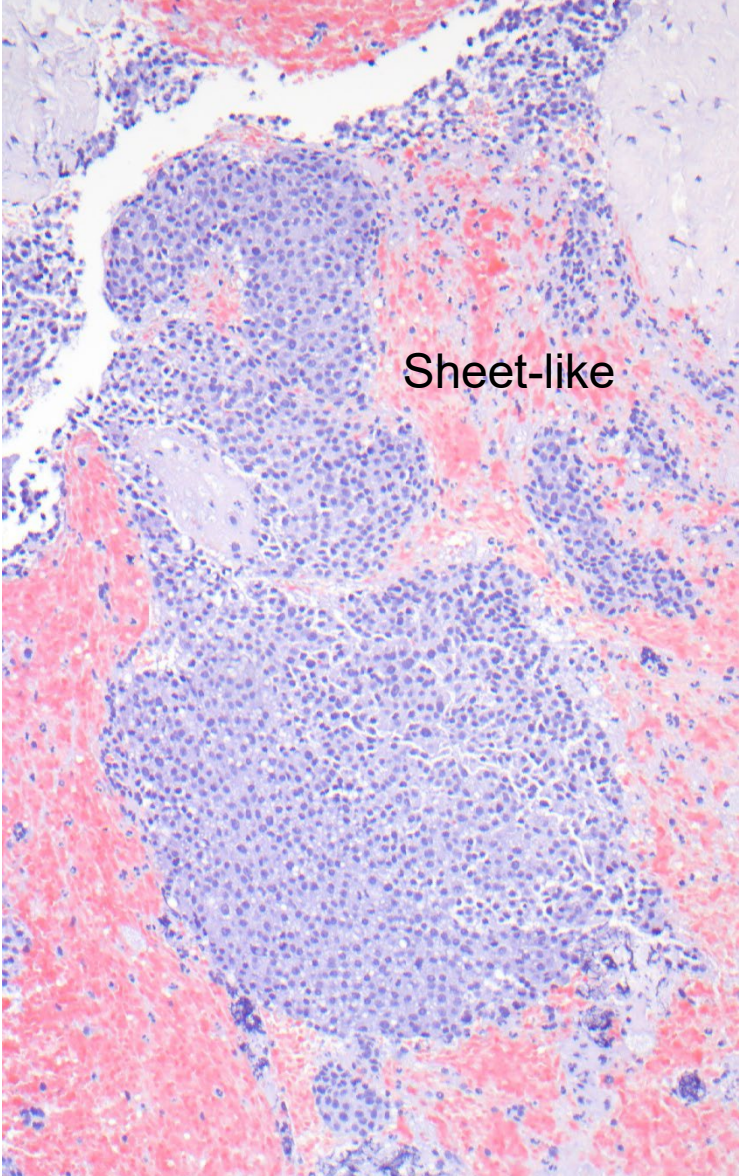
Pap Stain of Acinar Cell Carcinoma

Deceptively bland cytology with uniform nuclei and frequent nucleoli



Chromatin is subtly different from neuroendocrine tumor; usually stains are needed on cell block

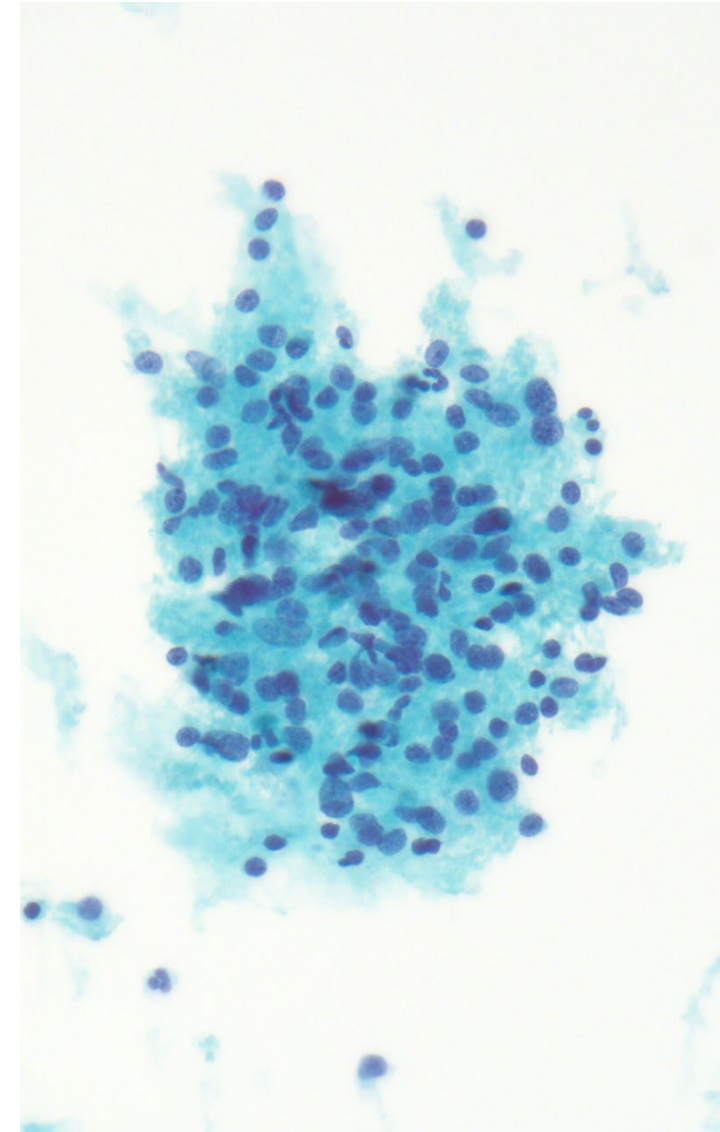
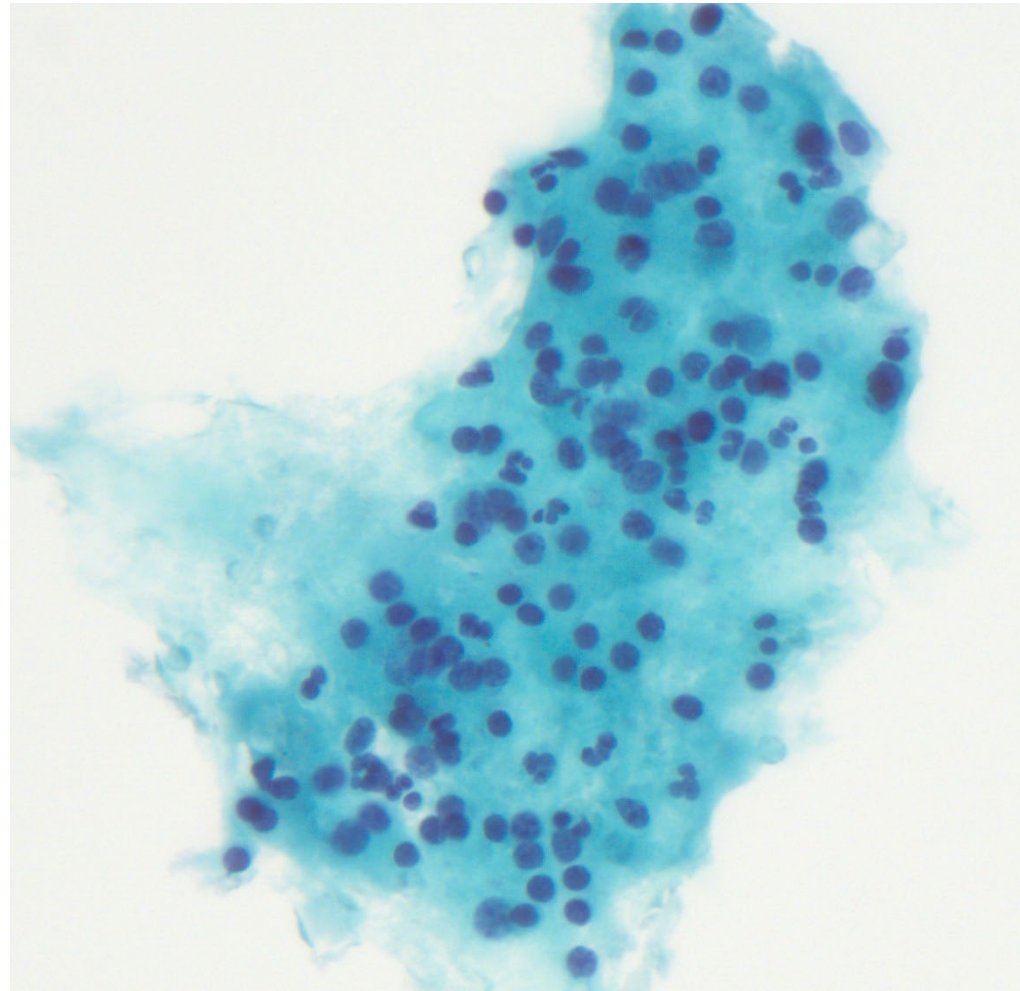
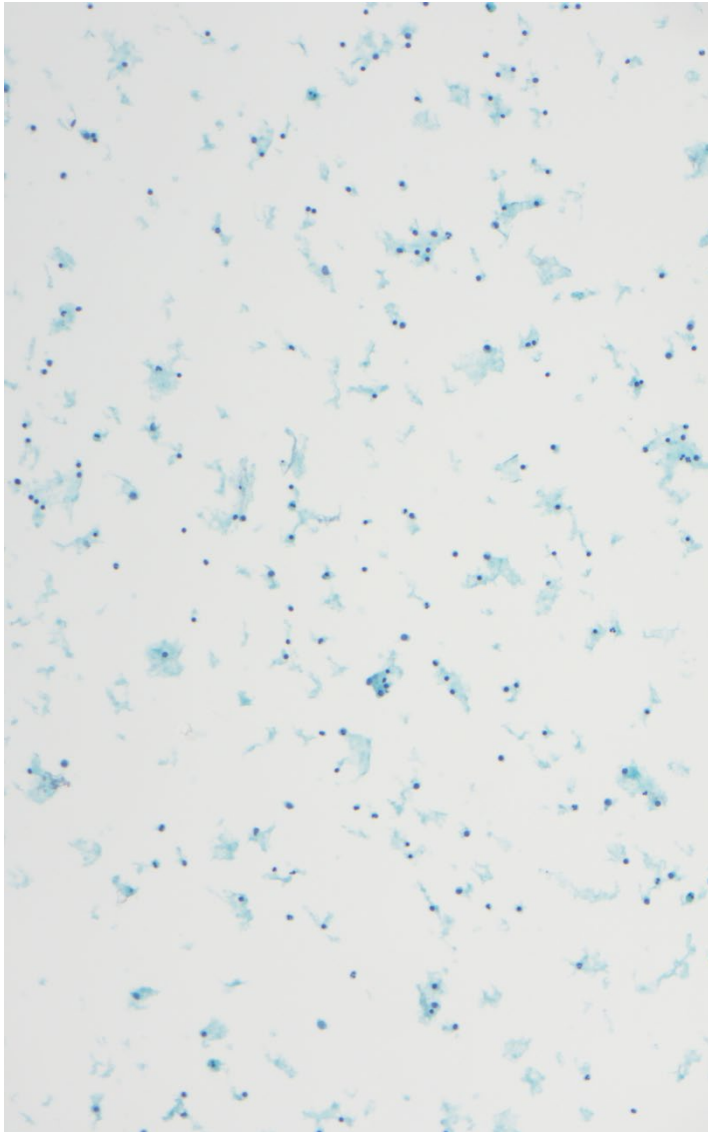
Acinar Cell Carcinoma Cell Block



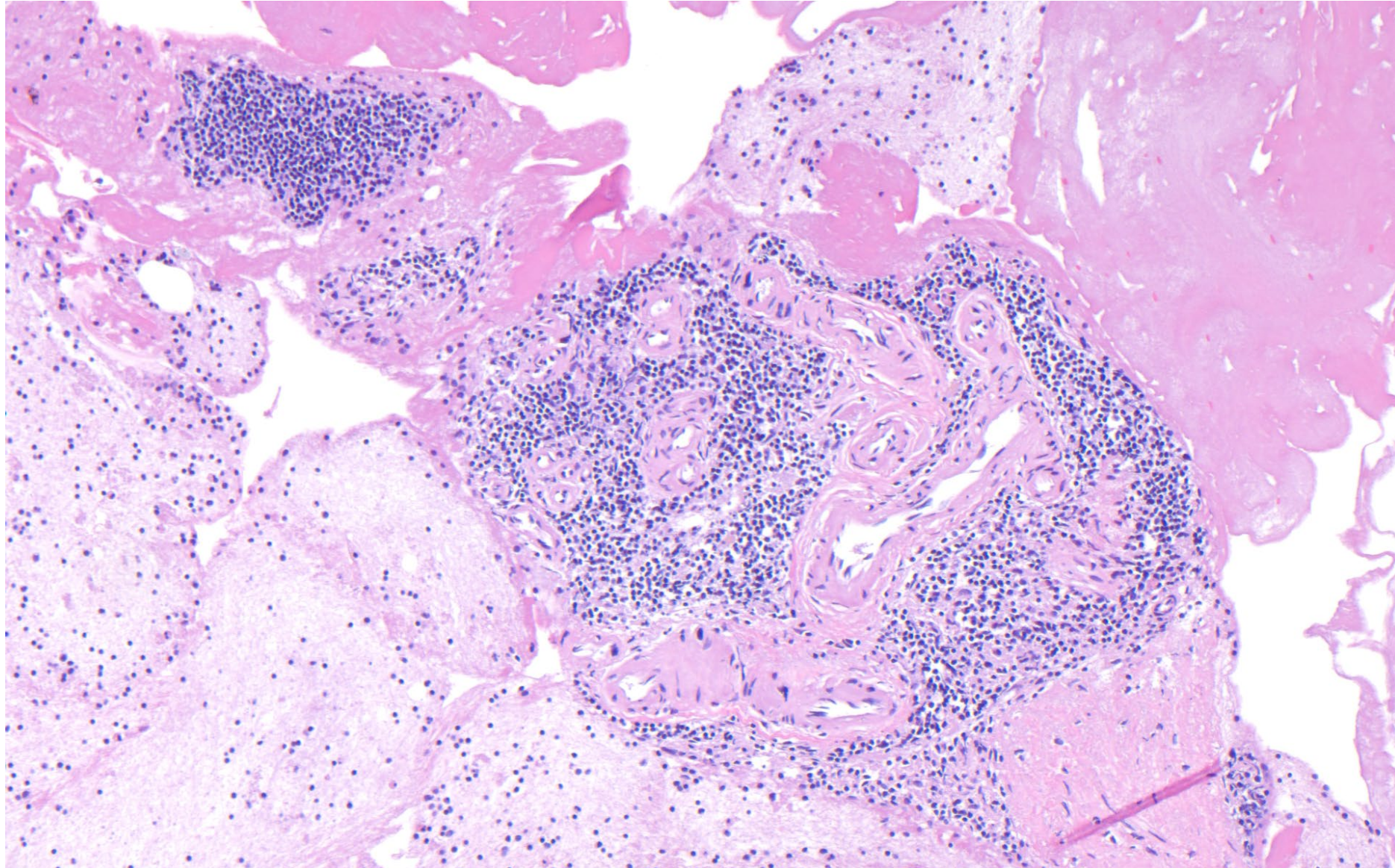
Work-up for Non-Ductal Solid Tumors

Tumor Type	Best Immunohistochemistry	Additional Issues
Well-differentiated Neuroendocrine Tumor	Synaptophysin, Chromogranin, INSM-1	Needs Ki-67 (Mib-1) count for grading
Solid Pseudopapillary Neoplasm	Beta-catenin	May stain with Synaptophysin
Acinar Cell Carcinoma	Trypsin	May be mixed with neuroendocrine tumor

Splenule – The Sore Thumb



Splenule Cell Block



- Surprisingly problematic
- Often not initially considered by radiologist or gastroenterologist
 - Frequently confused with neuroendocrine tumor
- Think splenule if:
 - Lack of epithelial cells in pancreas tail “solid tumor”
 - Many inflammatory cells in background
 - Germinal center-like groups
 - Prominent vascularity in cell block

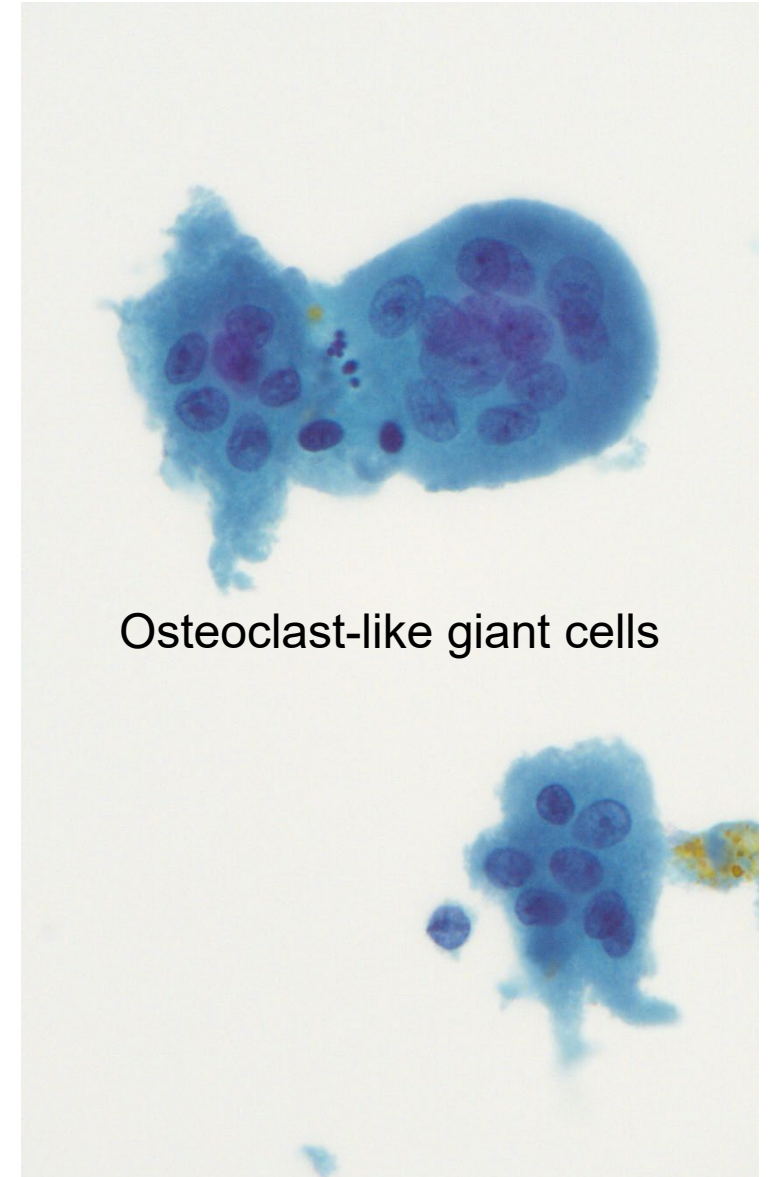
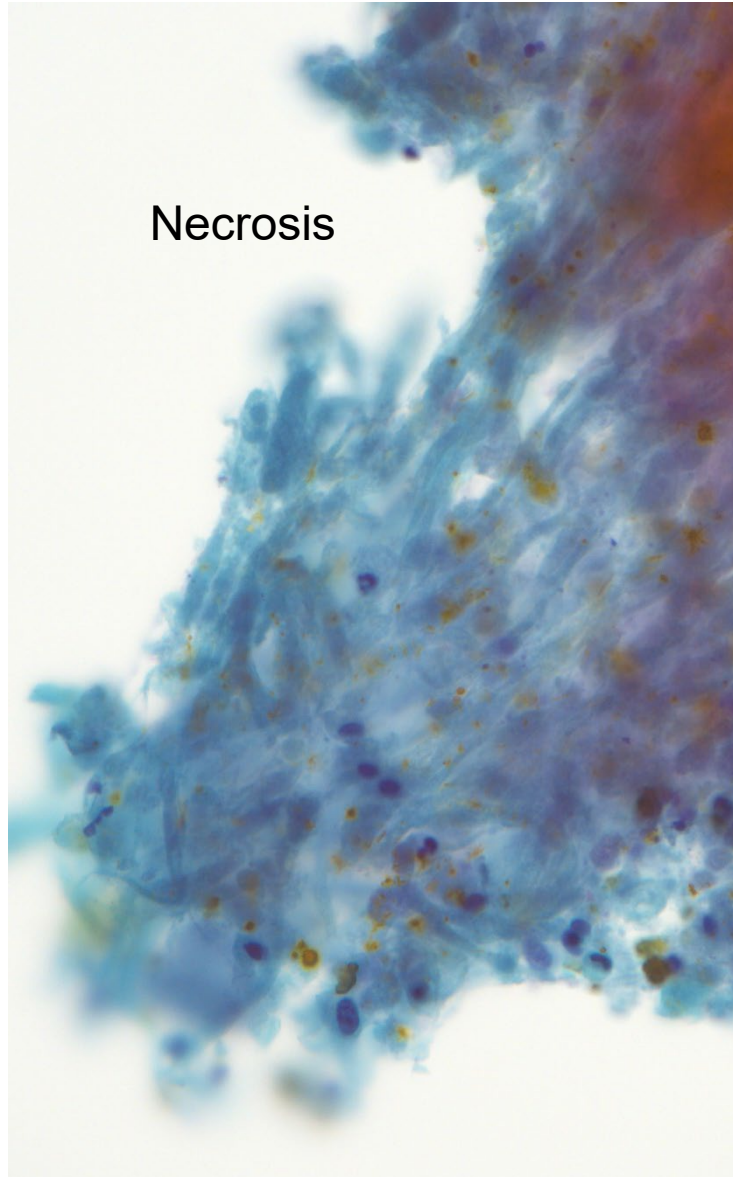
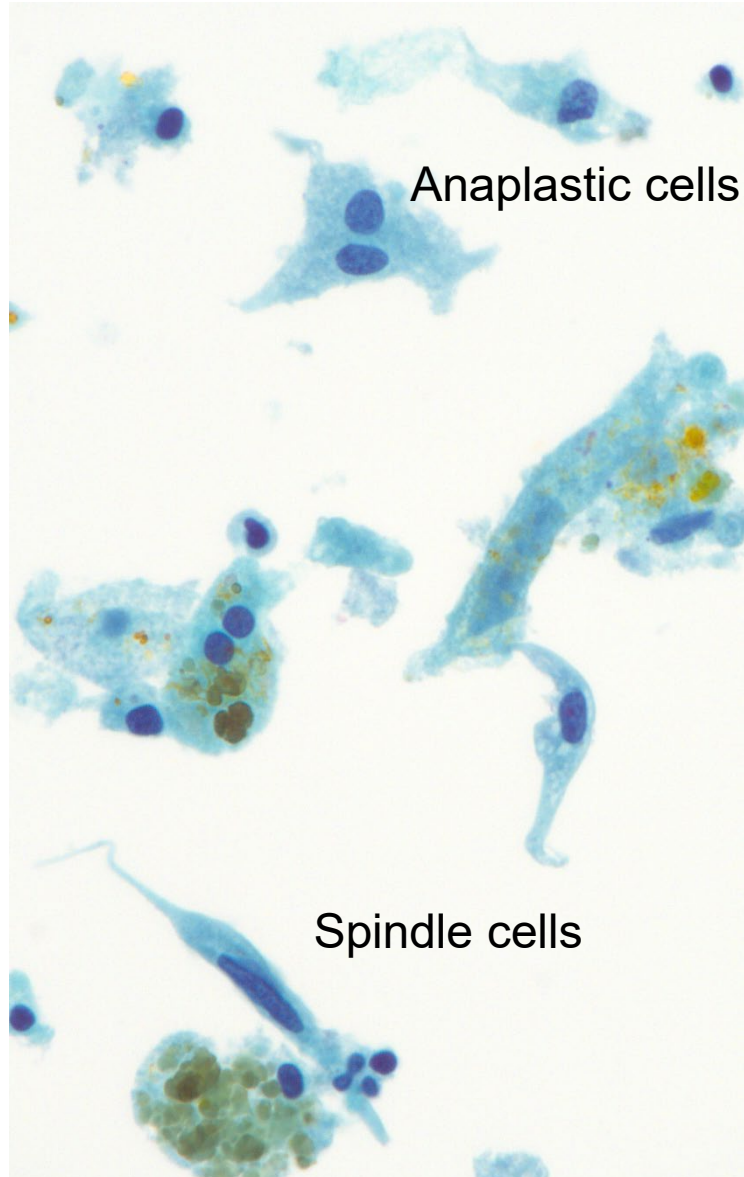
- High-grade / undifferentiated carcinomas
 - Undifferentiated carcinoma (anaplastic or sarcomatoid)
 - Neuroendocrine carcinoma
- Metastasis
 - Renal cell carcinoma most common
- Pediatric tumors
 - Pancreatoblastoma
 - Multicystic adenomatoid hamartoma
- Solid forms of usually cystic tumors
 - Serous cystadenoma
 - Intraductal papillary neoplasms

> [Diagn Cytopathol.](#) 2014 Sep;42(9):738-43. doi: 10.1002/dc.23114. Epub 2014 Feb 19.

