Salivary Gland Cytology: Diagnostic Limitations and Pitfalls

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FNA of Salivary Gland

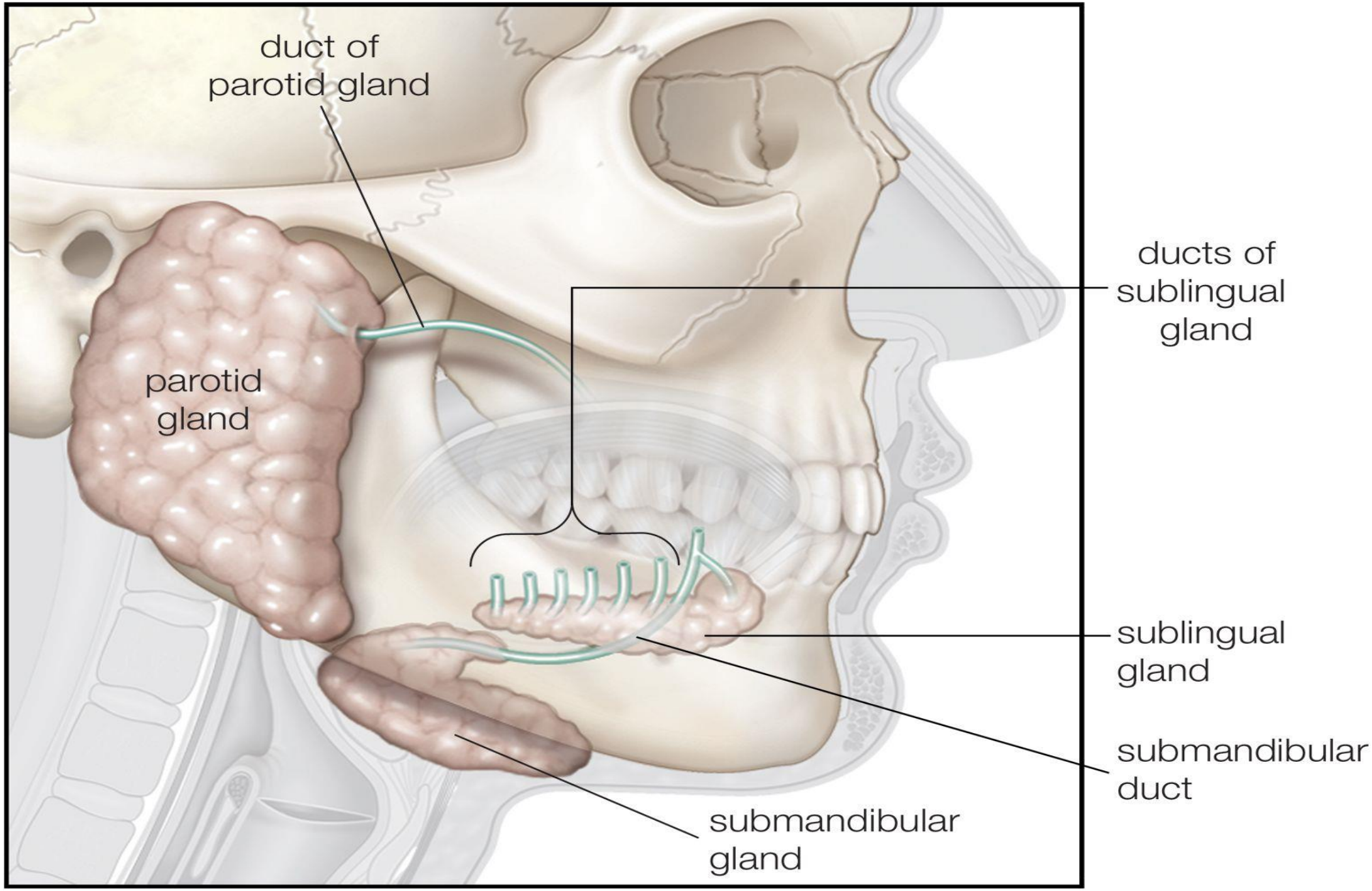
- Indications
  - Assessment of clinically suspicious masses
- Purpose
  - Provide information regarding need for surgery
- Adequacy
  - Defines the quality and quantity of sample
- Diagnostic utility
  - Neoplastic vs. non-neoplastic
  - If neoplastic, benign/low grade vs. high grade
- Potential Pitfalls
  - Benign vs. malignant basaloid/oncocytoid neoplasms
  - Cystic lesions

Indications for Salivary Gland FNA

- Distinguish non-neoplastic from neoplastic
- Modify management according to type of neoplasm
  - Benign – lobectomy or observation
    - Pleomorphic adenoma, Warthin tumor
  - Low-grade malignancy – lobectomy
  - High-grade malignancy – Total resection, lymph node sampling
- Distinguish tumors arising in submandibular gland/tail of parotid from enlarged lymph nodes
  - Primary tumor vs. reactive LN or lymphoma
- Document recurrence of neoplasm or metastases