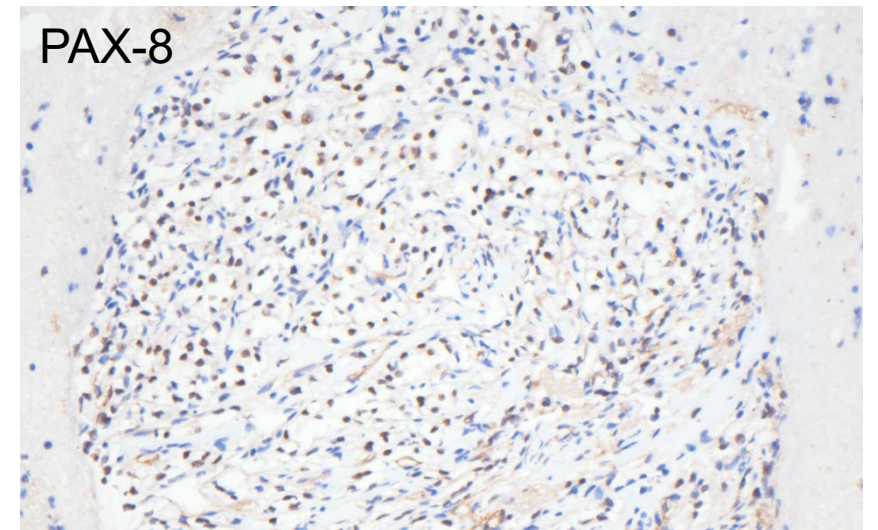
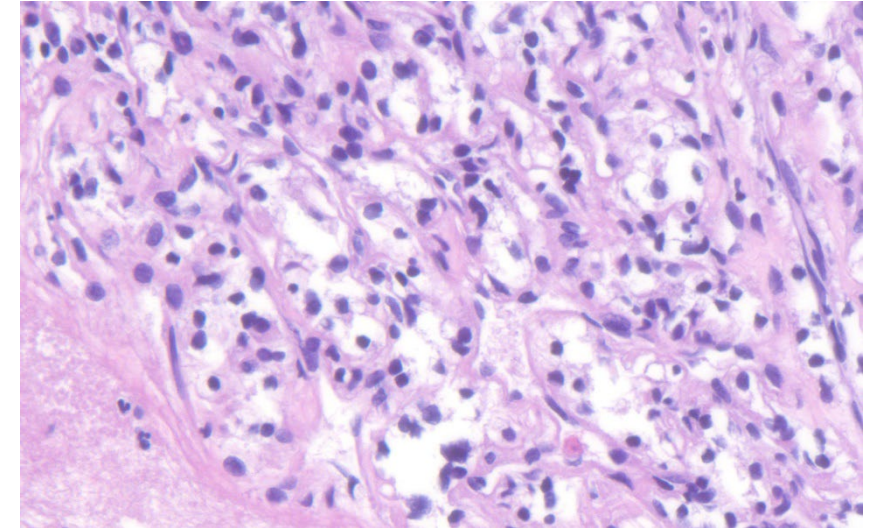
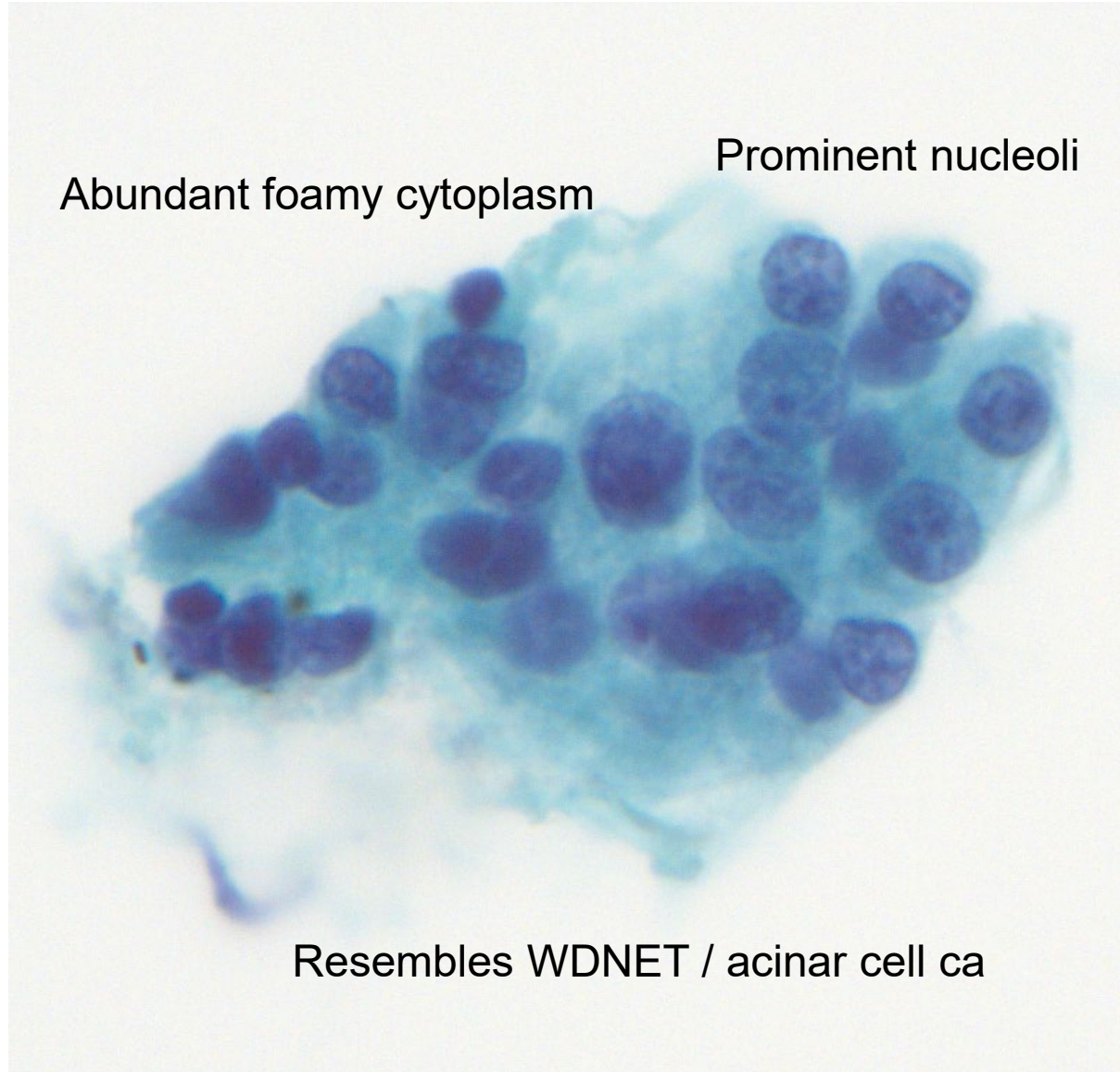
Secondary tumors of the pancreas diagnosed by endoscopic ultrasound-guided fine-needle aspiration: a 10-year experience

[Lindsay Waters](#)¹, [Quisheng Si](#), [Nancy Caraway](#), [Dina Mody](#), [Gregg Staerkel](#), [Nour Sneige](#)

Undifferentiated Carcinoma with Osteoclast-like Giant Cells



Metastatic Renal Cell Carcinoma



Pancreatoblastoma

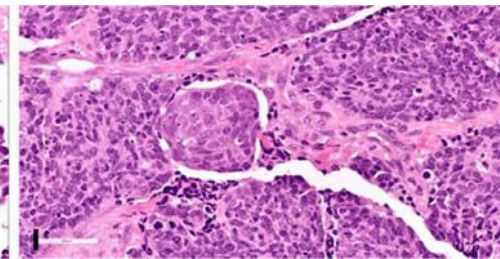
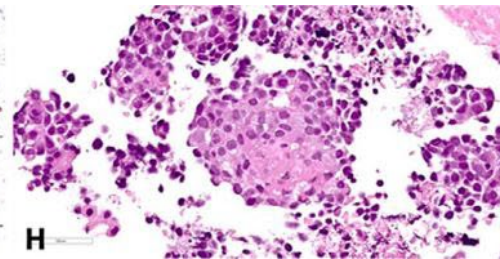
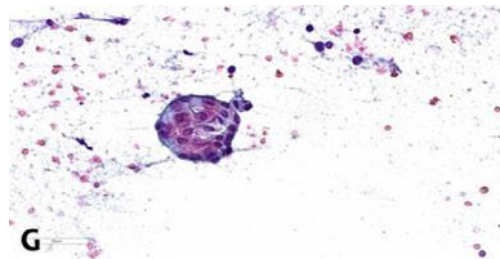
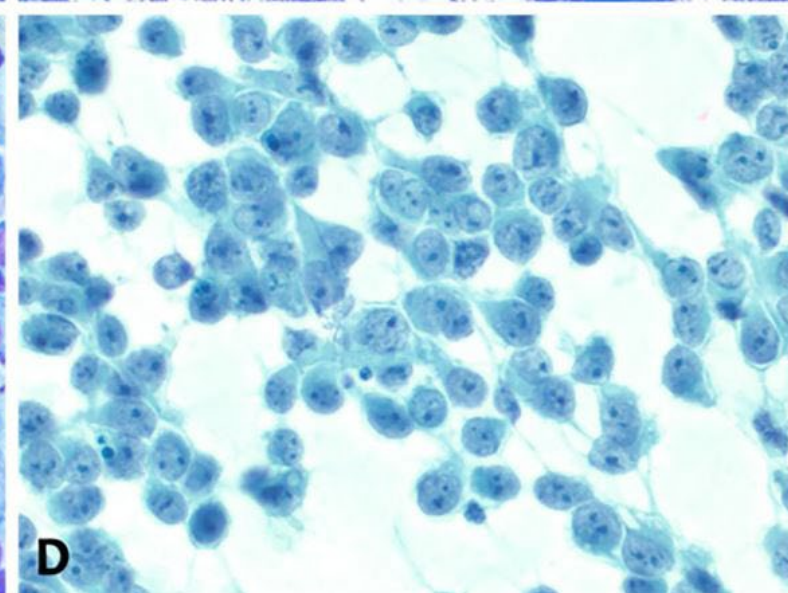
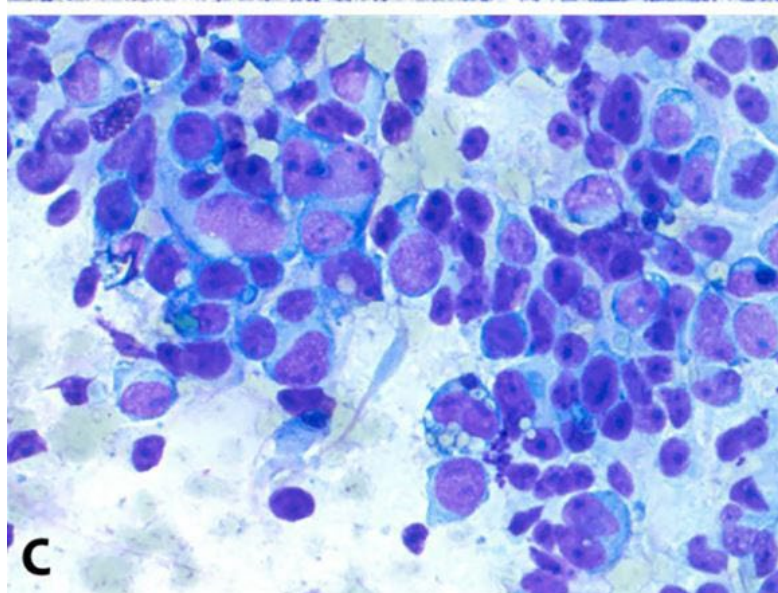
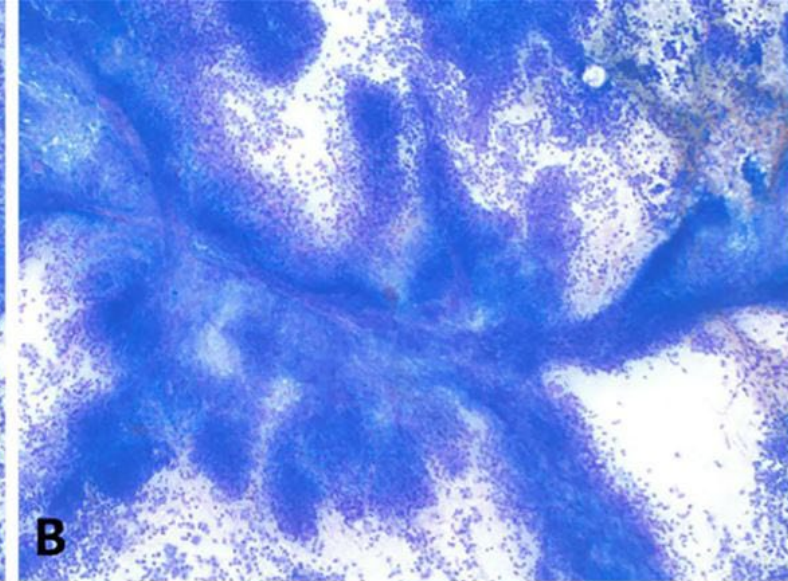
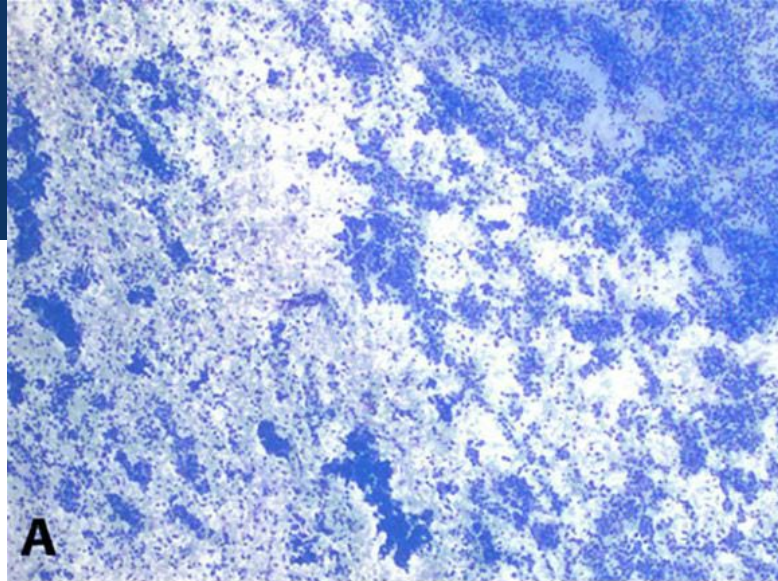
Reid *et al.* Cancer Cytopathol 2019;
127: 708-19.

Hypercellular smears with complex
pseudopapillary 3D architecture

Predominantly primitive blast-like cells
with prominent nucleoli

Intermixed larger squamoid cells, often
forming into morules

Frequently misdiagnosed as
neuroendocrine tumor when
encountered in adults



Pancreatic Cysts

Pancreatic Cysts

Mucinous cysts:

↑ CEA (>192)

↓ Glucose

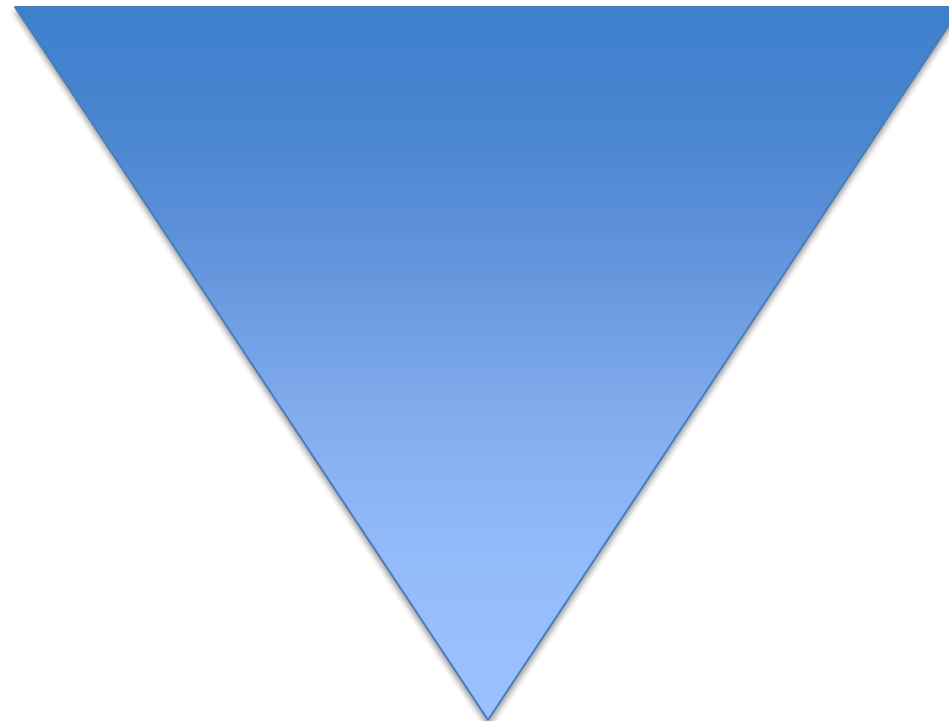
↓ Amylase

Serous cysts:

↓ CEA

↑ Glucose (>50)

↓ Amylase

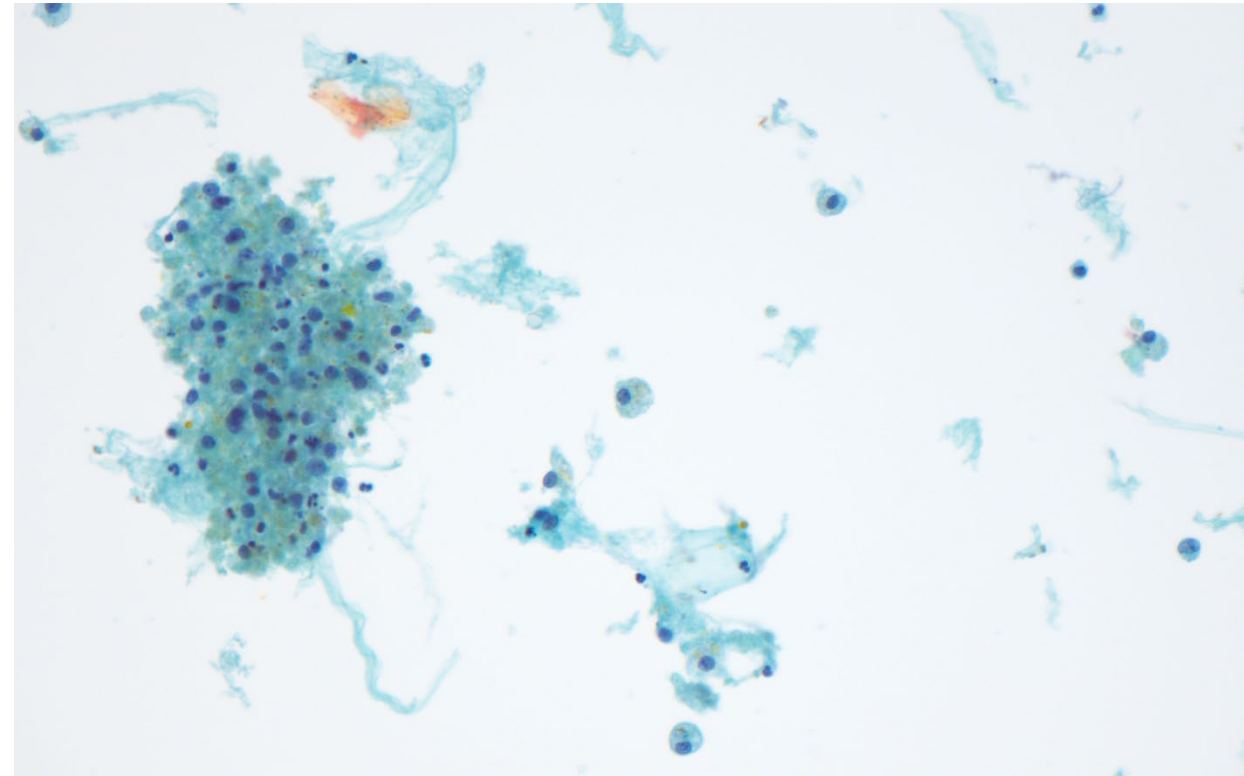
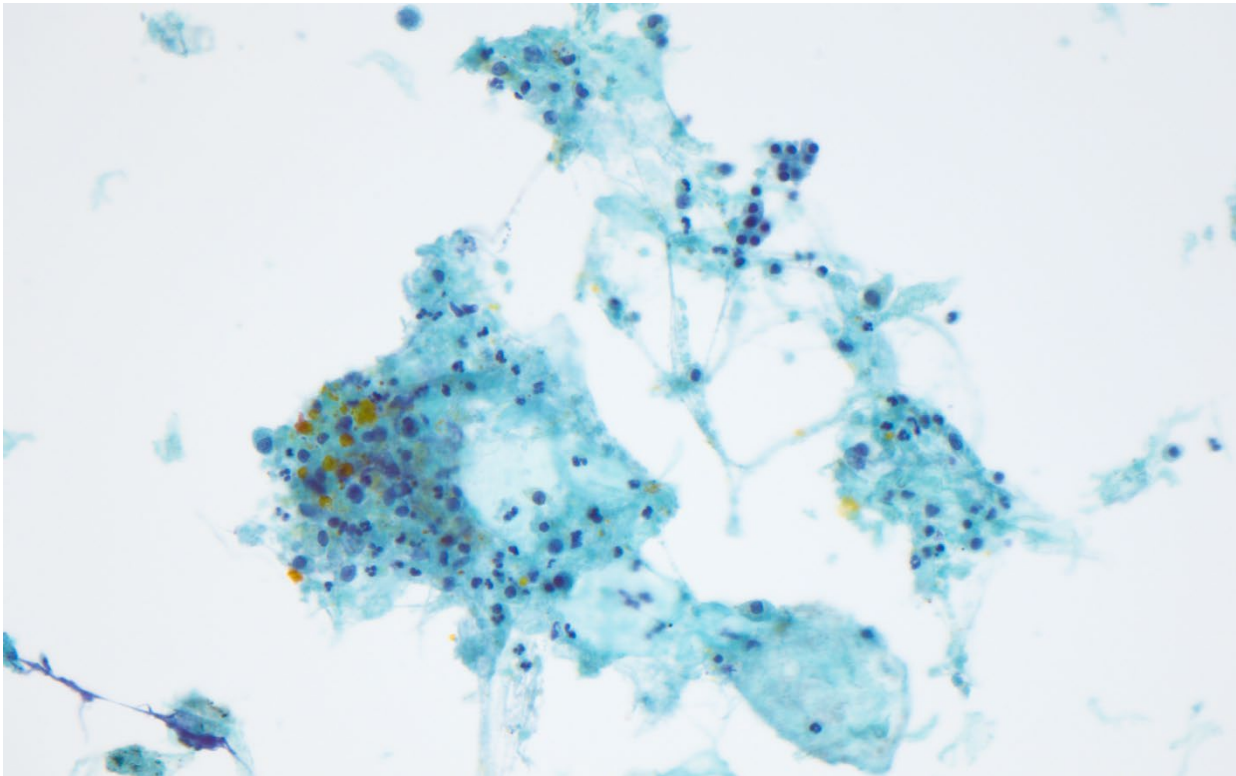


Pseudocysts: ↓ CEA ↓ Glucose ↑ Amylase

Pseudocyst Contents

Acute inflammation and bile

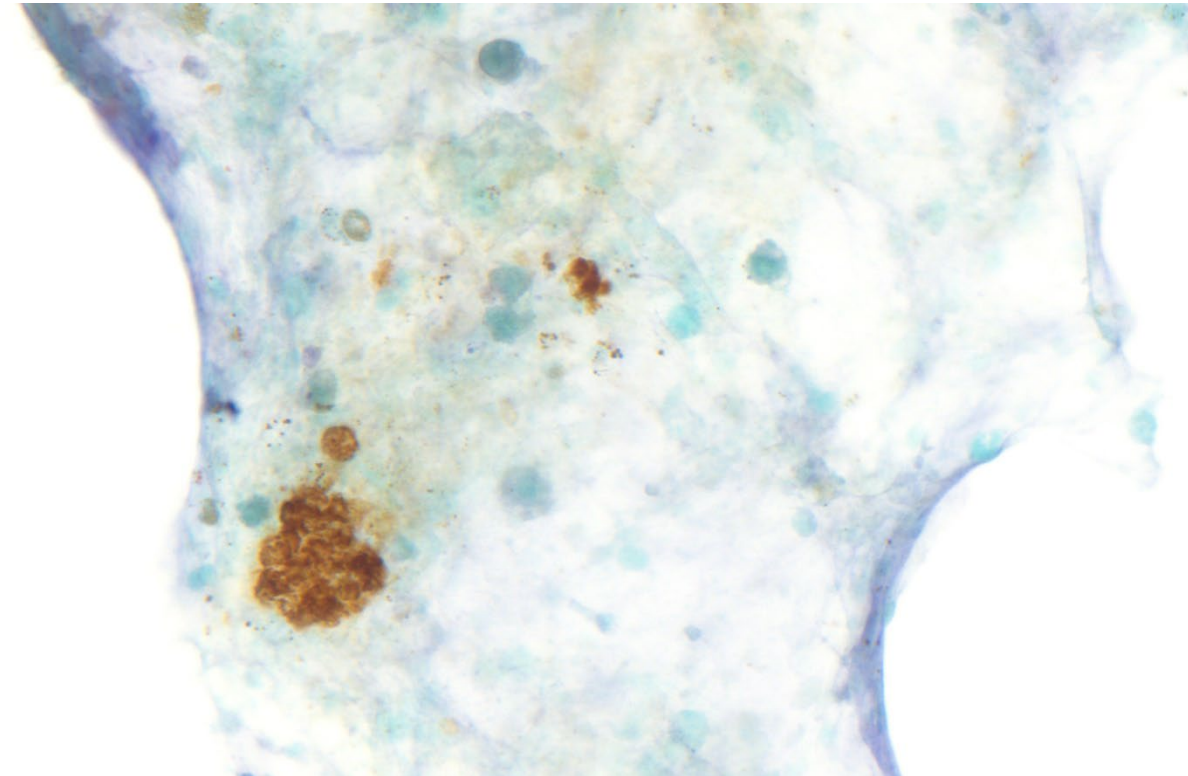
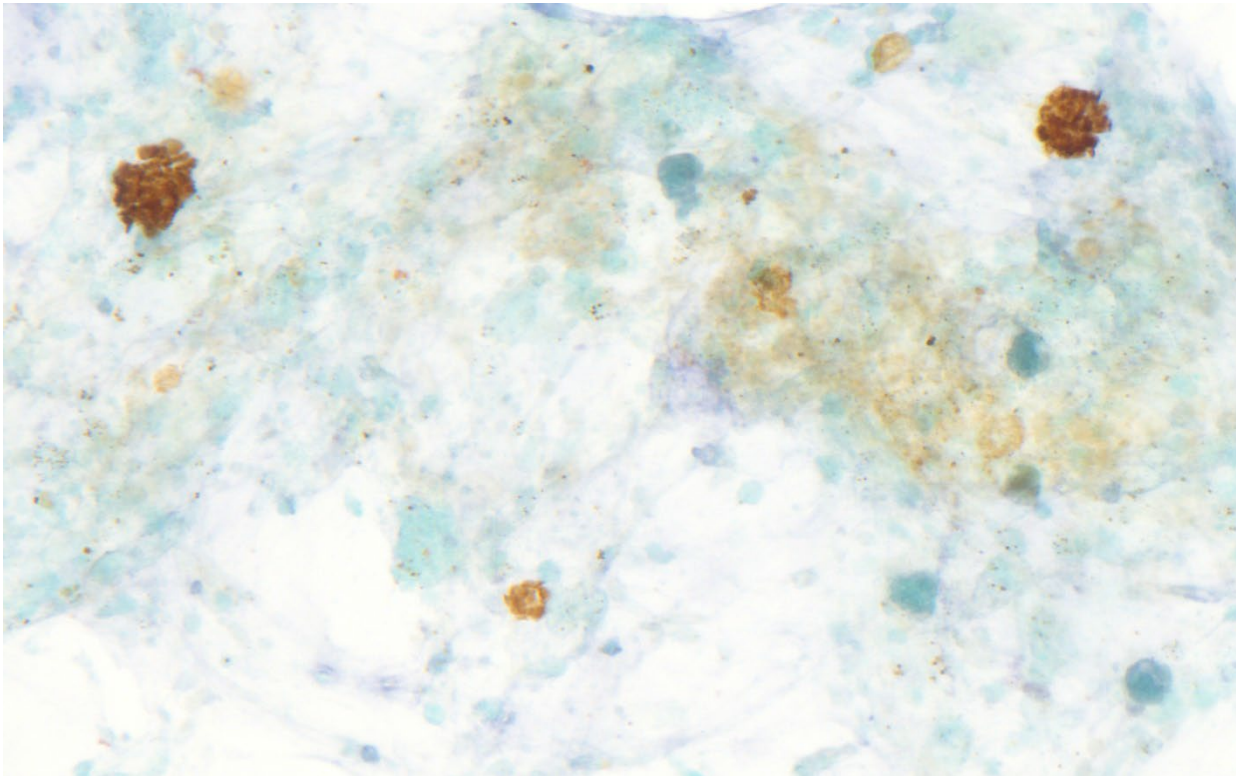
Macrophages



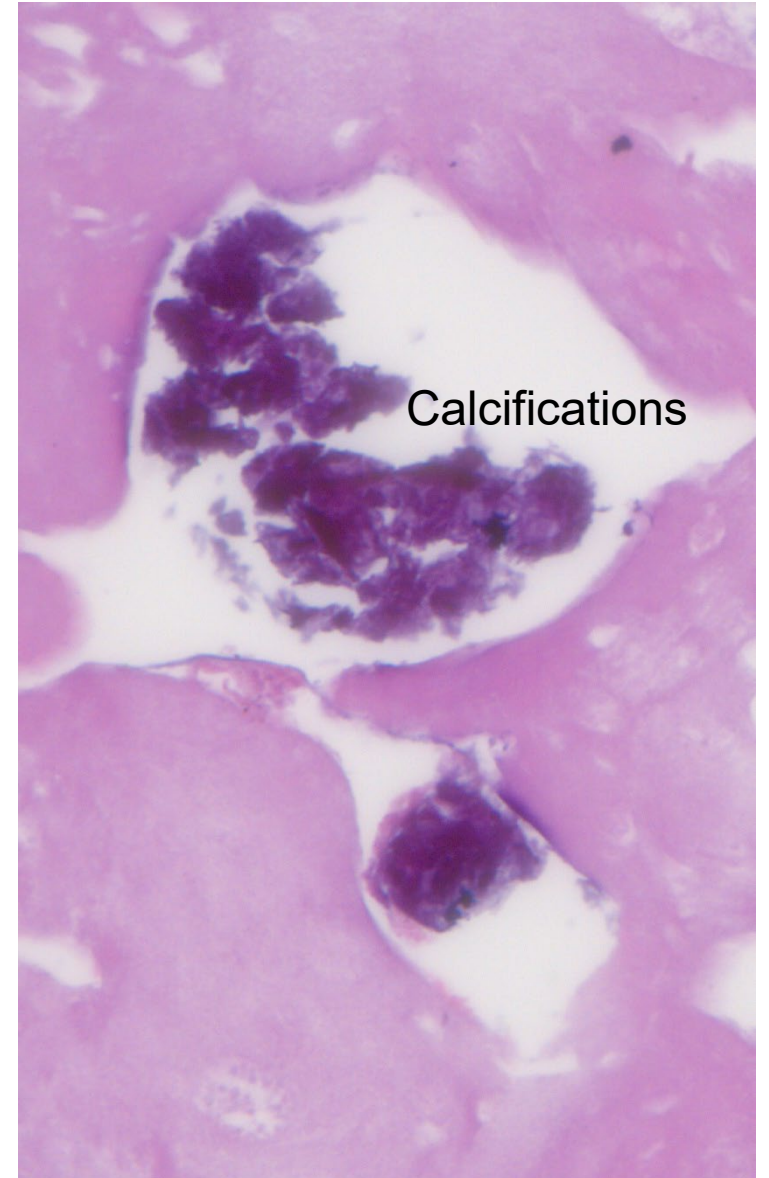
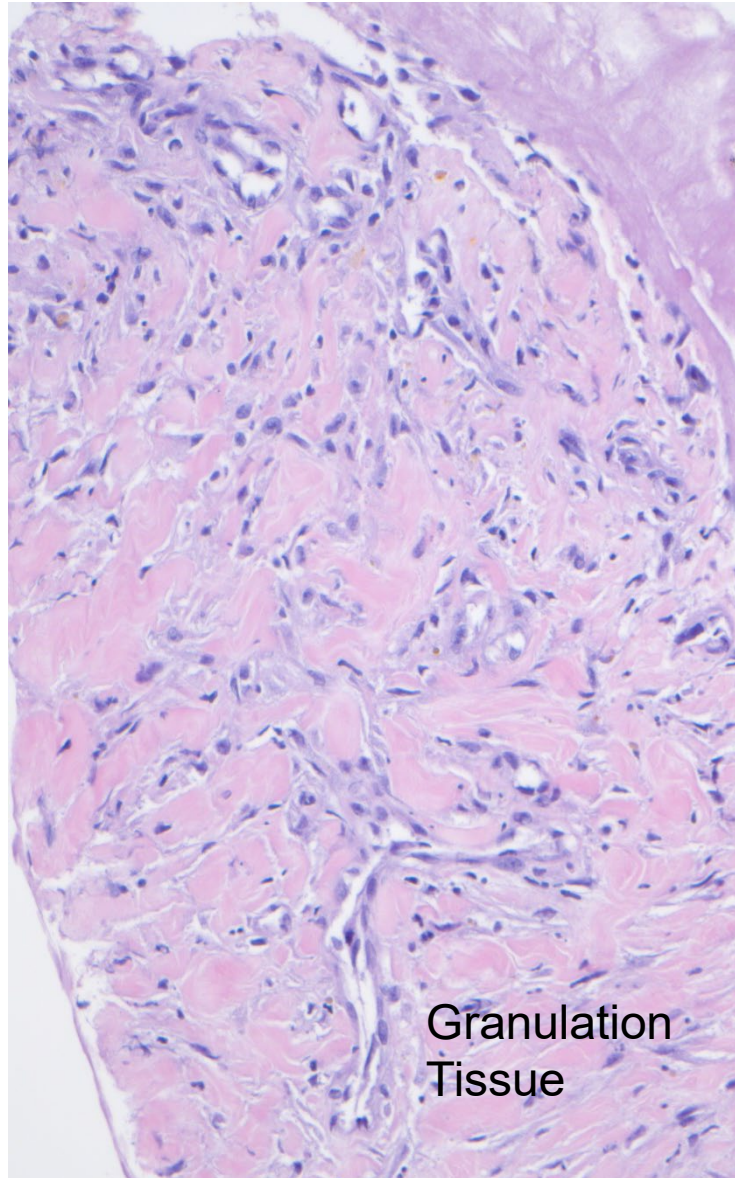
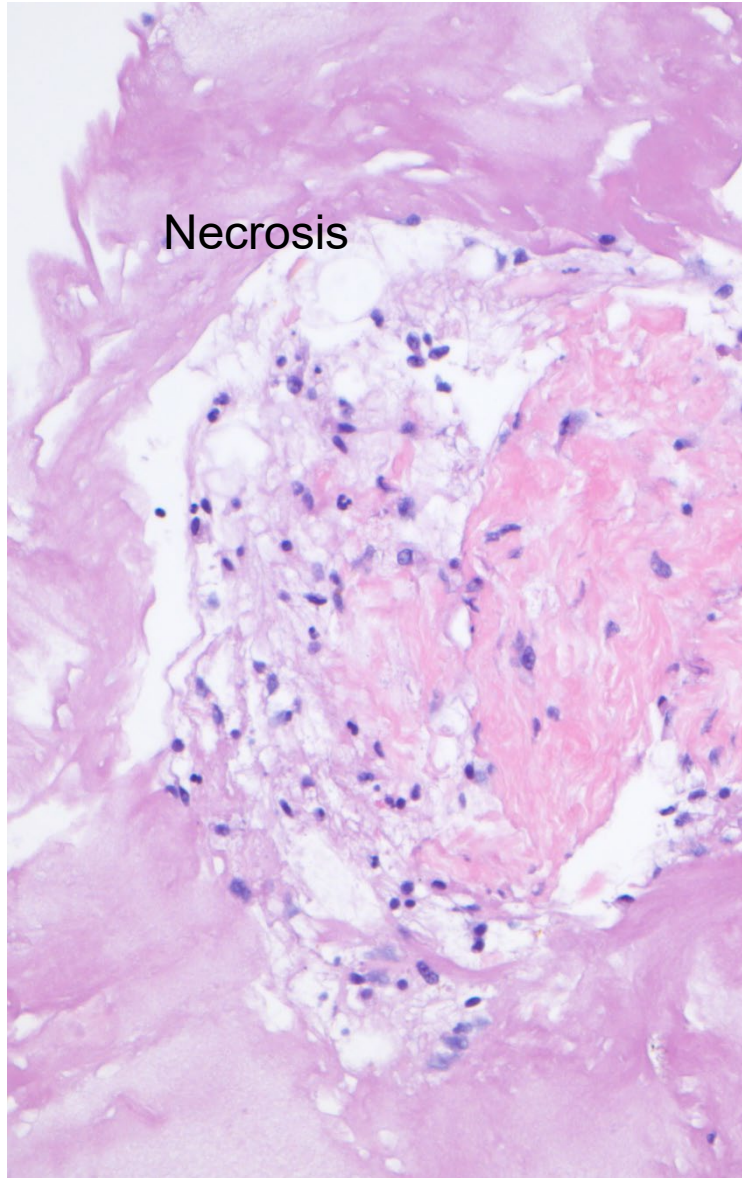
Pseudocyst Post Hemorrhage

Low cellularity, degenerative changes

Hemosiderin and debris



Pseudocyst Cell Block Findings



- Lack cyst lining cells
 - Epithelial elements are contaminants
 - Findings are non-specific
- Necrosis in a neoplasm can look identical
 - Including by radiology
- Clinical / endoscopic impression and chemistries are essential

Mucinous Cysts

- Two main types of mucinous cysts:
 - Intraductal papillary mucinous neoplasm (IPMN)
 - Mucinous cystic neoplasm (MCN)
- IPMN features:
 - Mixed male and female
 - More often in head of pancreas
 - Main duct (higher risk) versus branch duct (lower risk) types
- MCN features:
 - Usually women
 - Usually tail of pancreas
- All have potential to develop into invasive carcinoma

- Usually incidental detection by abdominal CT
- Treatment is complicated by the difficulty of pancreas surgery
 - Most have low malignant potential
 - How to monitor / manage is a dilemma
- Confirming mucinous cyst is the first step
 - Cytology and cyst fluid chemistries are a key component
 - Often low cellularity with non-specific cytology

Guidelines for Surveillance and Surgery

Surveillance of low-risk pancreatic cystic lesions

AGA 2015	<1 cm: MRI/CT at 1 y then every 2 y × 5 y
IAP 2017 (BD-IPMN only)	<1 cm: MRI/CT at 6 mo then every 2 y 1–2 cm: MRI/CT every 6 mo × 1 y, then every 1 y × 2 y, then every 2 y 2–3 cm: EUS in 3–6 mo, then EUS alternate with MRI every 1 y >3 cm: MRI alternate with EUS every 3–6 mo
ACR 2017 (for patients <65 y)	<1.5 cm: MRI/EUS/CT every 1 y × 5 y then every 2 y × 2 1.5–1.9 cm with MPD communication: MRI/CT/EUS every 1 y × 5 then every 2 y × 2 2.0–2.5 cm with MPD communication: MRI/CT/EUS every 6 mo × 4, then every 1 y × 2, then every 2 y × 3 >2.5 cm: MRI/CT/EUS every 6 mo × 4 then every 1y × 2 then every 2y × 3
ACG 2018	<1 cm: MRI every 2 y × 4 y 1–2 cm: MRI every 1 y × 3 y, then MRI every 2 y × 4 y 2–3 cm: MRI or EUS every 6–12 mo × 3 y, then MRI every 1 y × 4 y >3 cm: MRI alternate with EUS every 6 mo × 3 y, then MRI alternate with EUS every 1y × 4y
European 2018	MRI ± EUS every 6 mo × 1 y, than every 1 y until nonsurgical candidate

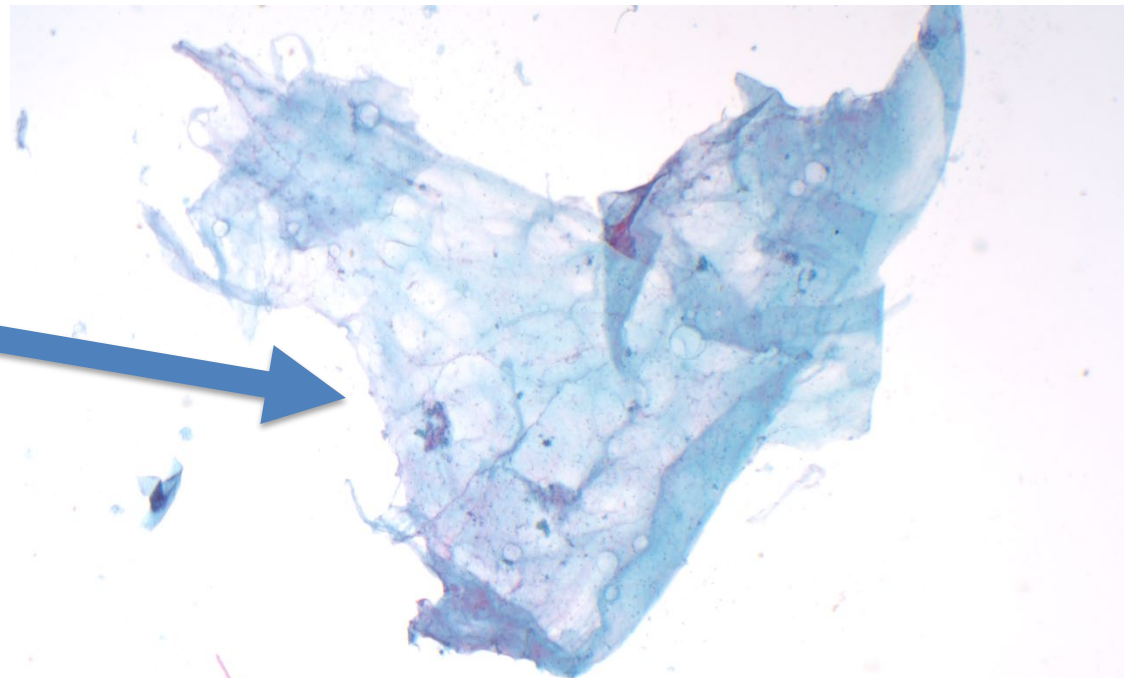
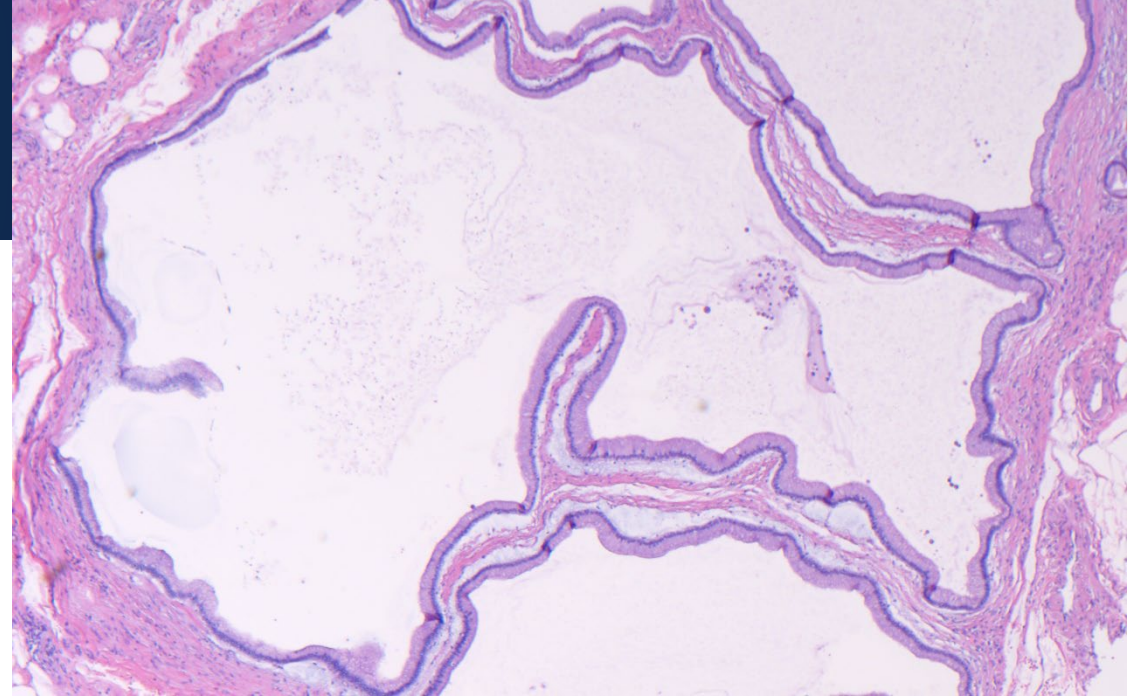
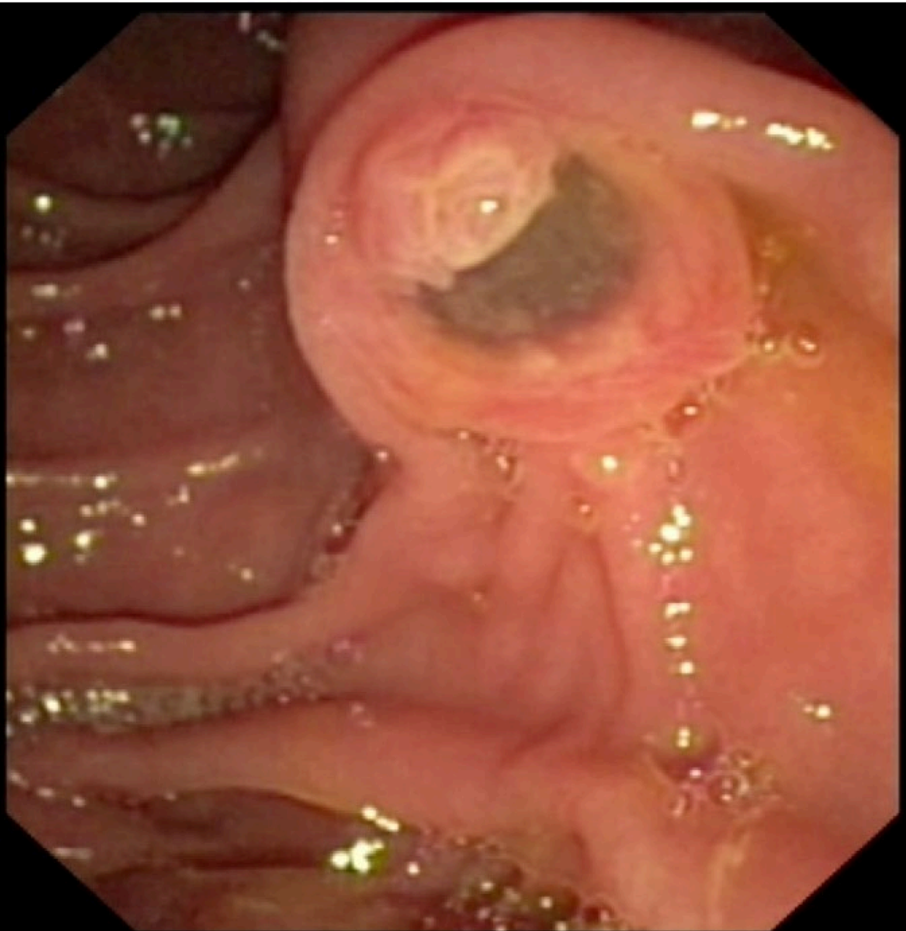
Indications for surgical resection