Cytologic Diagnosis of Salivary Gland Neoplasms

- Diagnosis of salivary gland neoplasms can be very challenging
- May not be able to render a specific diagnosis in many instances
- Rarity of neoplasms and unfamiliarity of pathologists with many of them
- Diversity and overlapping cytologic & histologic features amongst various tumors and within same tumor



FNA of Salivary Gland

- Rate of malignancy varies by gland
  - Parotid: 20-25%
  - Submandibular: 40-50%
  - Sublingual and minor: Up to 80%

FNA of Salivary Gland

- Some diagnoses are more common in particular sites:
  - Warthin tumor – only parotid/periparotid lymph nodes
  - Acinic cell carcinoma – mostly parotid gland
  - Basal cell adenoma/adenocarcinoma – mostly parotid gland
  - Cannalicular adenoma – minor glands
  - Polymorphous adenocarcinoma/CAMSG – palate and tongue

Tumor Phenotype

- Biphasic tumors
  - Pleomorphic adenoma
  - Basaloid neoplasms
  - Epithelial-myoepithelial carcinoma
  - Adenoid cystic carcinoma
- Acinar differentiation = acinic cell carcinoma
- Apocrine differentiation is a hallmark of SDC

- Immunostains help define/confirm tumor cell phenotype

Effectiveness of FNA

- Effectiveness of cytomorphology alone:
  - Overall sensitivity: 86-100%
  - Overall specificity: 48-94%
- Accuracy based on grade:
  - Benign/low-grade versus high-grade malignant: 81-100%
- Misinterpretation mostly associated with limited cellularity or bland-appearing neoplasms
- Diagnostic accuracy improves with experience and education

The Milan System for Reporting Salivary Gland Cytopathology II (2023)

Diagnostic category	% ROM	Comment	Management
I. Non-Diagnostic	15	Non-lesional	Clinical & radiologic correlation/repeat FNA
II. Non-Neoplastic	11		Clinical & radiologic correlation/repeat FNA
III. Atypia of undetermined significance (AUS)	30	Indefinite for neoplasm: reactive changes vs. poorly sampled neoplasm	Repeat FNA or surgery
IV. Neoplasm			
A. *Benign*	<3	PA, WT, etc.	Surgery or clinical FU
B. *Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)*	35	Cellular benign neoplasm vs. low-grade malignancy	Surgery
V. Suspicious for malignancy (SFM)	83	Highly suggestive but not diagnostic of malignancy	Surgery
VI. Malignant	98	Specify low-grade vs. high-grade malignancy	Surgery

Potential Pitfalls: Morphologic look-alikes

- Basaloid lesions
- Oncocytic lesions
- Clear cell neoplasms

Potential Pitfalls

- Basaloid lesions
  - Pleomorphic adenoma, basal cell adenoma/carcinoma, adenoid cystic carcinoma, polymorphous adenocarcinoma, carcinoma ex pleomorphic adenoma
- Oncocytic lesions
  - Warthin tumor, mucoepidermoid carcinoma, acinic cell carcinoma, secretory carcinoma, salivary duct carcinoma, squamous cell carcinoma
- Clear cell neoplasms
  - Myoepithelial neoplasms, hyalinizing clear cell carcinoma, metastatic renal cell carcinoma

FNA of Salivary Glands

- More commonly encountered diagnostic problems
  - Cellular benign neoplasms
  - Low-grade and high-grade malignancies
  - Cystic lesions
  - Atypical lymphoid and inflammatory changes
  - Unusual cytologic presentation of common lesions
  - Rare unusual lesions

- Strict and well defined cytologic and architectural criteria

Traditional Approach to Evaluation of SG Tumors

- **Non-neoplastic**
- **Benign neoplasms**
  - Pleomorphic adenoma
  - Basal cell adenoma
  - Warthin tumor
- **Malignant neoplasms**
  - LG mucoepidermoid carcinoma
  - Acinic cell carcinoma
  - Adenoid cystic carcinoma
  - High grade and undifferentiated carcinoma

Practical Diagnostic Approach

- Low power architecture
- Cell size
- Amount of cytoplasm
- Degree of cytologic atypia
- Background