AGA 2015	Dilated MPD, cyst >3 cm, solid cystic component, worrisome cytology
IAP 2017	BD-IPMN with MPD >10 mm or MD involvement, jaundice, mural nodule >5 cm, worrisome cytology MD-IPMN with MPD >10 mm, jaundice, mural nodule, worrisome cytology
ACR 2017 (for patients <65 y)	Worrisome features cyst diameter >3 cm, thickened cyst wall, nonenhancing mural nodule, MPD >7 cm High-risk stigmata jaundice, enhancing solid component, MPD <10 mm, worrisome cytology
ACG 2018	New diabetes, jaundice, acute pancreatitis due to cyst, elevated carbohydrate antigen 19-9, cyst growth >3 mm/y, mural nodule, MPD dilation >5 mm, focal dilation of MPD, IPMN or MCN >3 cm, worrisome cytology
European 2018	Relative growth rate >5 mm/y, carbohydrate antigen 19-9 >37 U/mL. MPD 5–9 mm, cyst >4 cm new onset diabetes, acute pancreatitis, enhancing nodule <5 mm Absolute indications MPD >10 mm, worrisome cytology, solid mass, jaundice, enhancing nodule >5 mm

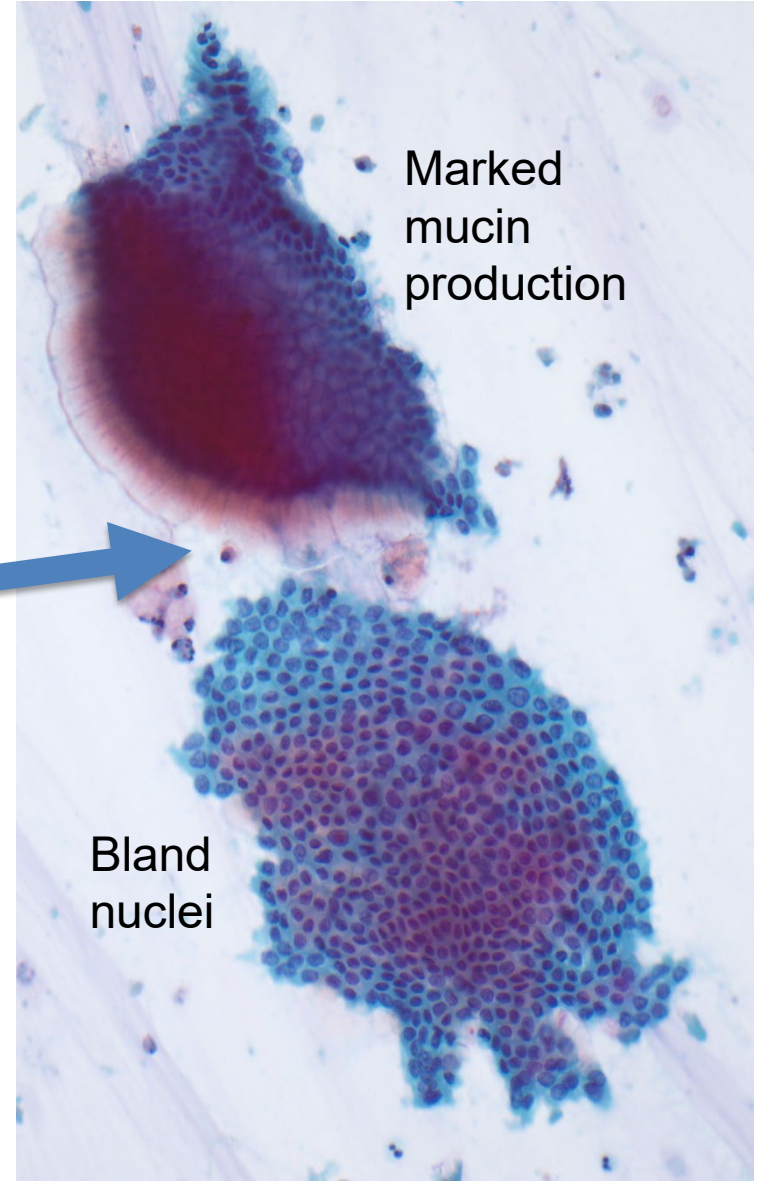
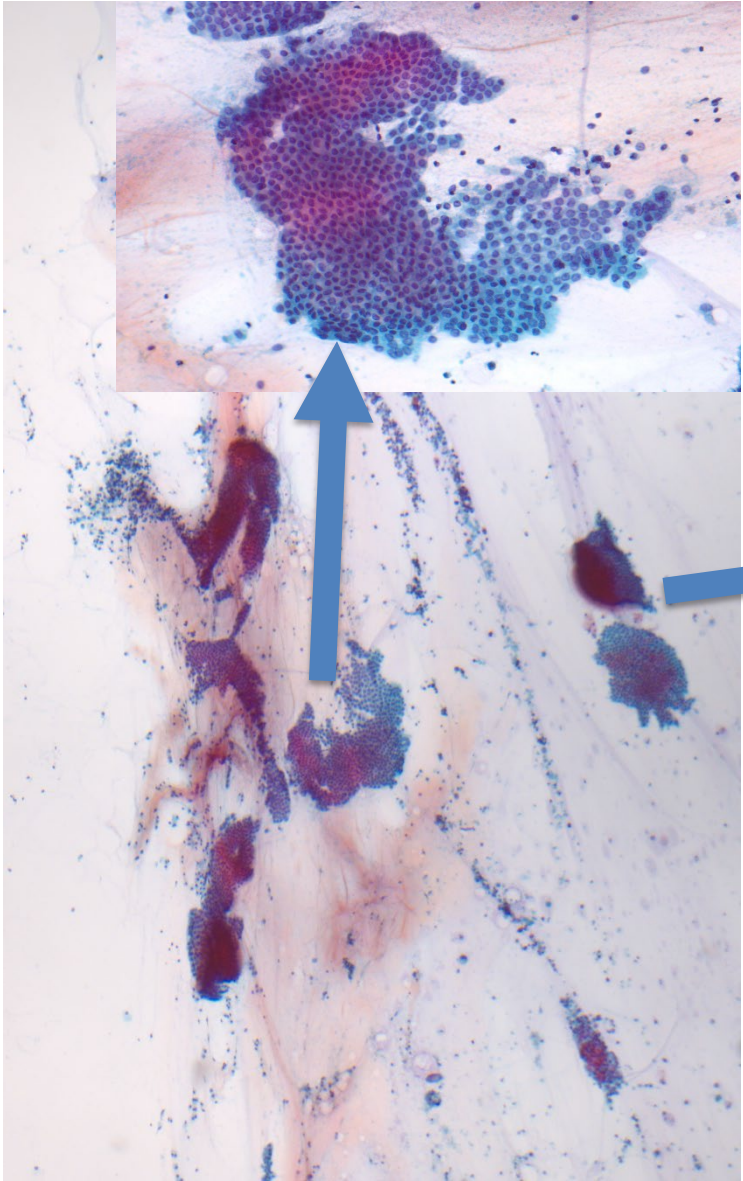
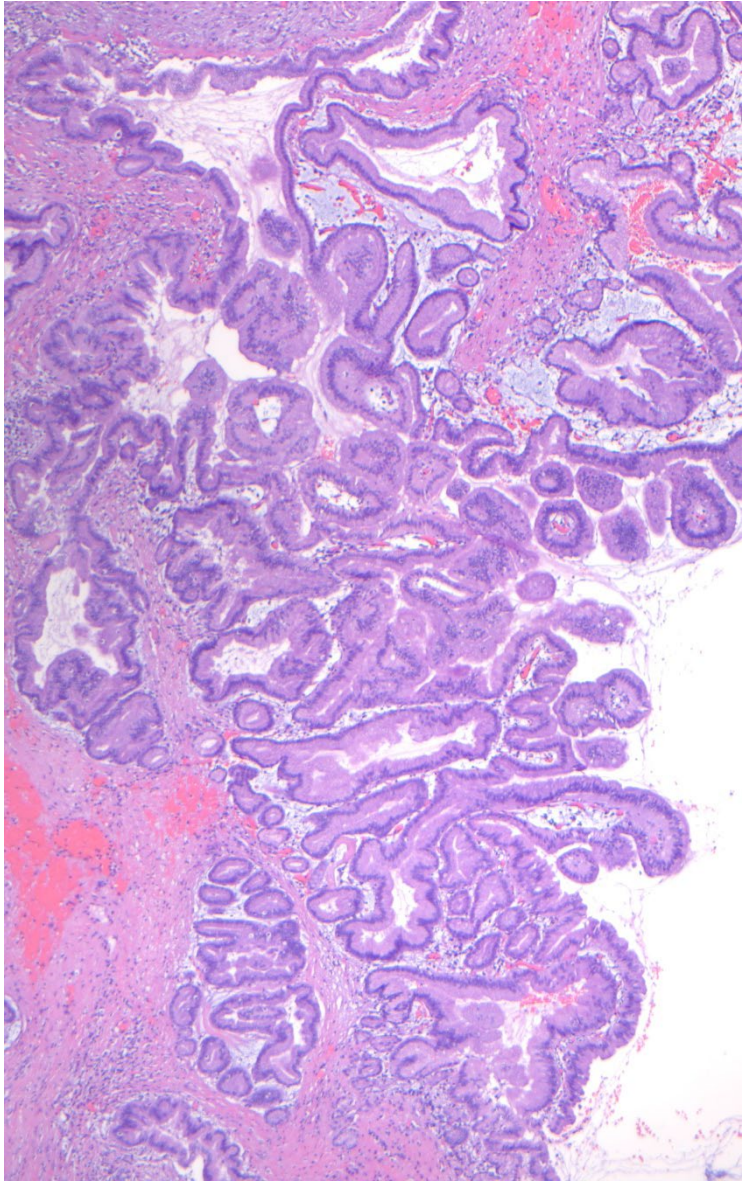
- Pan-Low (Pancreaticobiliary neoplasm, low-risk/grade)
 - Includes mucin-only samples or elevated CEA (>192)
 - Bland mucinous epithelial elements
 - Cells the size of duodenal enterocytes
 - Polarized nuclei
 - Intranuclear inclusions may be seen
 - Smooth nuclear contours and even chromatin
 - Absent or inconspicuous nucleoli
- Pan-High (Pancreaticobiliary neoplasm, high-risk/grade)
 - High-grade epithelial elements: dysplasia vs. invasive carcinoma

Abundant Mucin

“Fish mouth” ampulla



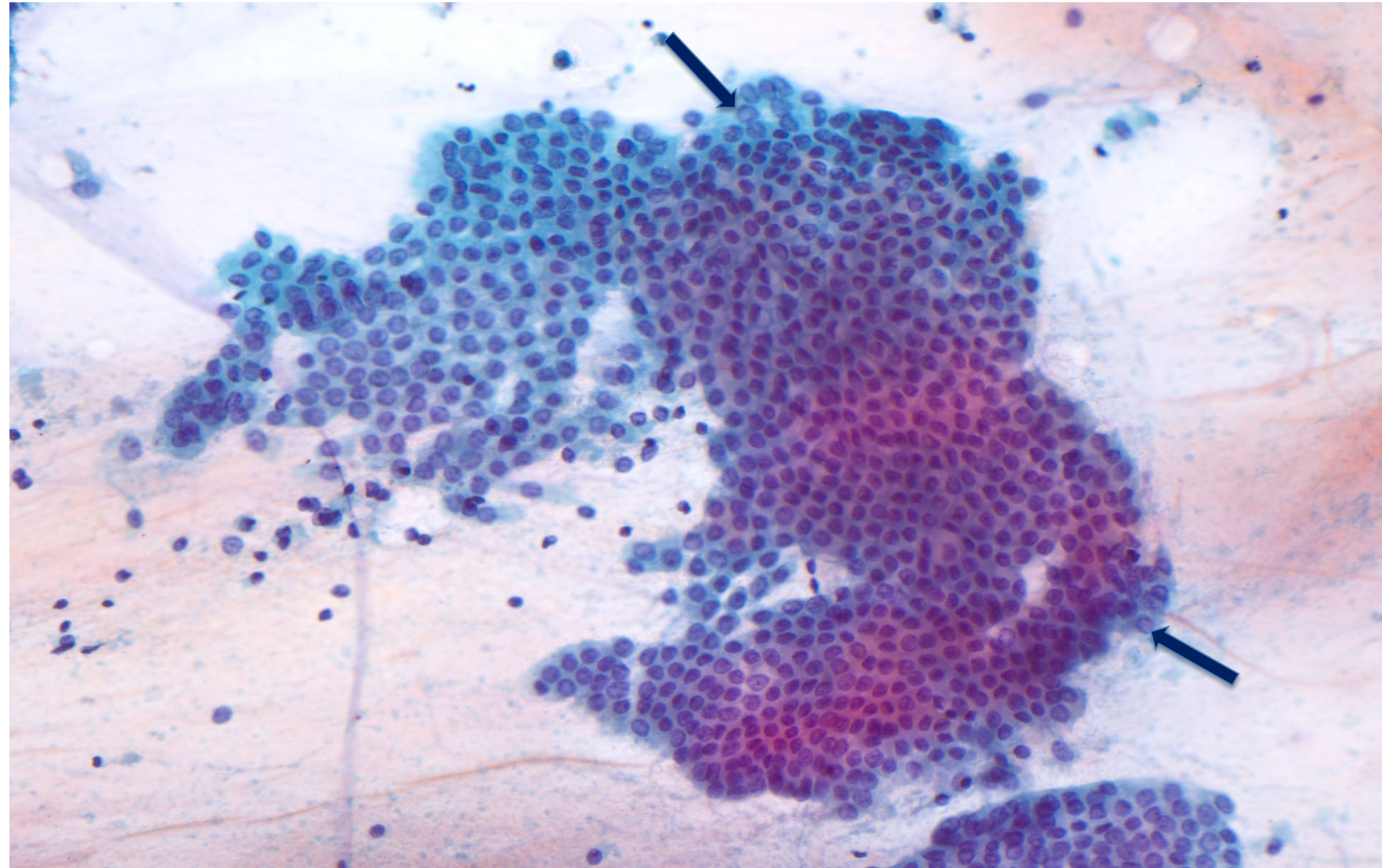
High Cellularity IPMN (↑ Risk)



Marked
mucin
production

Bland
nuclei

IPMN Nuclear Features



Occasional
pseudo-
inclusions

Uniform
nuclear size
and
chromatin

Mild nuclear
contour
irregularities

Gastric-Type IPMN Vs Normal Foveolar Mucosa

Normal

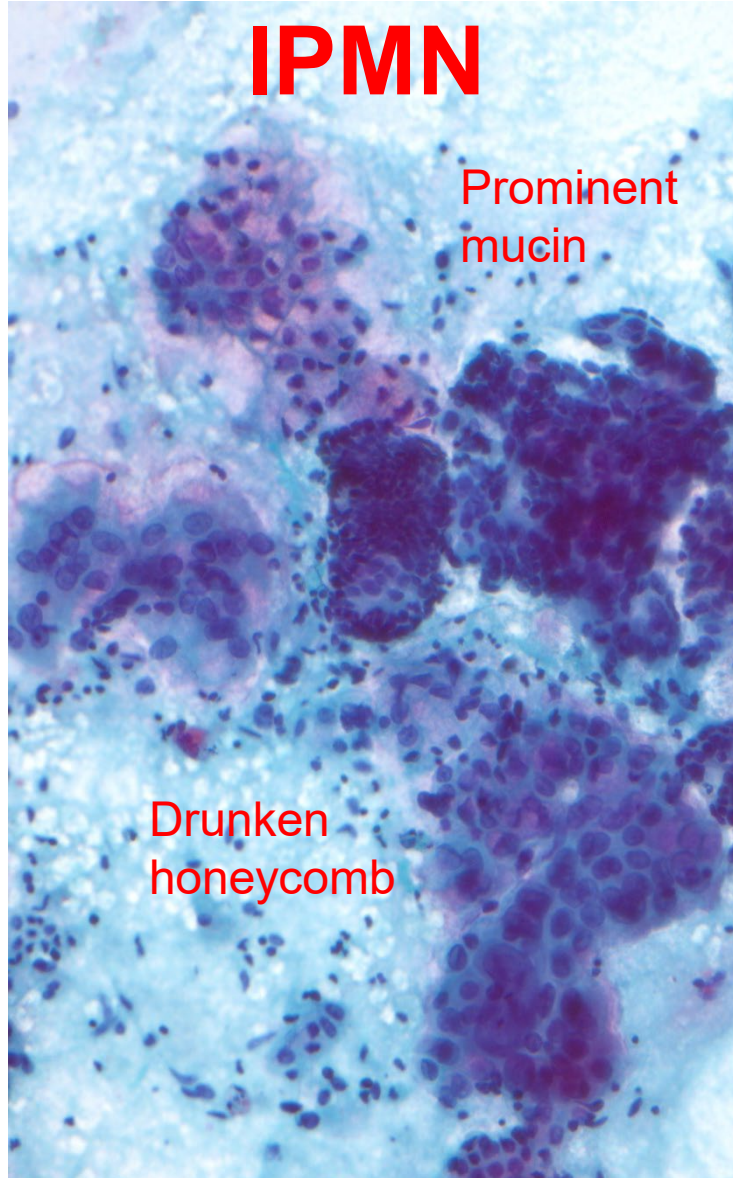
Uniform
basal
nuclei



IPMN

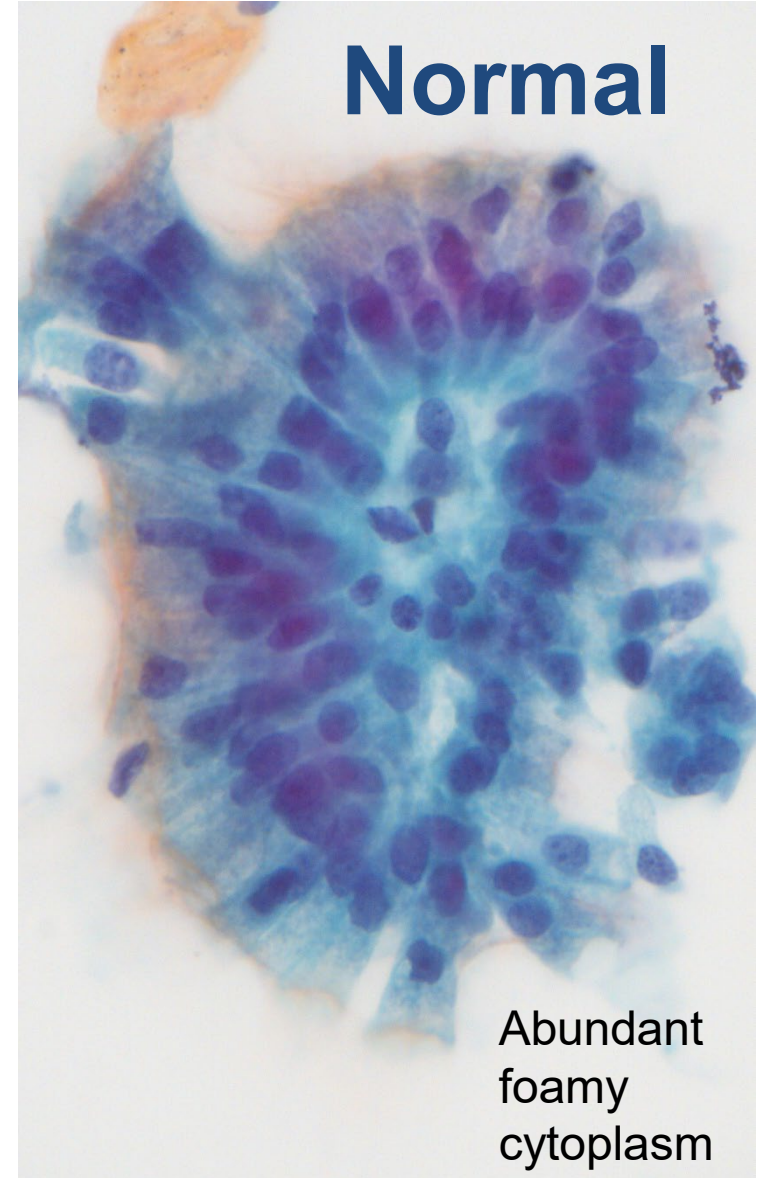
Prominent
mucin

Drunken
honeycomb



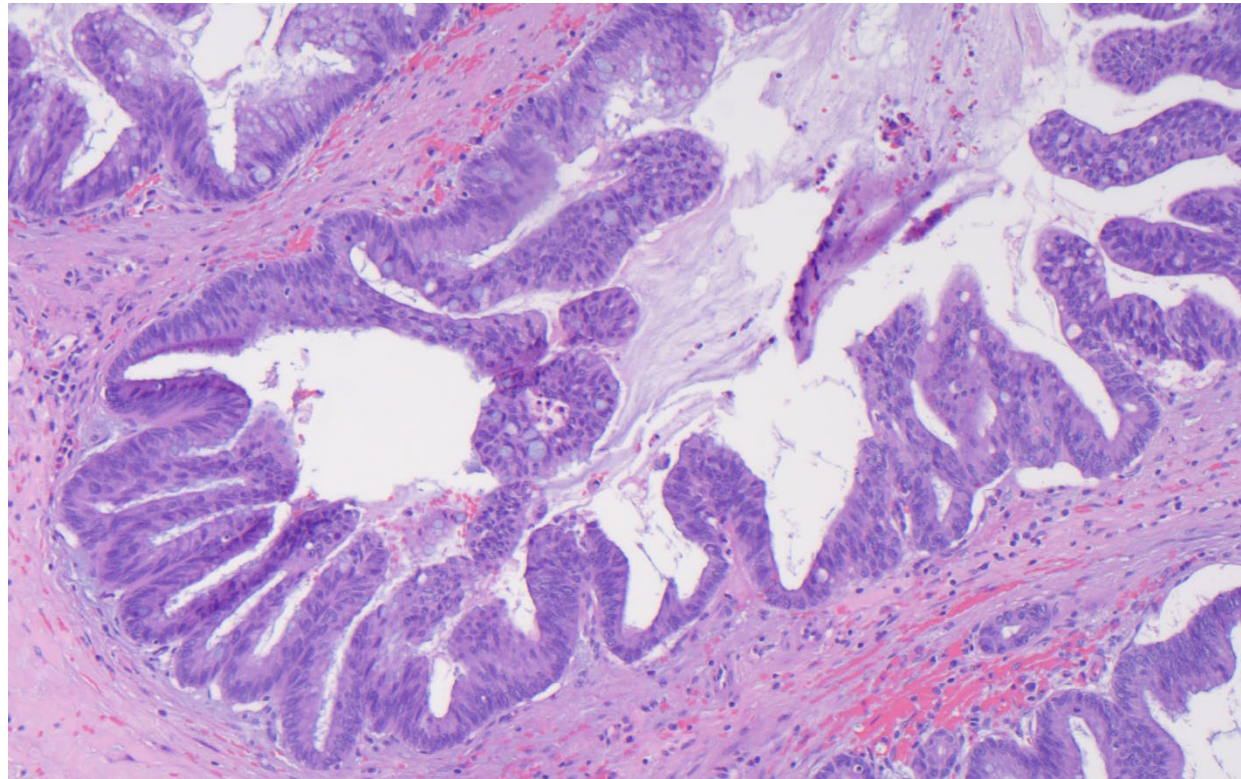
Normal

Abundant
foamy
cytoplasm

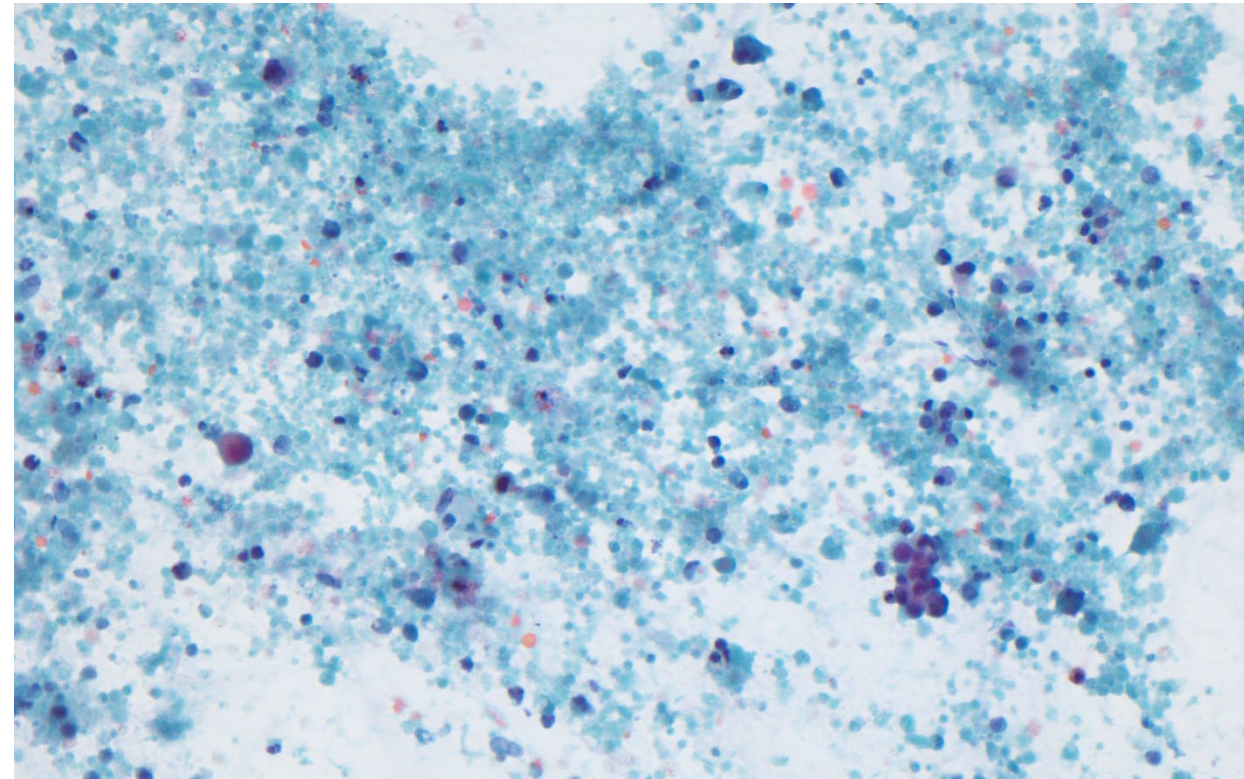


Intestinal-Type IPMN with HGD

High-grade dysplasia, luminal necrotic debris

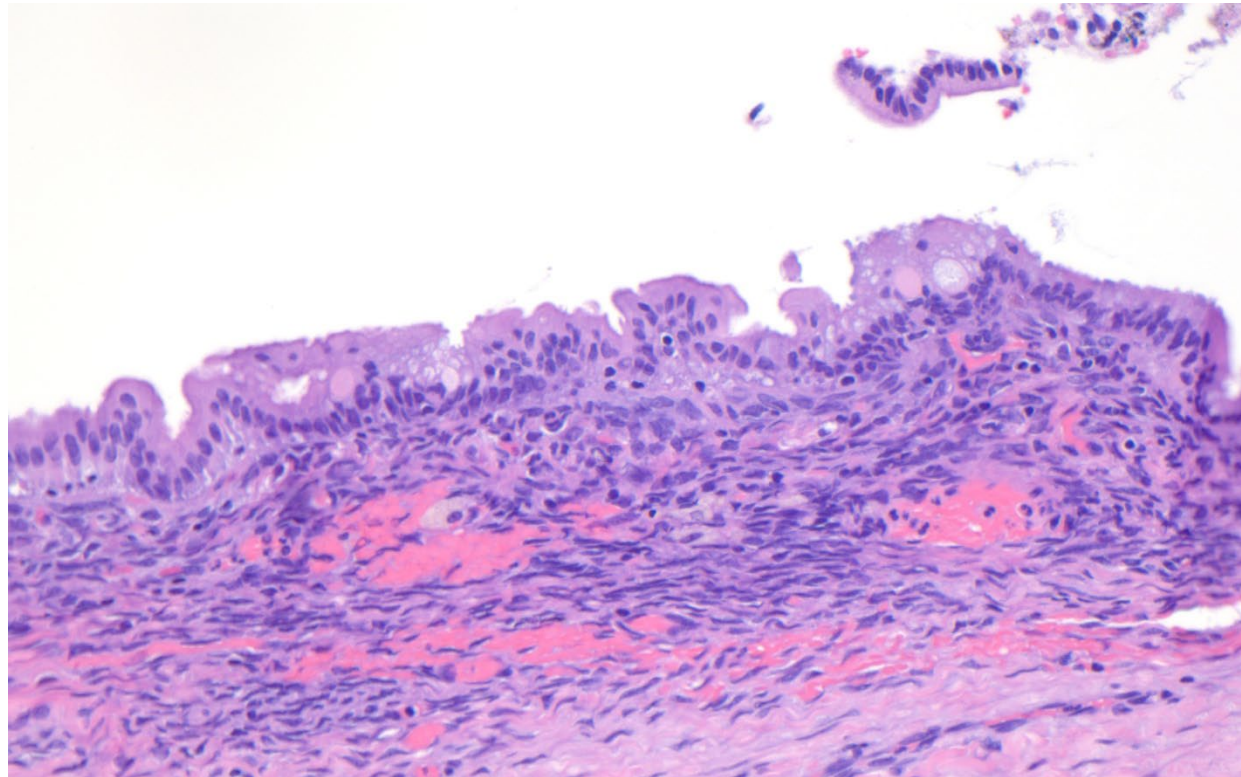


Necrosis and malignant cells

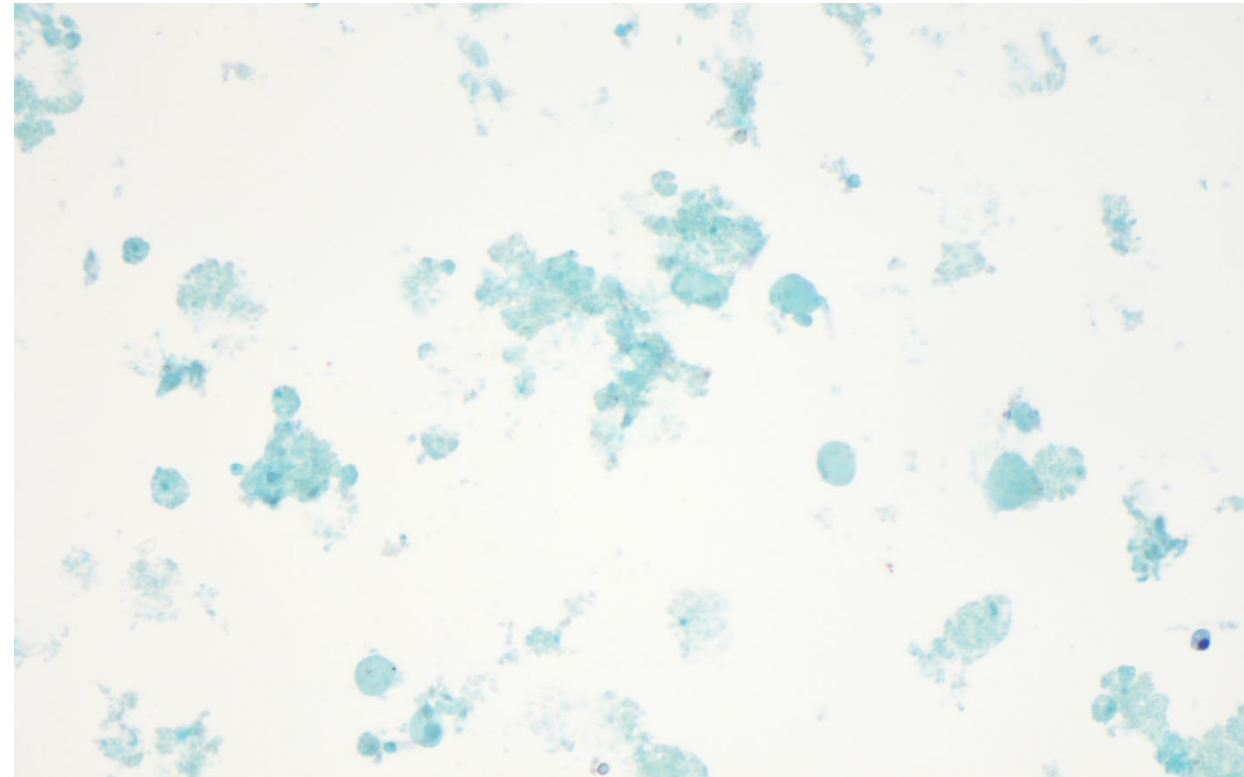


Mucinous Cystic Neoplasm (MCN)

Ovarian-type stroma; simple lining in this example



Degenerated macrophages and debris

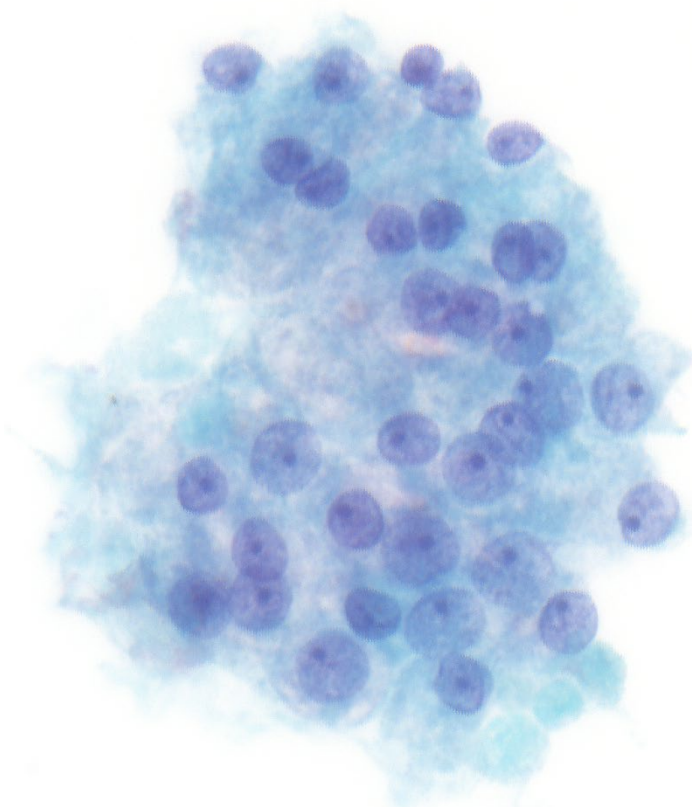


- Females
 - Usually in the tail
 - Usually simple
- Cytomorphologically similar to IPMN
 - Same range from paucicellular / cyst contents to obvious malignancy
- Ovarian-type stroma needed for diagnosis
 - Not seen by cytology, rare in cell block

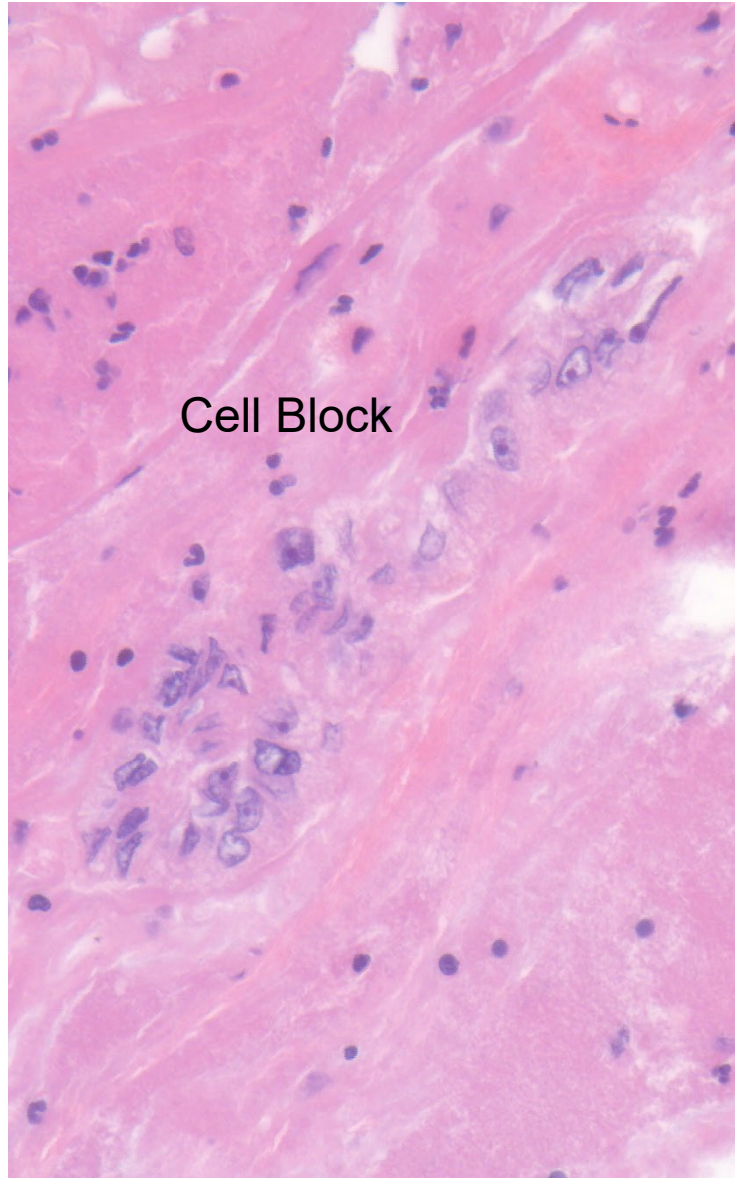
- Intraductal Papillary Oncocytic Neoplasm (IPON)
 - Low malignant potential
 - Simple architecture
 - Bland oncocytic cells
- Intraductal Tubulopapillary Neoplasm (ITPN)
 - High malignant potential
 - Complex architecture
 - High N:C ratio cells, often dysplastic / malignant