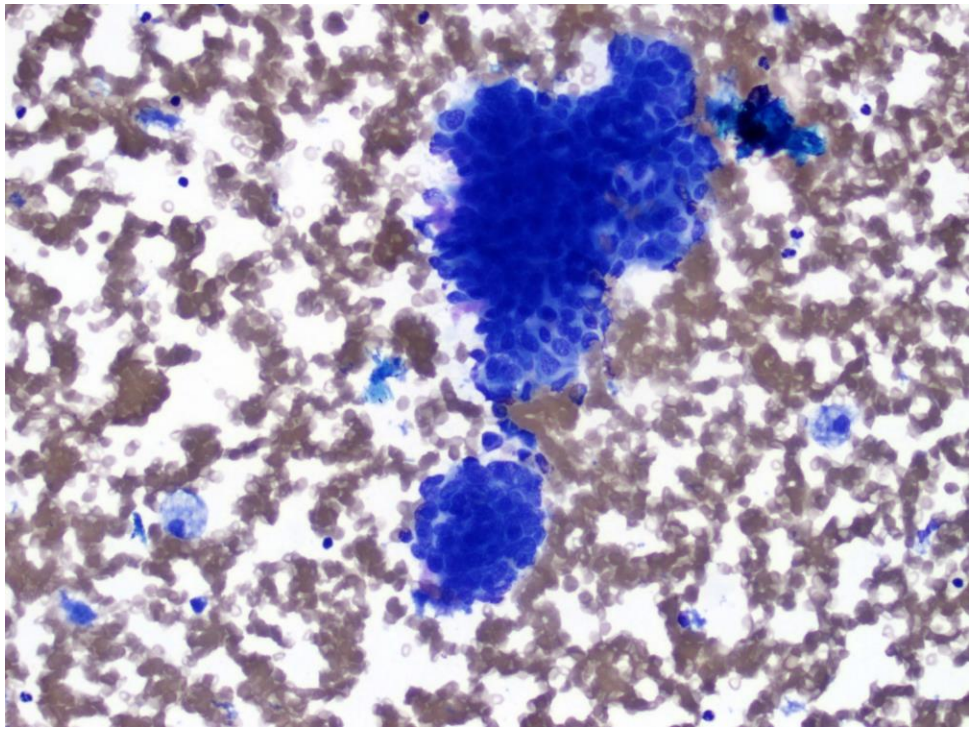
Practical Diagnostic Approach

- Basaloid cell features
- Intermediate size cells with bland cytology
- Large cells with abundant cytoplasm
- Many cytologic faces of PA
- Lymphoid-rich lesions

- Emphasizes DDx of tumors that share similar cytomorphologic features
- Helps identify limitations and pitfalls associated with FNA

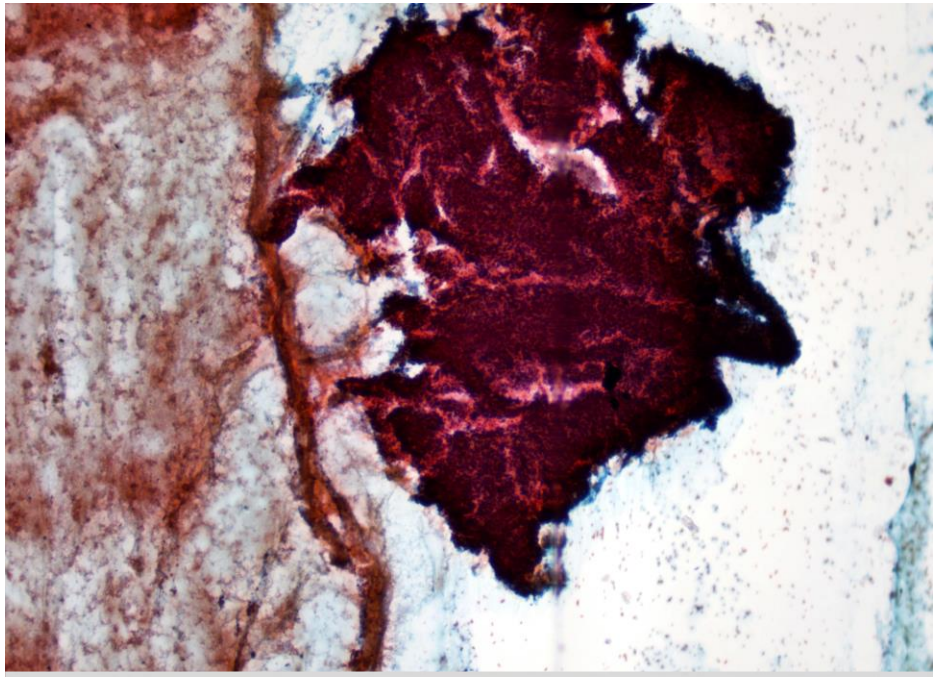
Salivary Gland Neoplasms with Basaloid Features

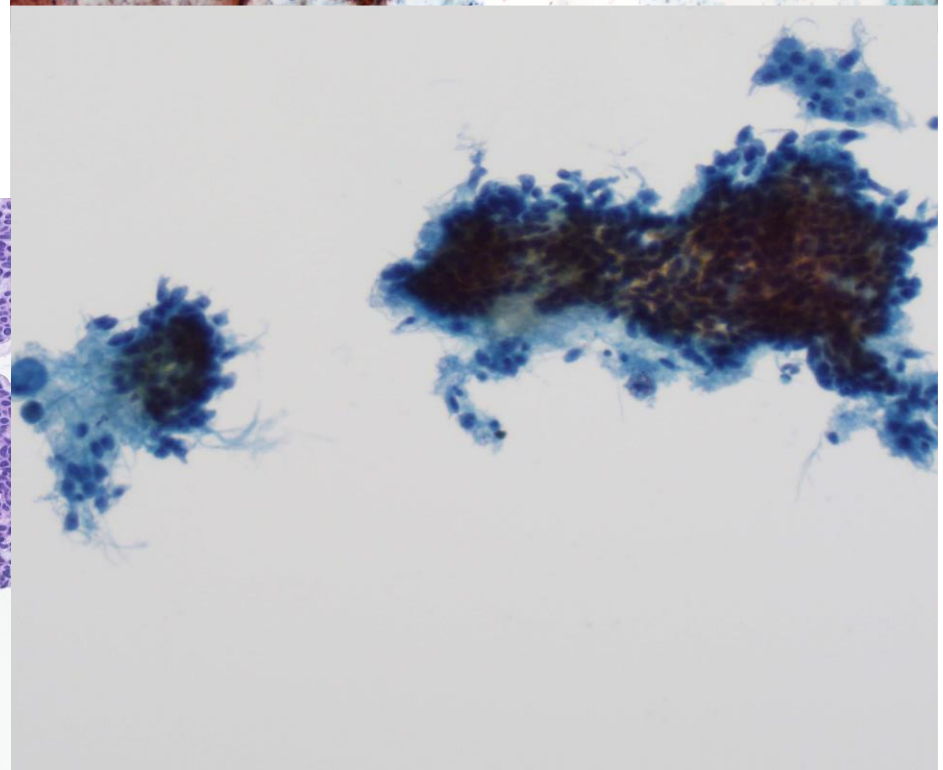
- Small sized cells, scant cytoplasm
  - Basal cell adenoma
  - Basal cell adenocarcinoma
  - Adenoid cystic carcinoma
  - Basaloid squamous cell carcinoma
  - Neuroendocrine carcinoma
  - Pleomorphic adenoma