Intraductal Papillary Oncocytic Neoplasm

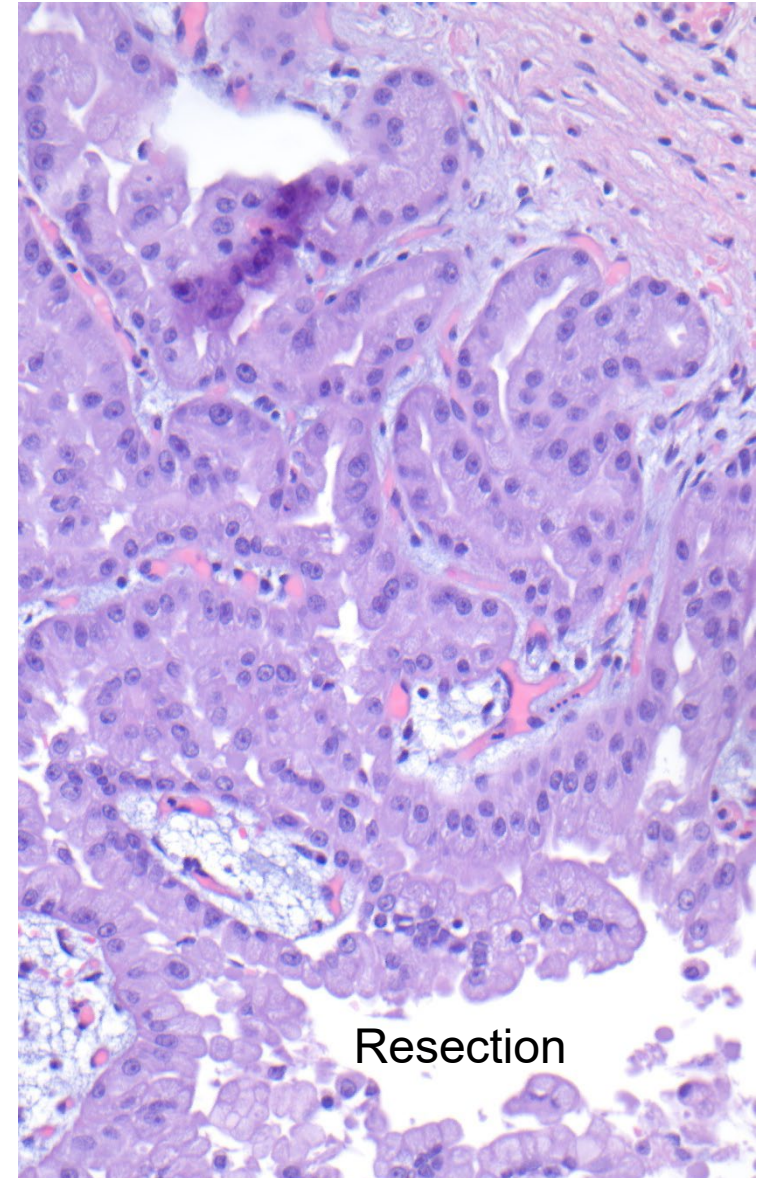
ThinPrep



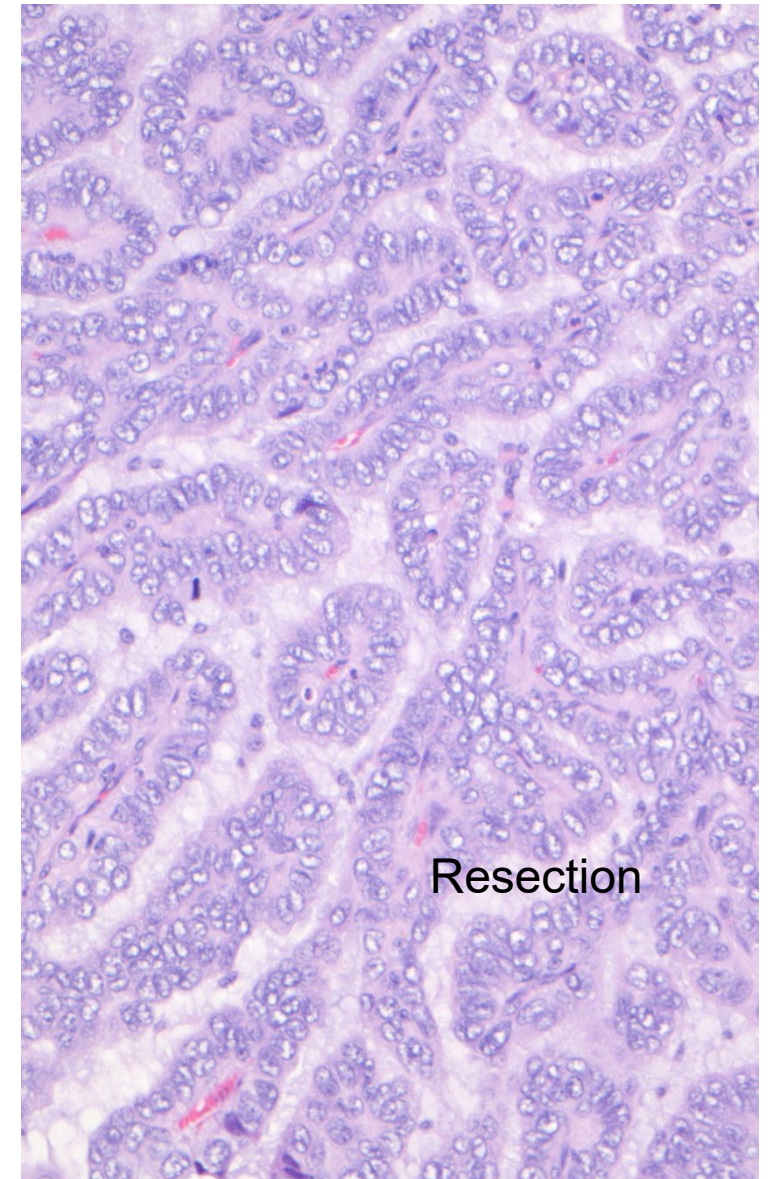
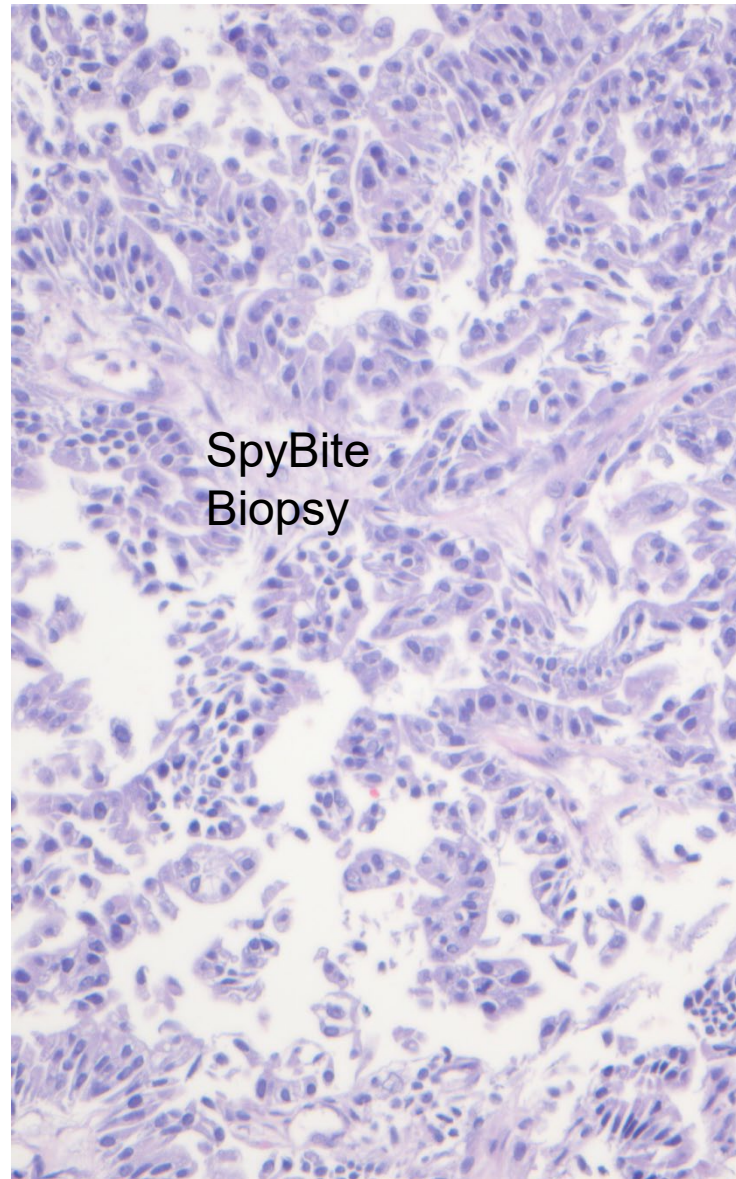
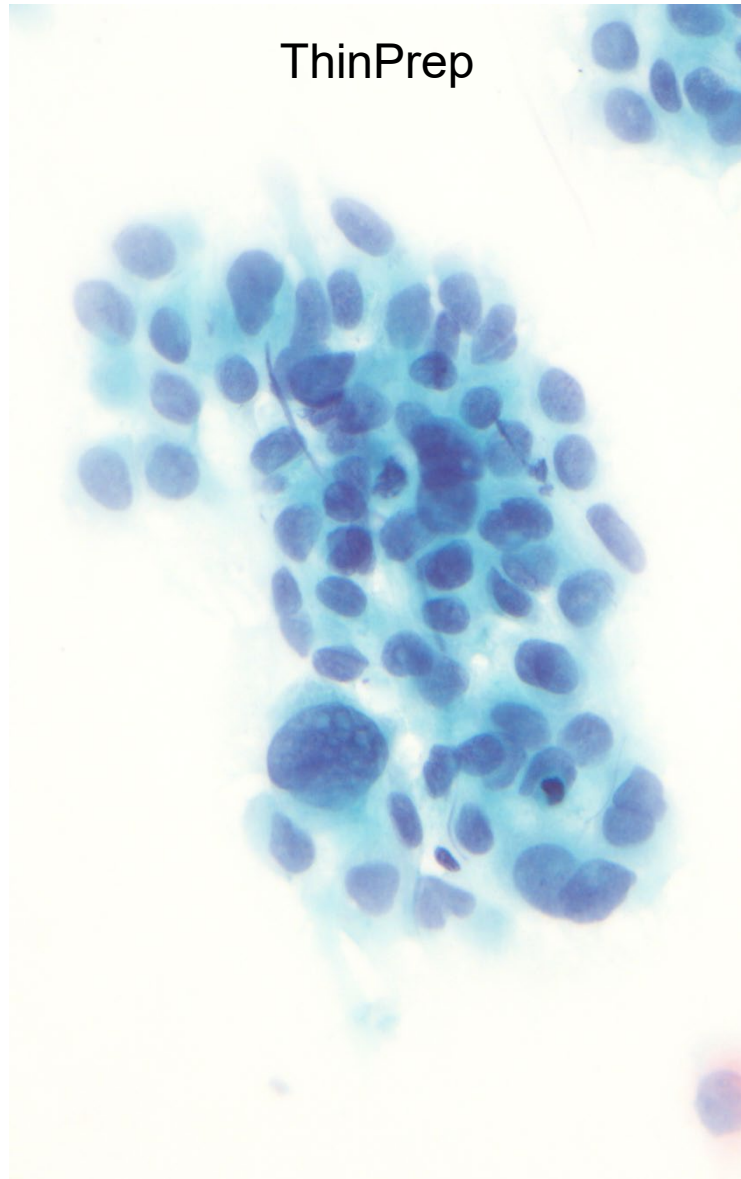
Cell Block



Resection

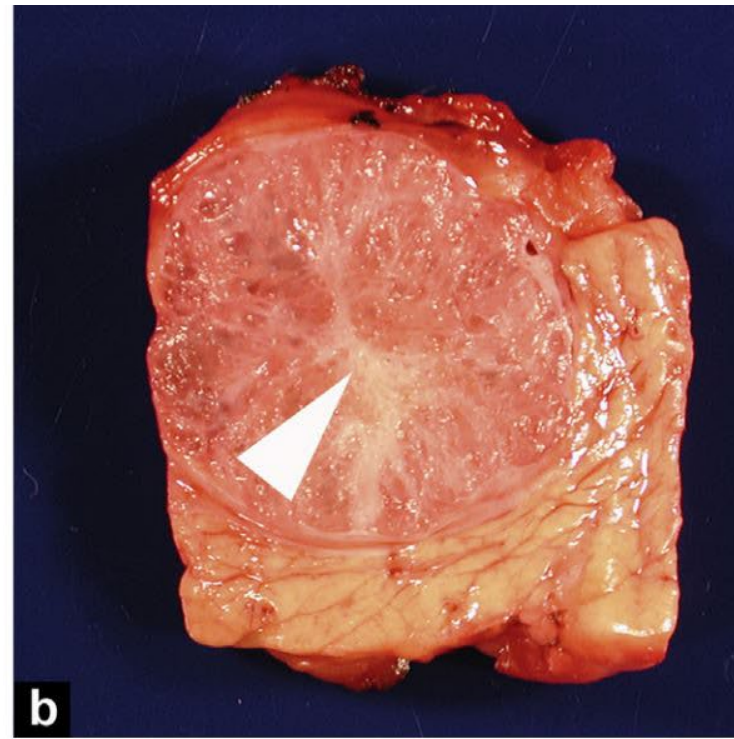


Intraductal Tubulopapillary Neoplasm



Serous Cystadenoma

- Usually diagnosed by radiology
- Biopsy only needed in subset
- May be paucilocular – very difficult to diagnose
- Female predominance, may be any part of the pancreas



Chu *et al.* Diagn Interv Imaging
2017; 98: 191-202.

ThinPrep of Serous Cystadenoma

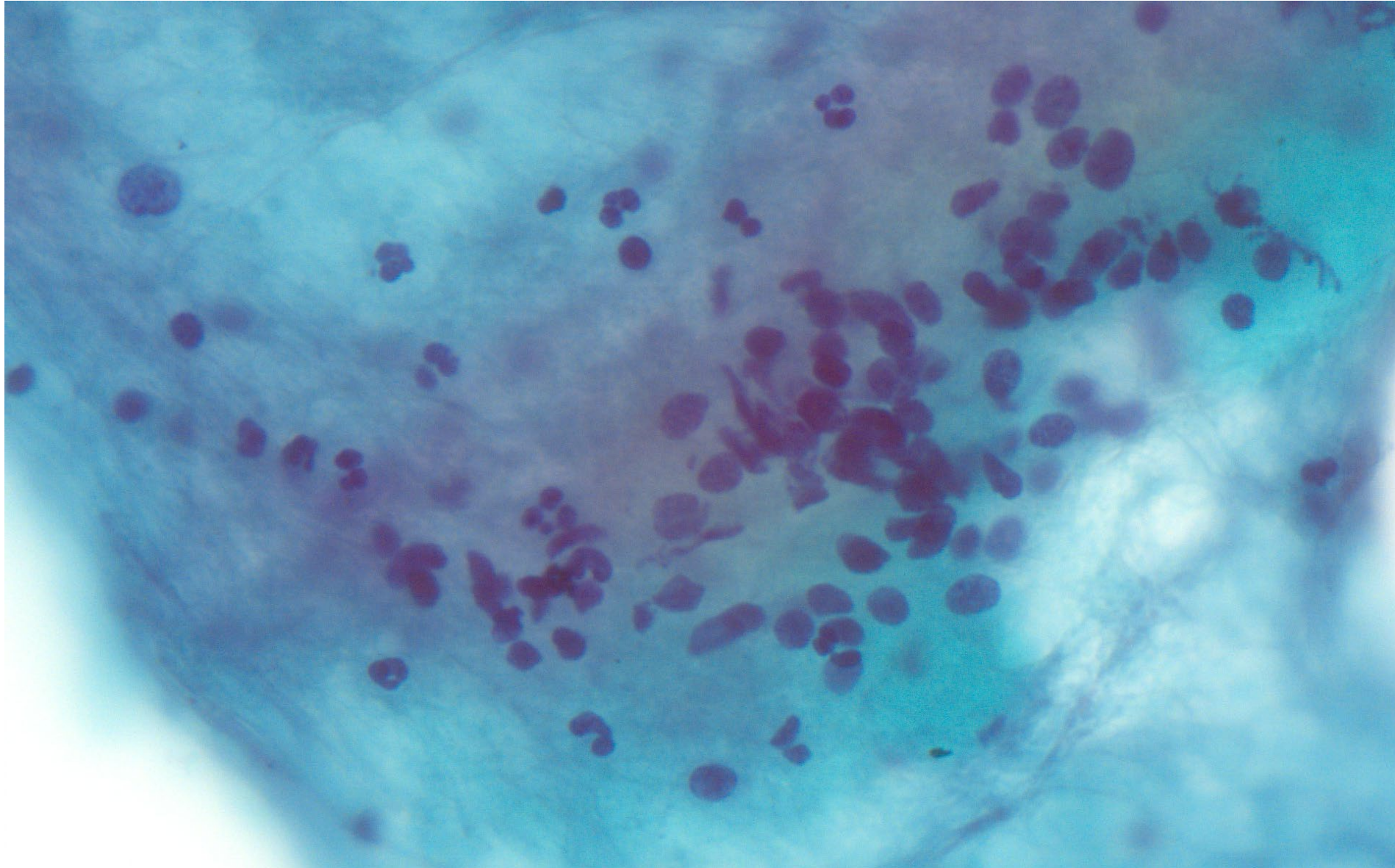
Flat sheets

Scant
cellularity

Abundant
cytoplasm

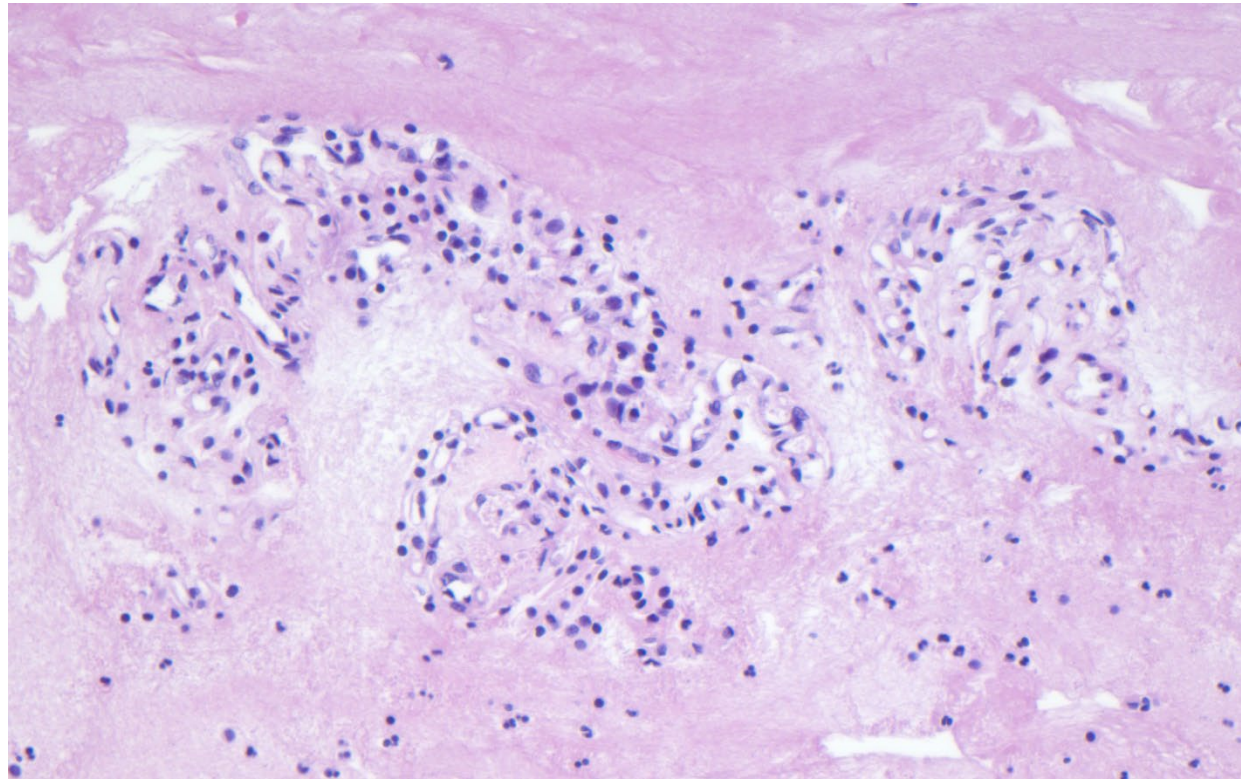
Bland
uniform
nuclei

Abundant Proteinaceous Material

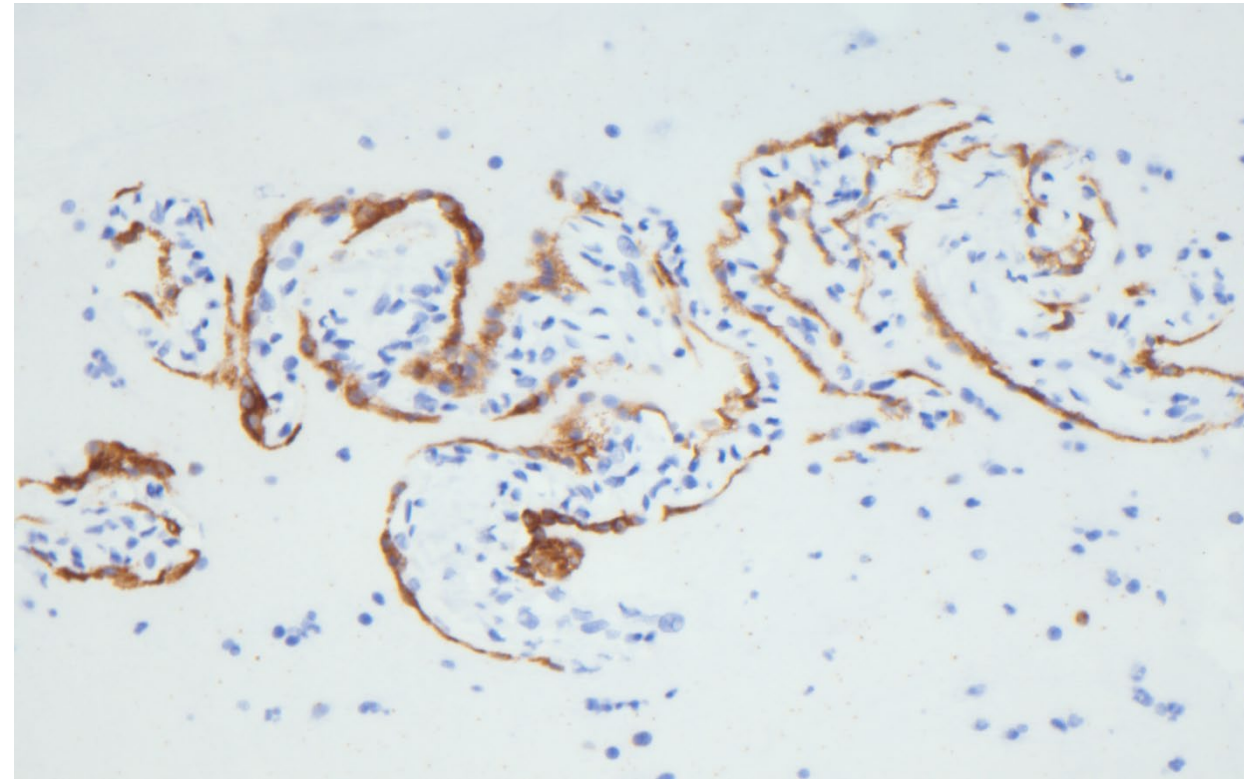


Cell Block – Often More Diagnostic

Cell block



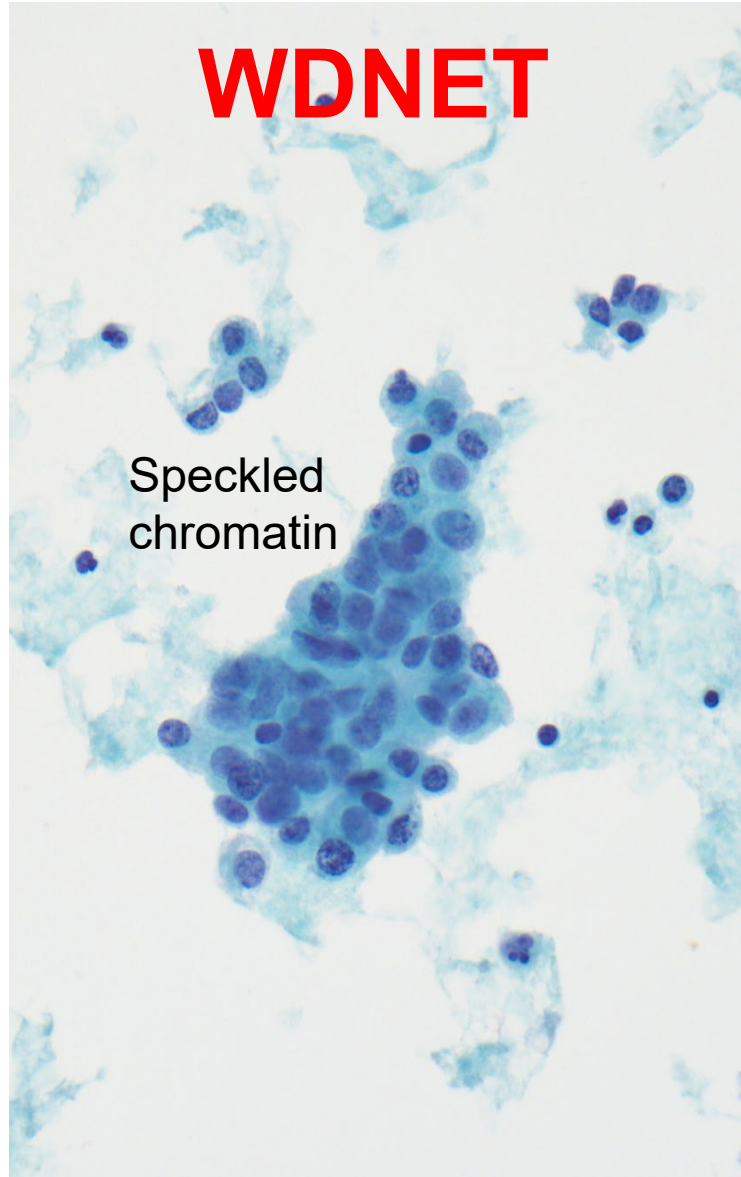
Inhibin



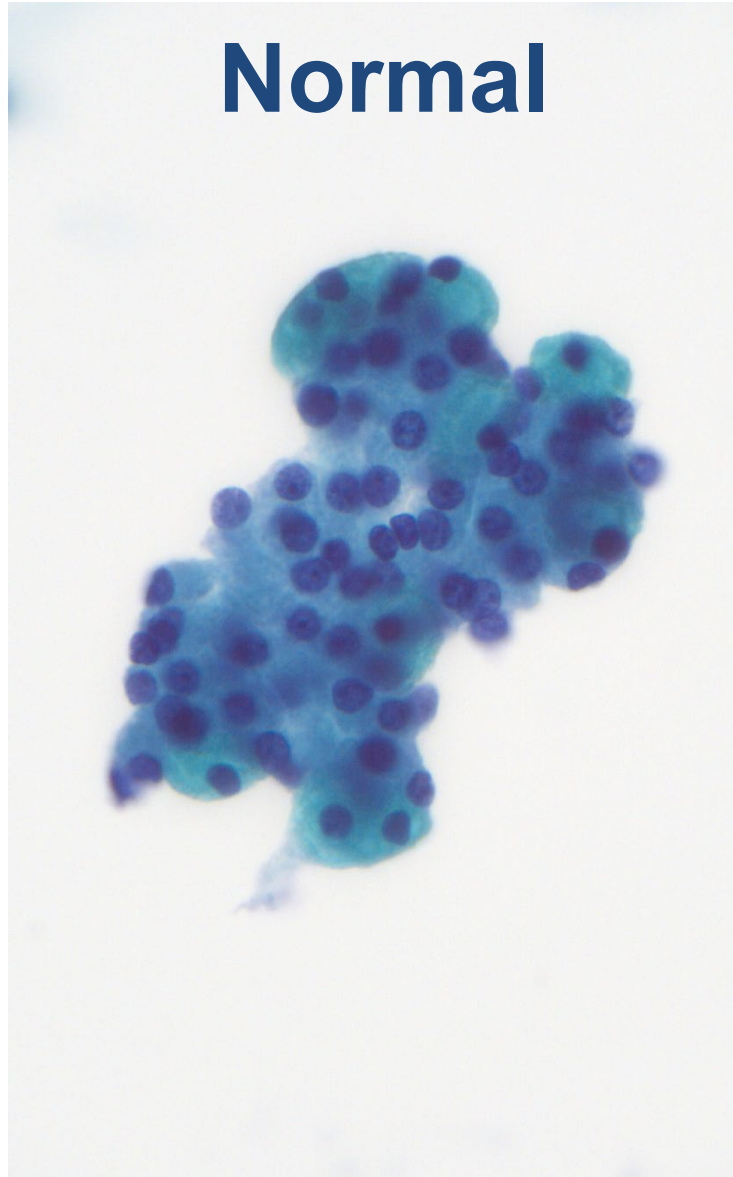
- WNET or acinar cell carcinoma may be cystic
 - Difficult to identify when paucicellular
- Cystic necrosis
 - Ductal adenocarcinoma
 - Metastasis
- Lymphoepithelial cyst
 - Bland squamous cells (similar to esophageal contaminant)
 - Lymphocytes
 - Difficult to diagnose by cytology alone