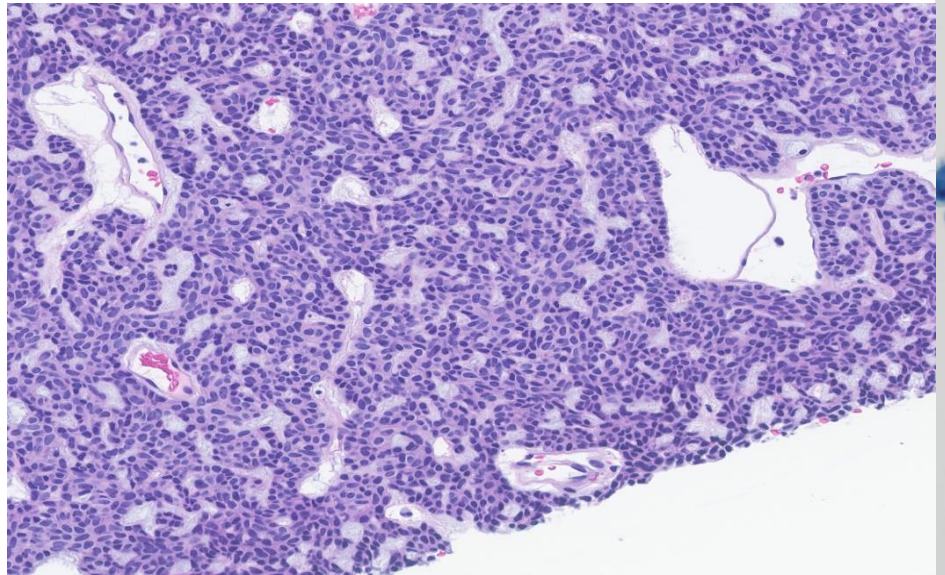


Basal Cell Adenoma

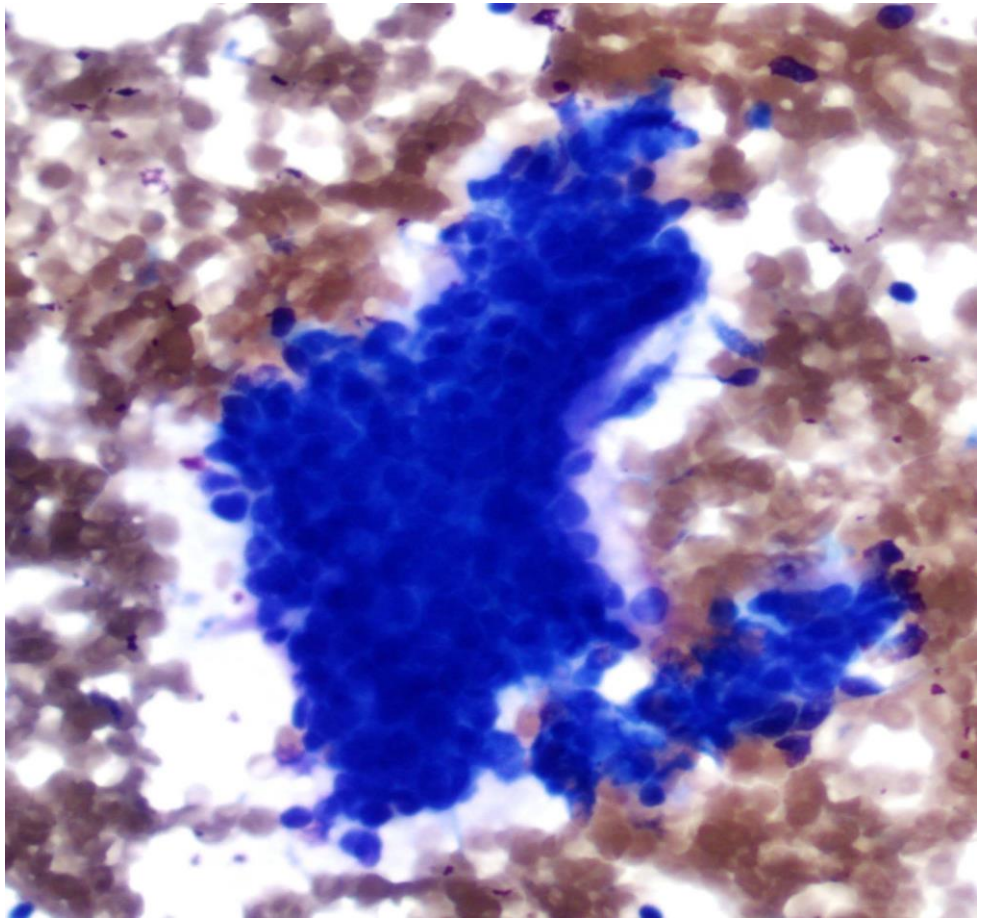
- Tightly cohesive clusters
- Branching, budding, trabeculae
- Sharp community borders
- Many naked nuclei