Cystic WDNET Vs Benign Pancreatic Acini

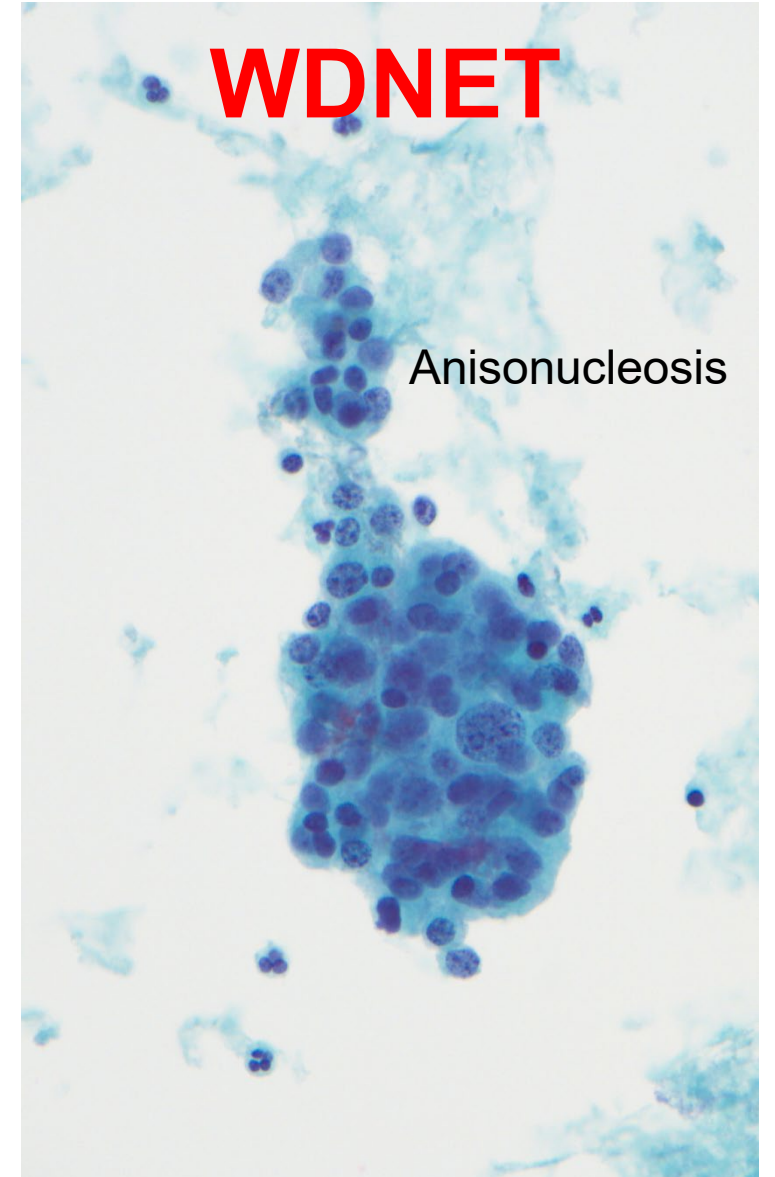
WDNET



Normal



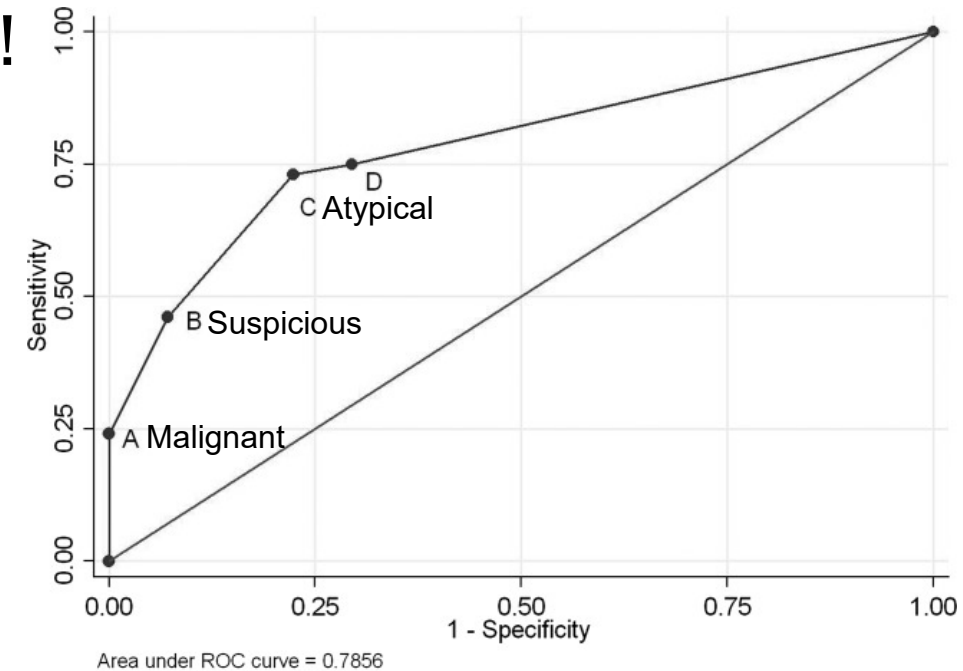
WDNET



Bile Duct Brushing Cytology

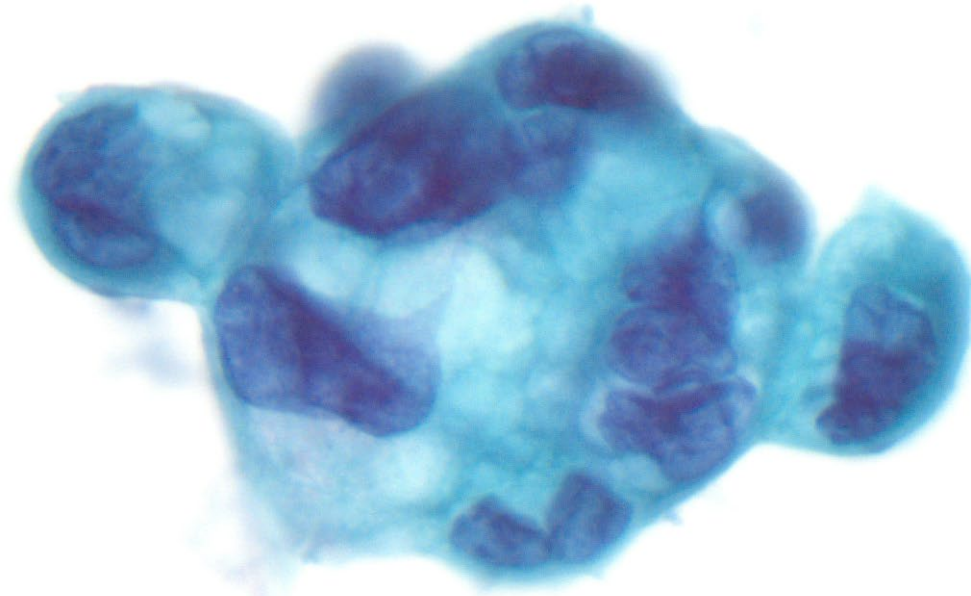
Bile Duct Brushings

- One of the most difficult specimens in cytology!
- Large degree of overlap:
 - Reactive vs.
 - Low-grade dysplasia vs.
 - High-grade dysplasia / carcinoma
- Clinical / radiologic correlation is key:
 - Check CT / ERCP
 - Check endoscopy note for degree of suspicion
 - “SpyGlass” enables visualization of stricture area

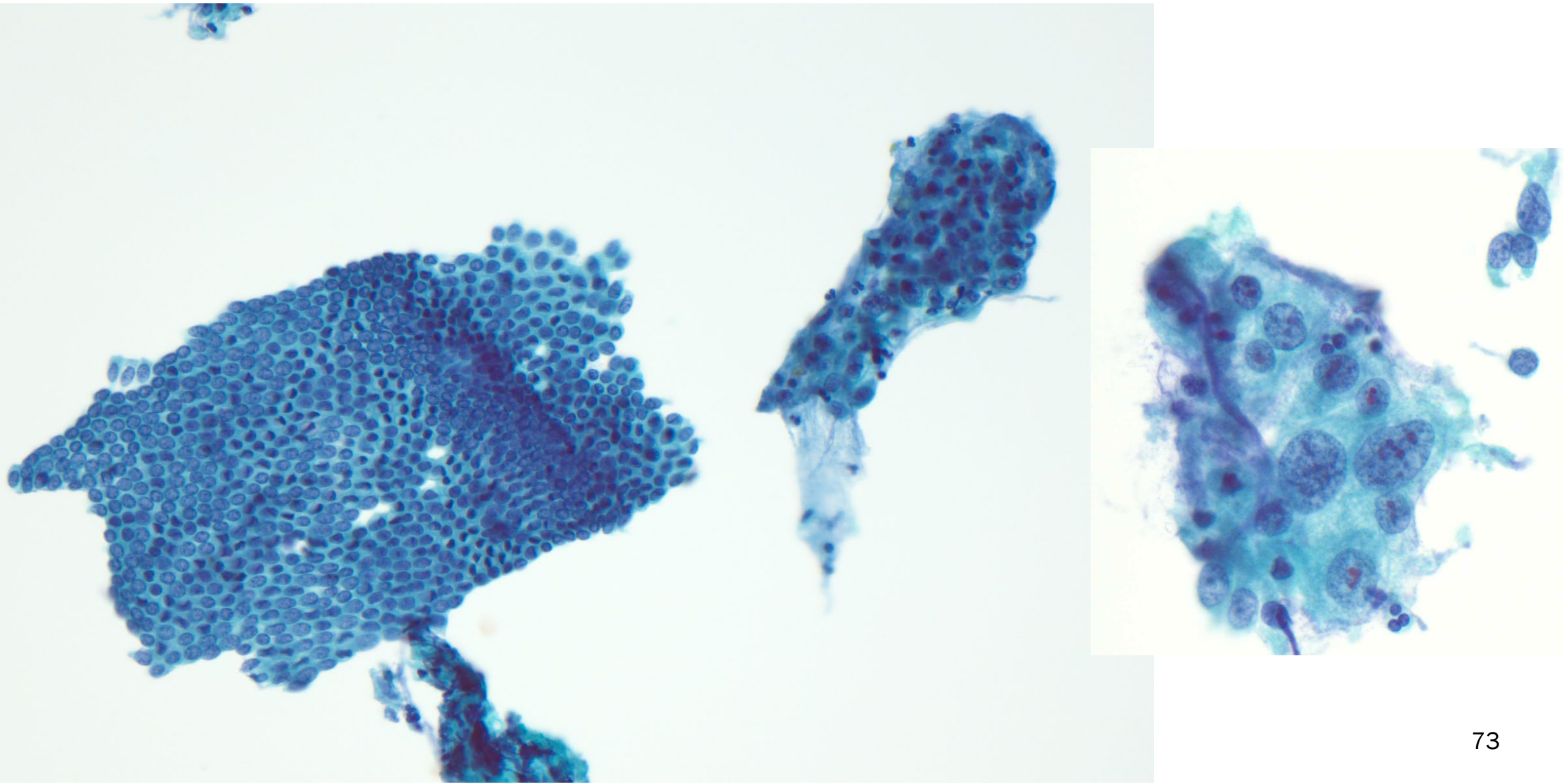


Chadwick *et al.*
Diagn Cytopathol
2014; 42: 285-91.

Some Bile Duct Brushings Are Obviously Malignant

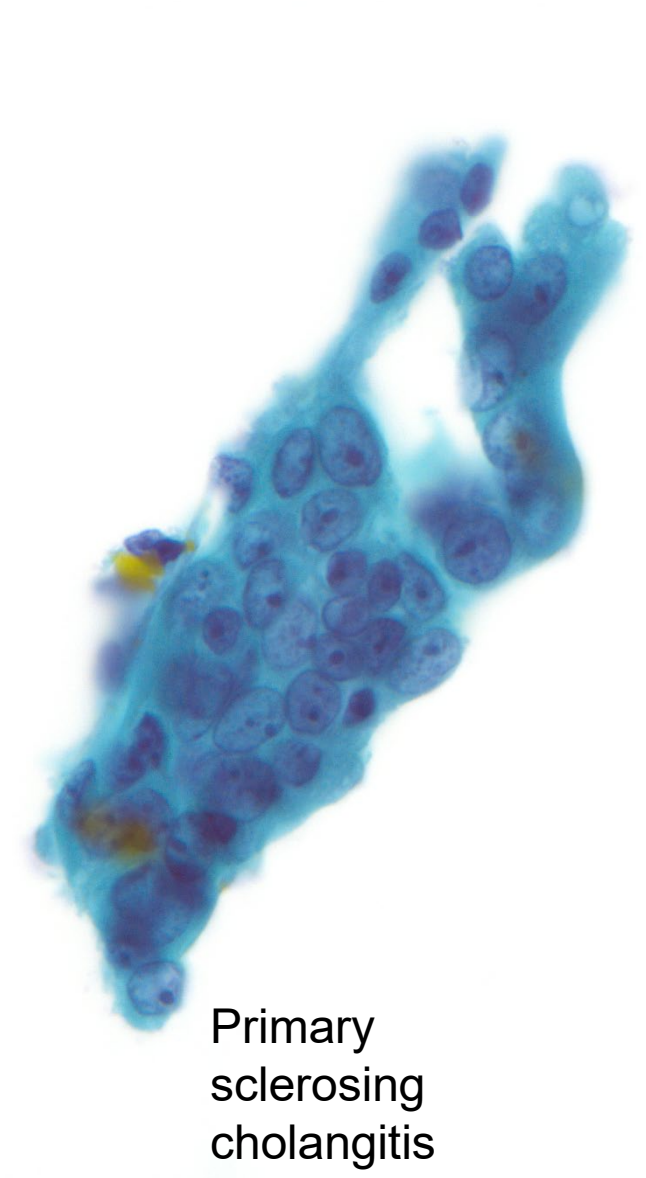
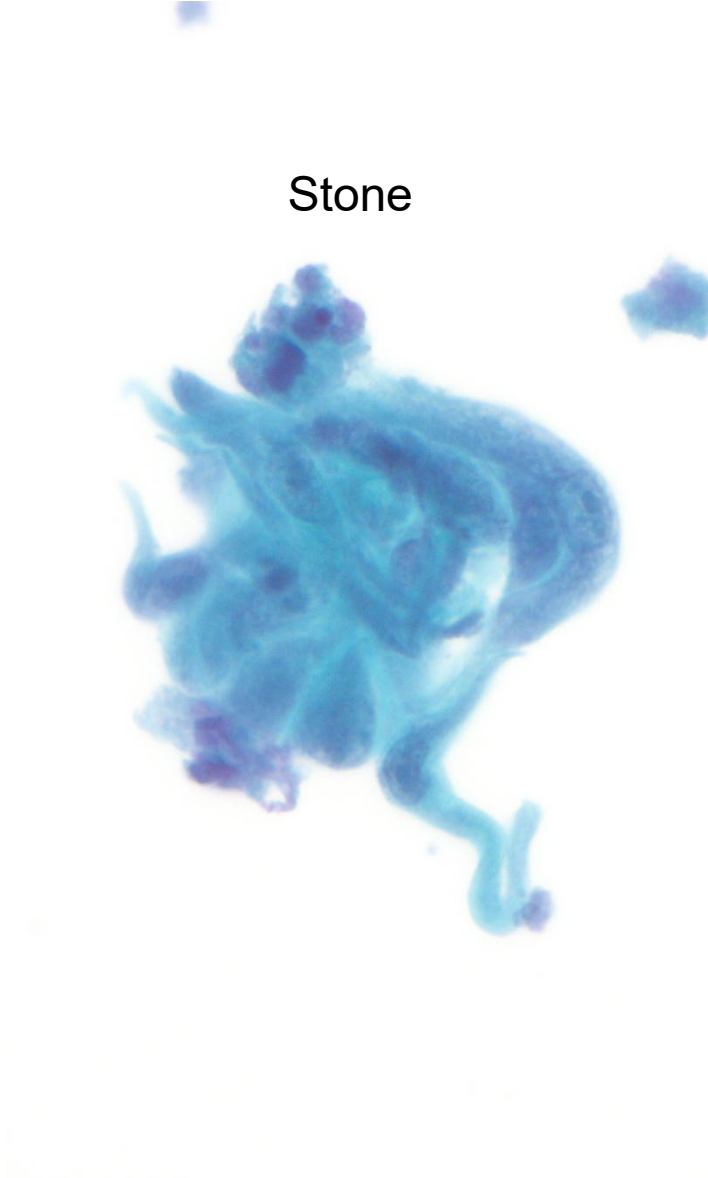
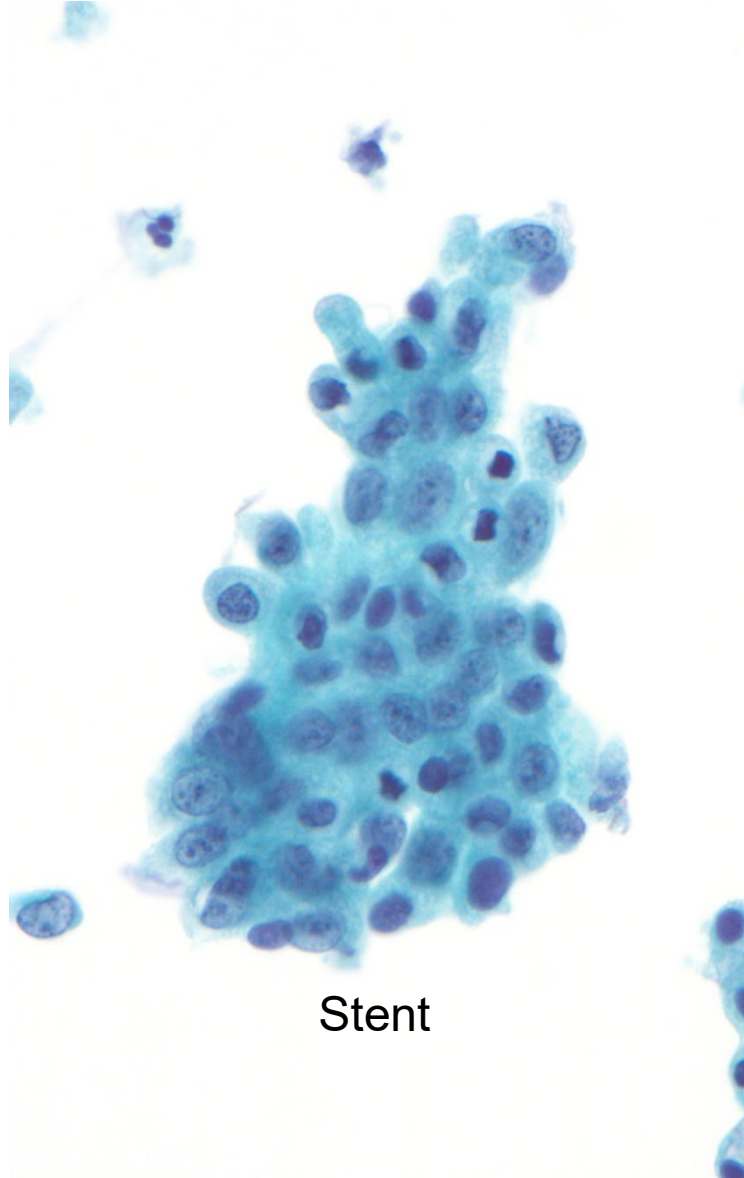


Comparison of Malignant Vs Normal Bile Duct Lining Cells

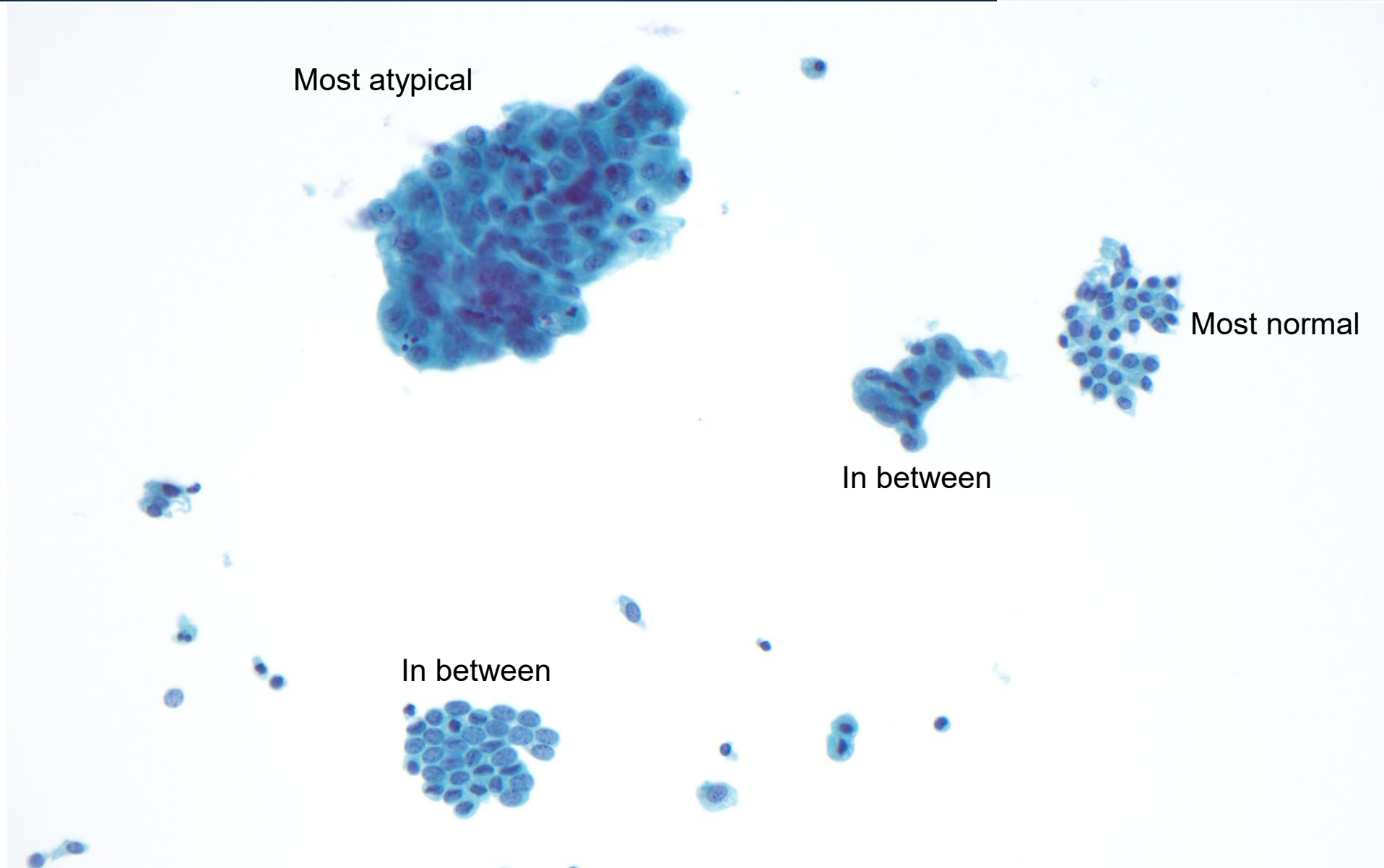


- Many “Atypical” bile duct brushings
- Is this helpful or just defensive?
- Can be sent for fluorescence in-situ hybridization (FISH)
 - Invented at Mayo Clinic to deal with primary sclerosing cholangitis population
 - Originally used same probes as UroVysion
 - Now have pancreaticobiliary-specific probes
 - Frequently negative even in high-suspicion cases
 - Often cells of interest are a small subset difficult to see by DAPI stain
 - FISH is inherently difficult with insufficient laboratory personnel