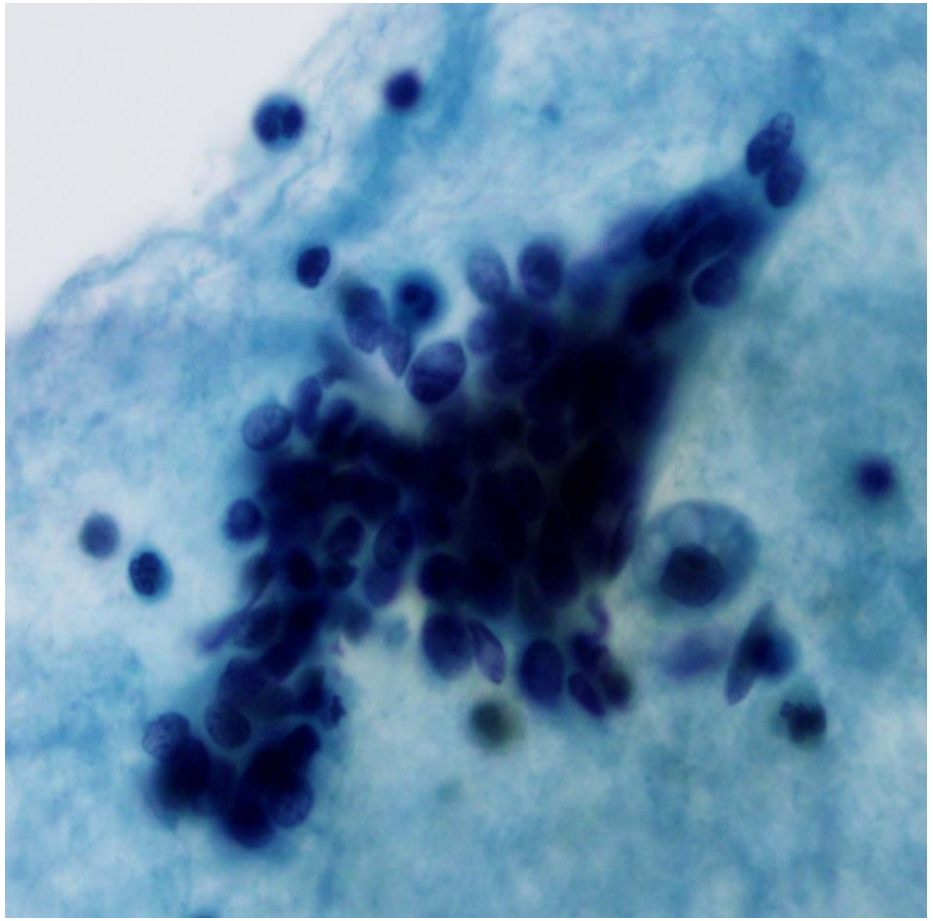




Basal Cell Adenoma



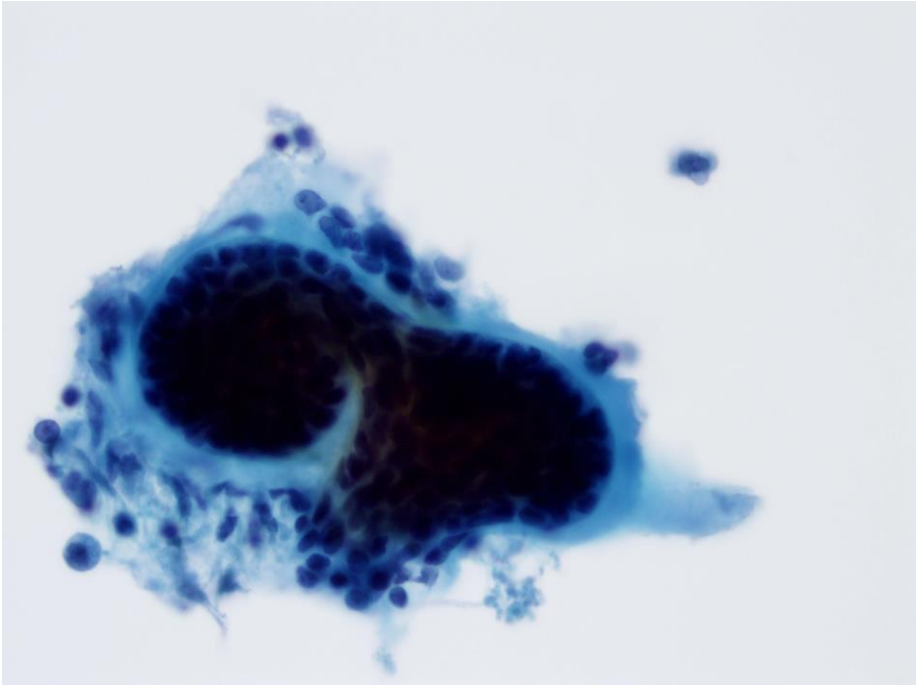
- Small cells
- Minimal cytoplasm
- Naked nuclei