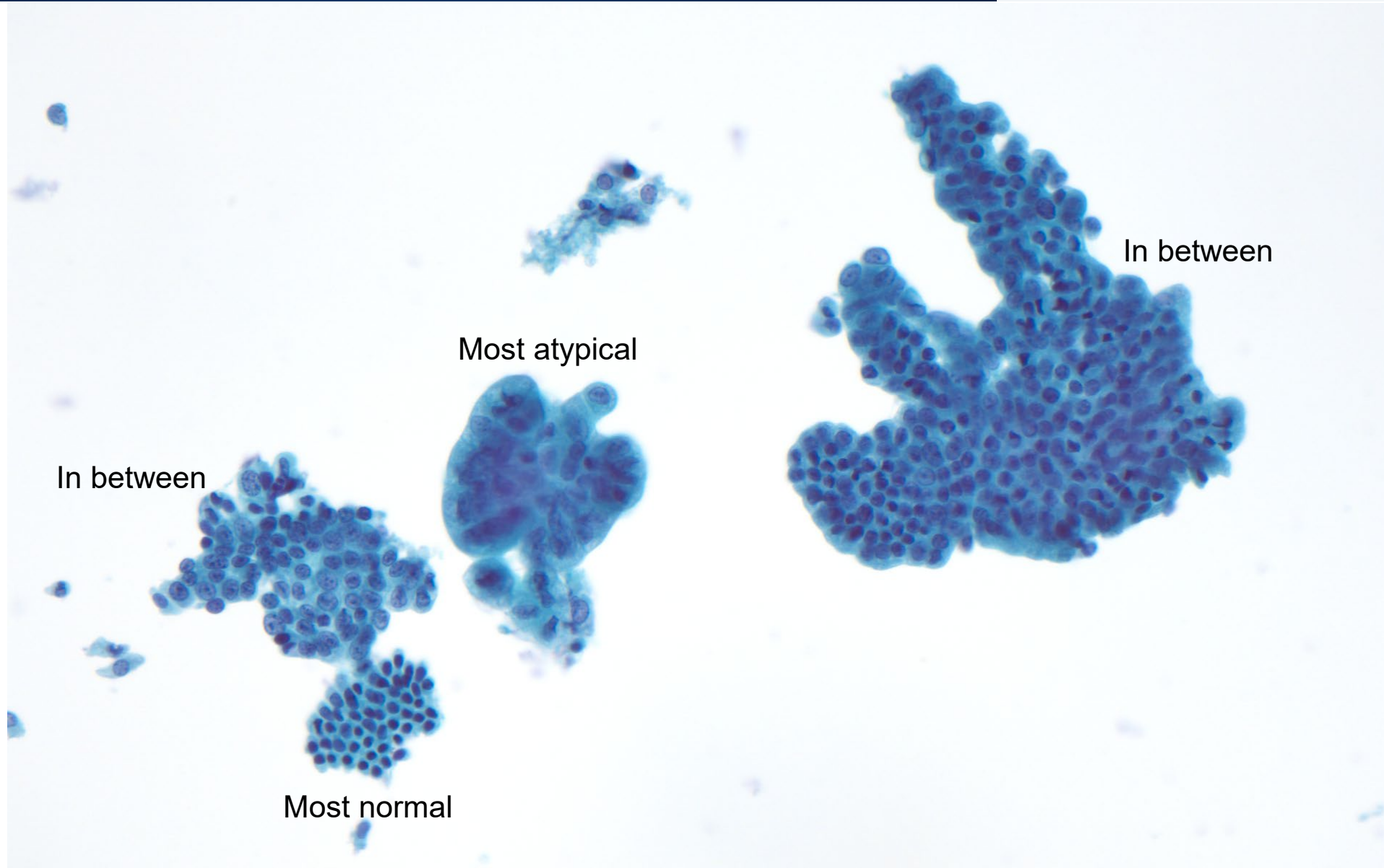
“Atypical” Bile Duct Brushings – All 3 Were Negative by FISH



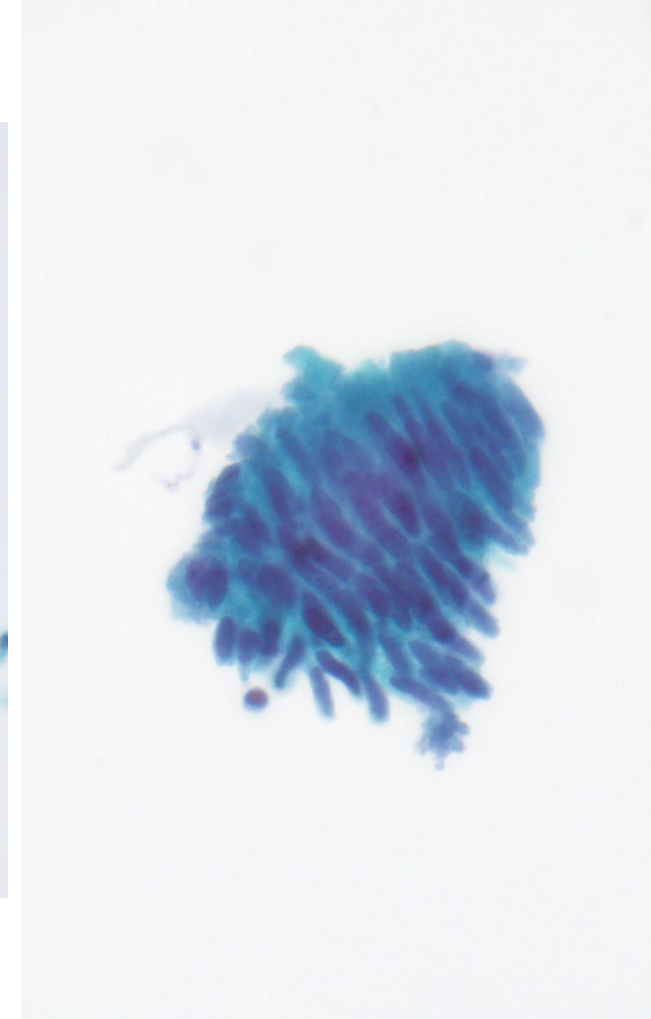
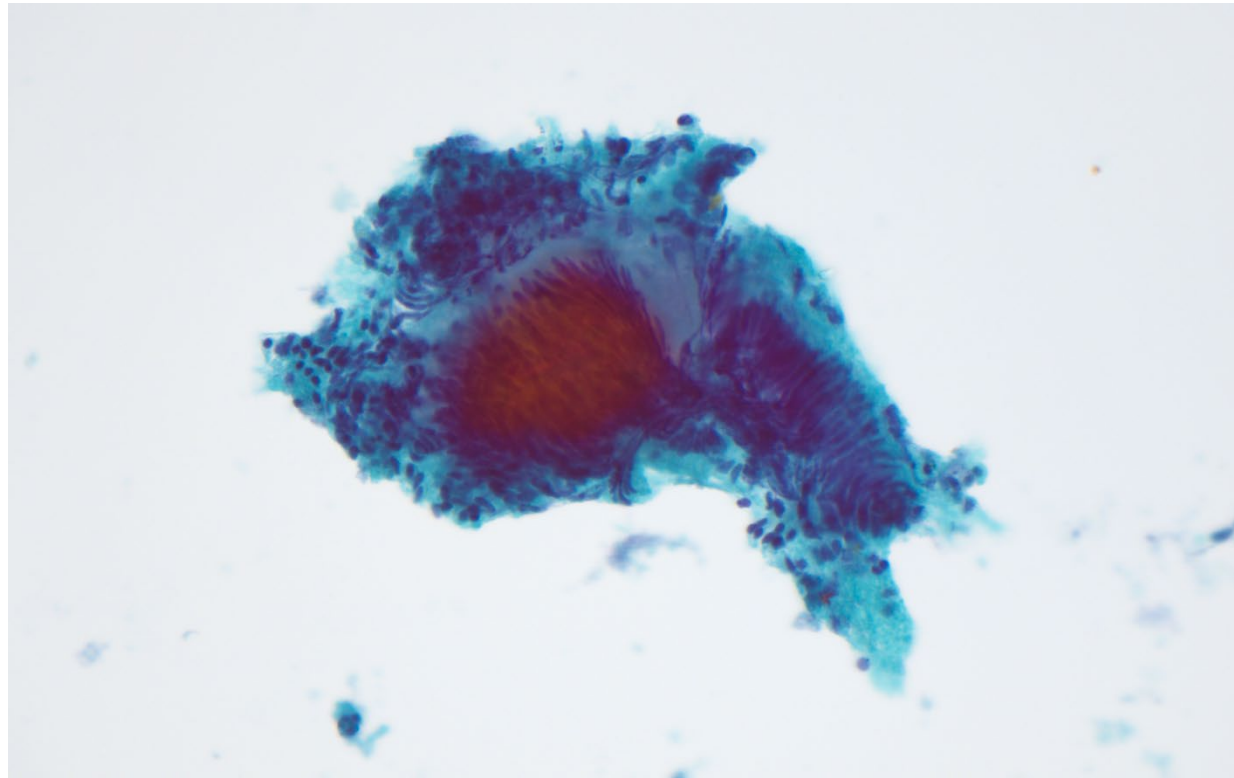
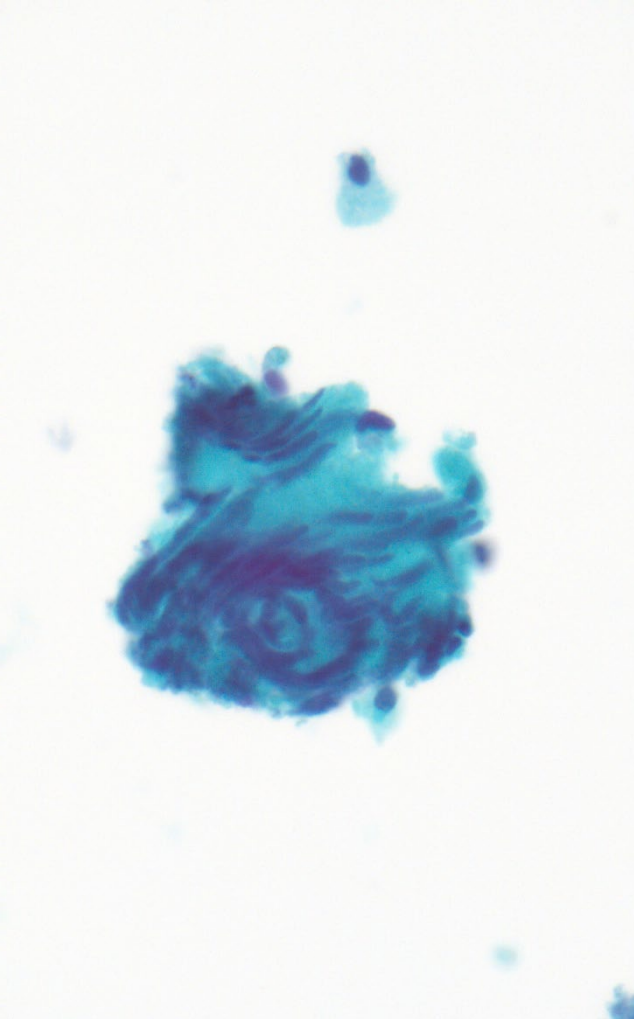
Spectrum of Changes in “Atypical”



Spectrum of Changes in “Atypical”



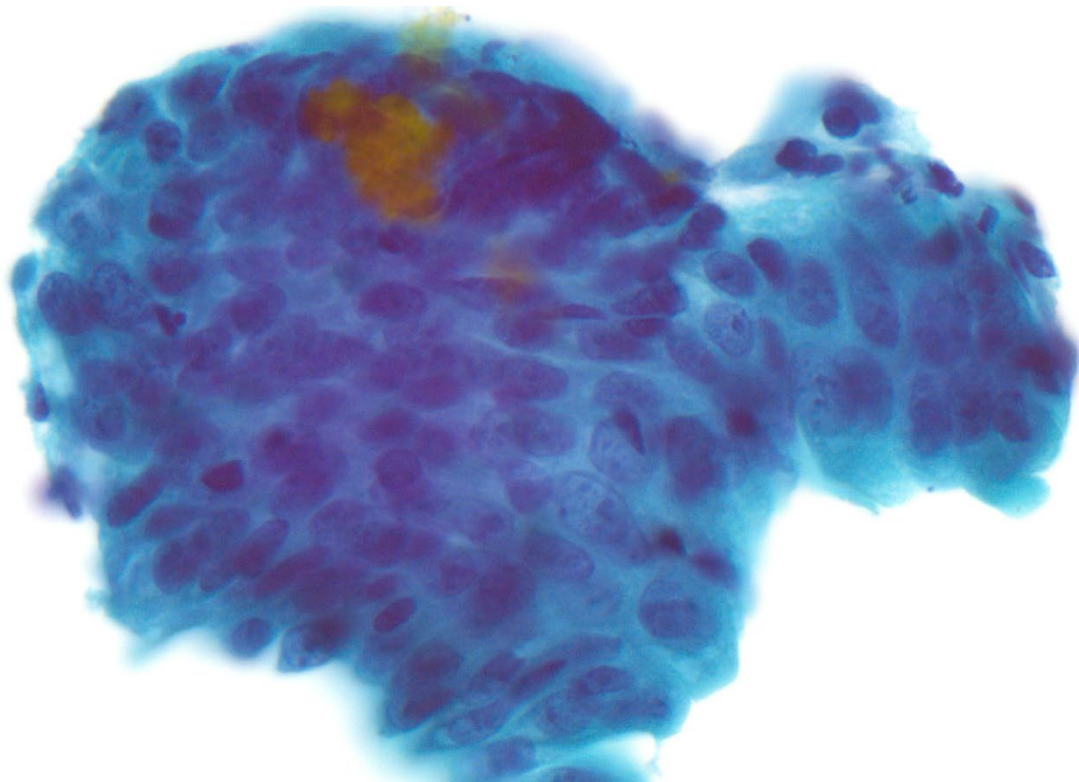
Not “Atypical”: Cautery Artifact



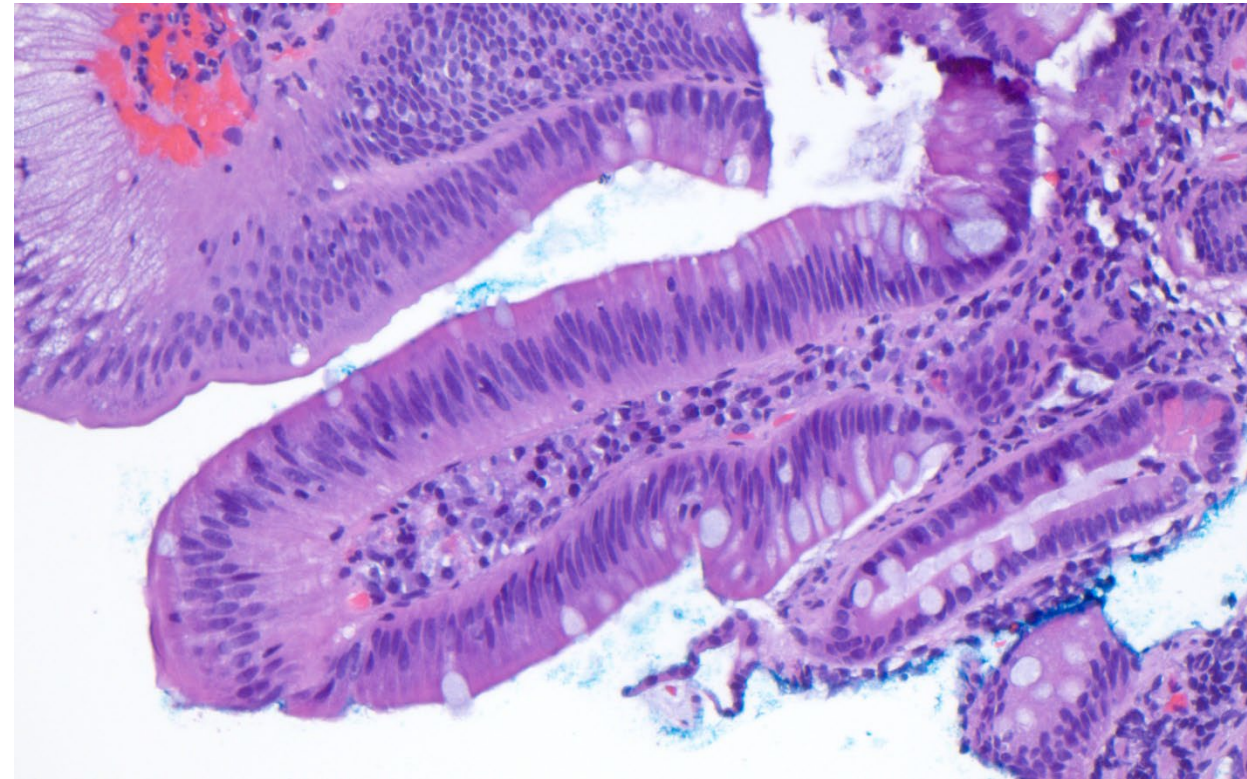
- Ampullary adenoma
 - Similar to colorectal tubular adenoma in appearance
 - Usually elongate, dark, mildly pleomorphic nuclei (low-grade dysplasia)
 - May become high-grade or invasive
- Intra-ampullary papillary-tubular neoplasm (IAPN)
 - Even more rare
 - Often mixed ductal and intestinal-type epithelial elements
 - Usually mixed low- and high-grade areas
 - Complex papillary and tubular architecture
 - May become invasive

Ampullary Adenoma (Low-Grade)

Brushing (ThinPrep)

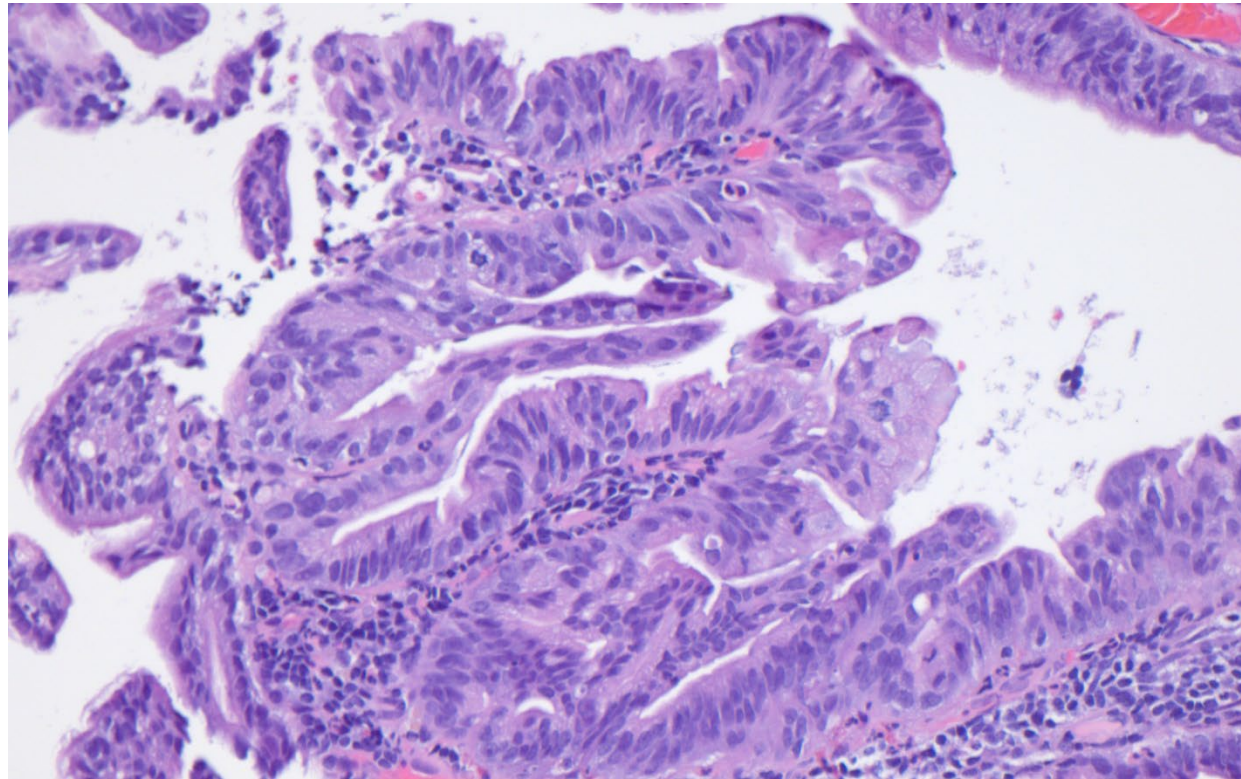


Forceps biopsy

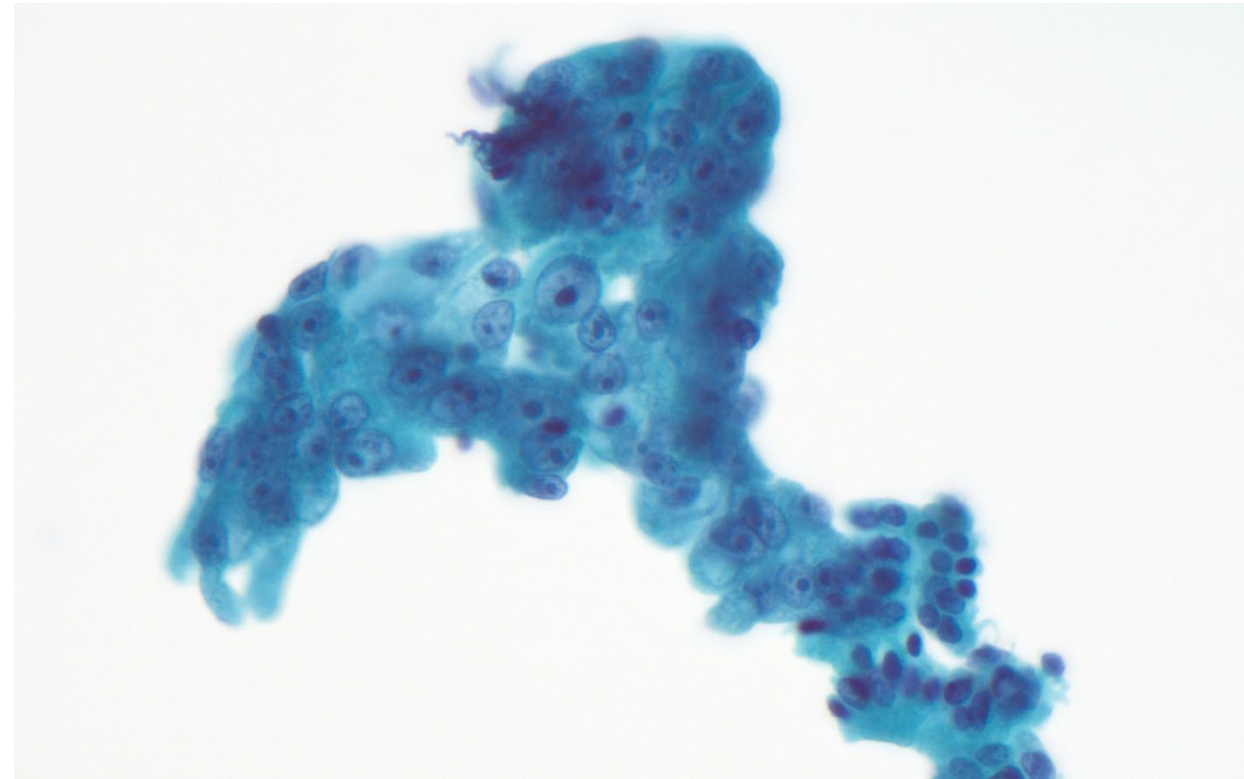


Intra-Ampullary Papillary-Tubular Neoplasm (IAPN)

Forceps biopsy



Brushing (ThinPrep)



Summary

- Most pancreaticobiliary tumors are ductal adenocarcinoma
 - Usually easy to diagnose if well-sampled
- Non-ductal solid tumors are more problematic
 - Overlapping morphologic features
 - Immunohistochemistry essential
- Pancreatic cysts require correlation with ultrasound and chemistry
 - Range from paucicellular, to scant and bland, to obviously malignant
- Bile duct brushings are problematic
 - High “atypical” rate
 - Reflex FISH testing not always available or reliable

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