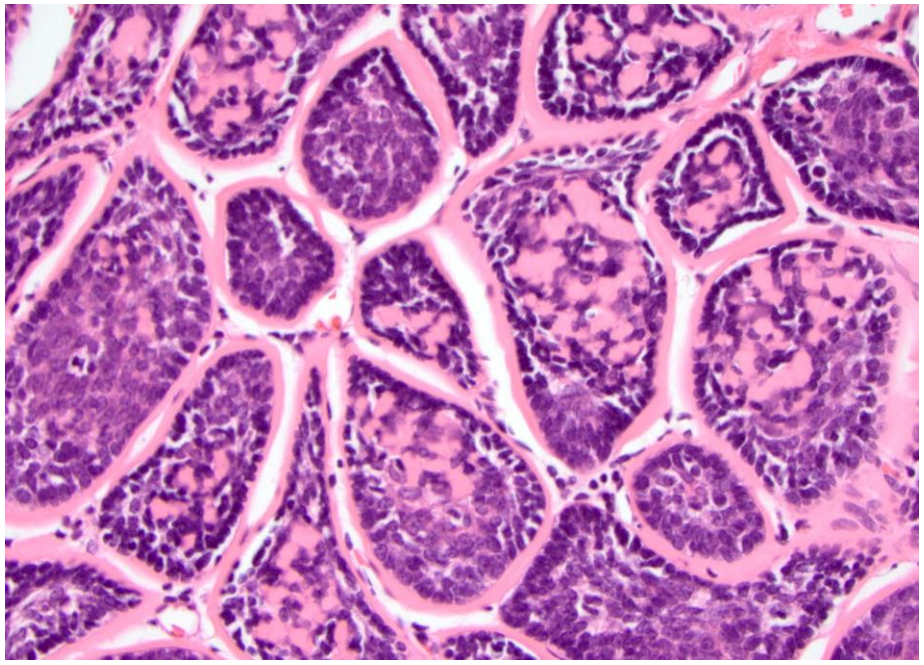


- Round to oval nuclei
- Fine-coarse chromatin
- No distinct nucleoli

Membranous basal cell adenoma

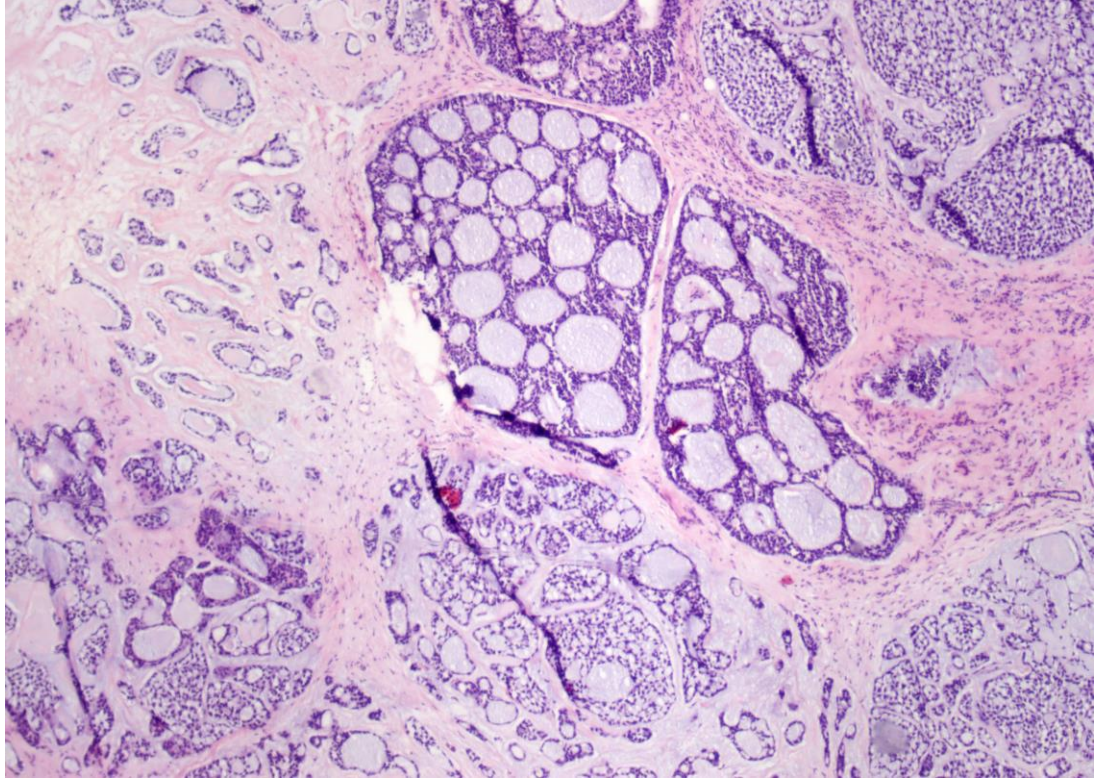
- Amorphous extracellular hyaline material may be seen at the periphery of cell clusters
- Acellular hyaline material
- Sharp interface between cells and hyaline material
- Not specific for membranous BCA

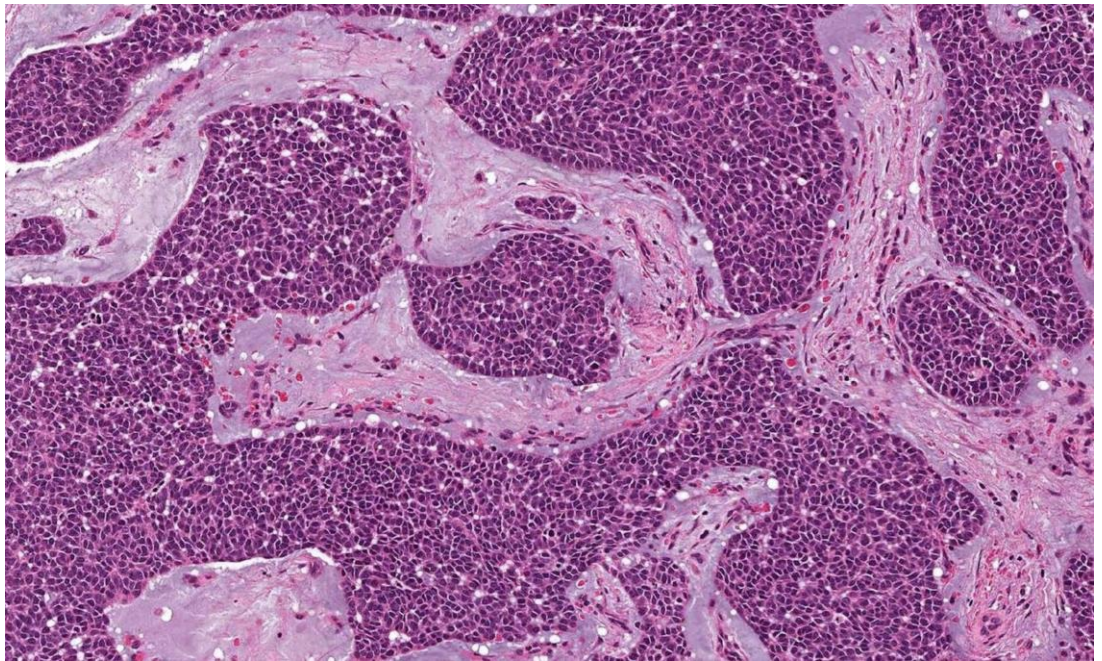




Adenoid Cystic Carcinoma

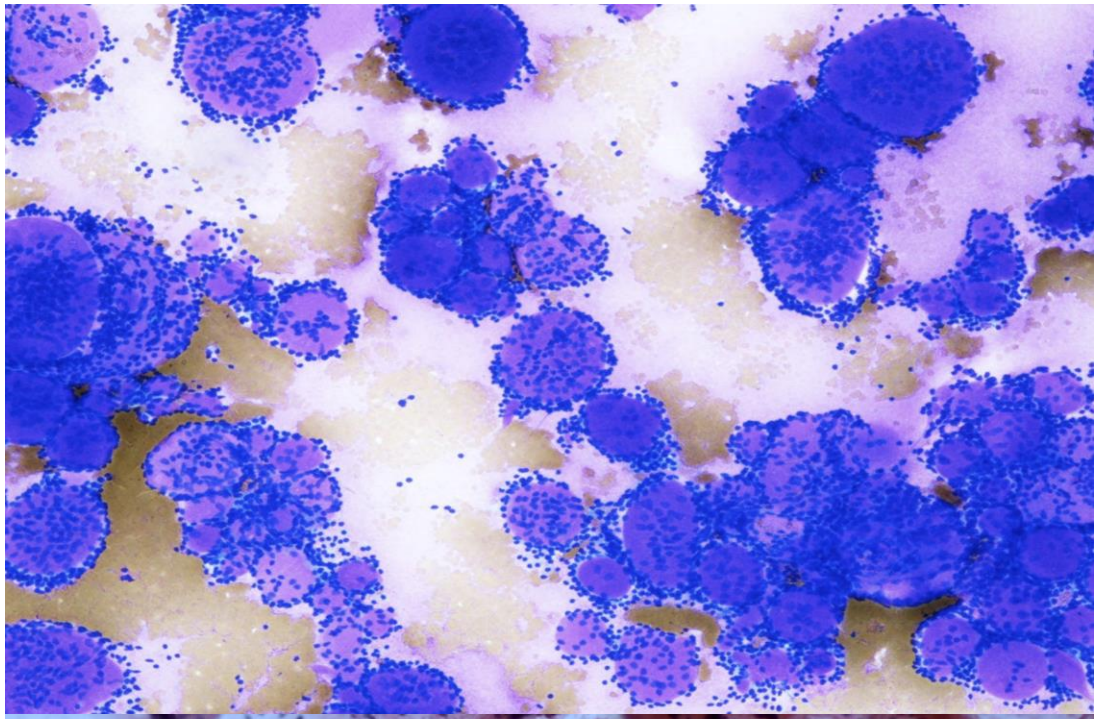
- Cribriform (Well Differentiated)
  - Clusters and branching multilayered groups of basaloid cells with cystic spaces
  - Globules of homogeneous acellular material
- Solid (Poorly Differentiated)
  - Loosely cohesive groups of cells with/out increased nuclear atypia and prominent nucleoli
  - Hyaline globules lacking

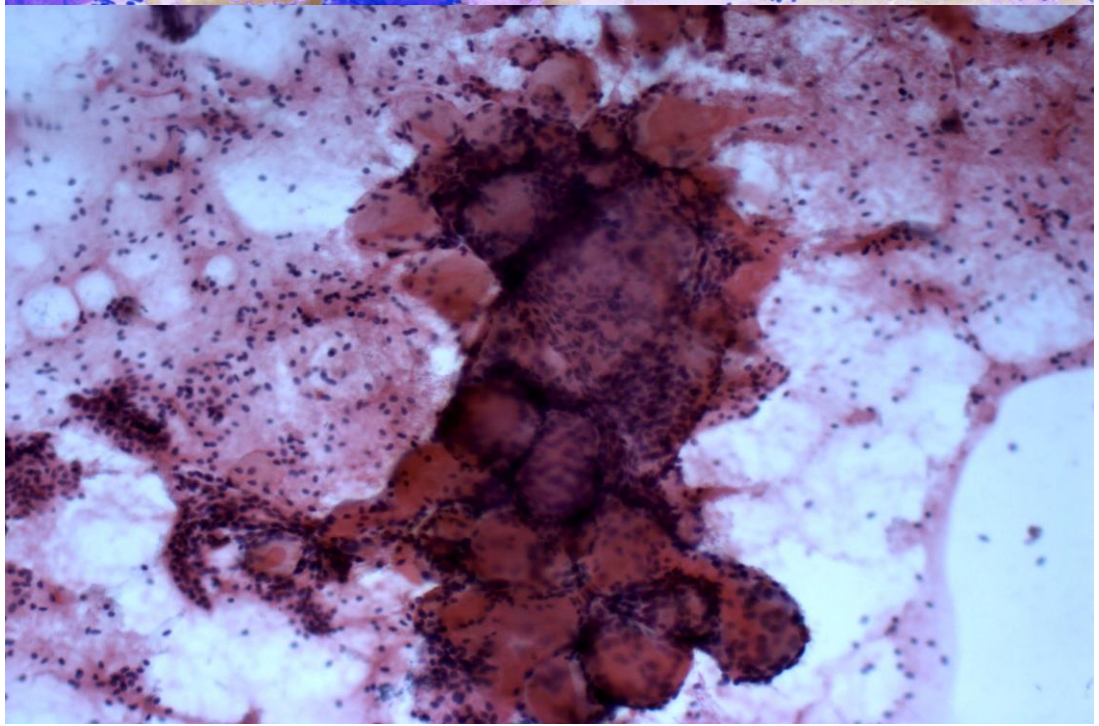




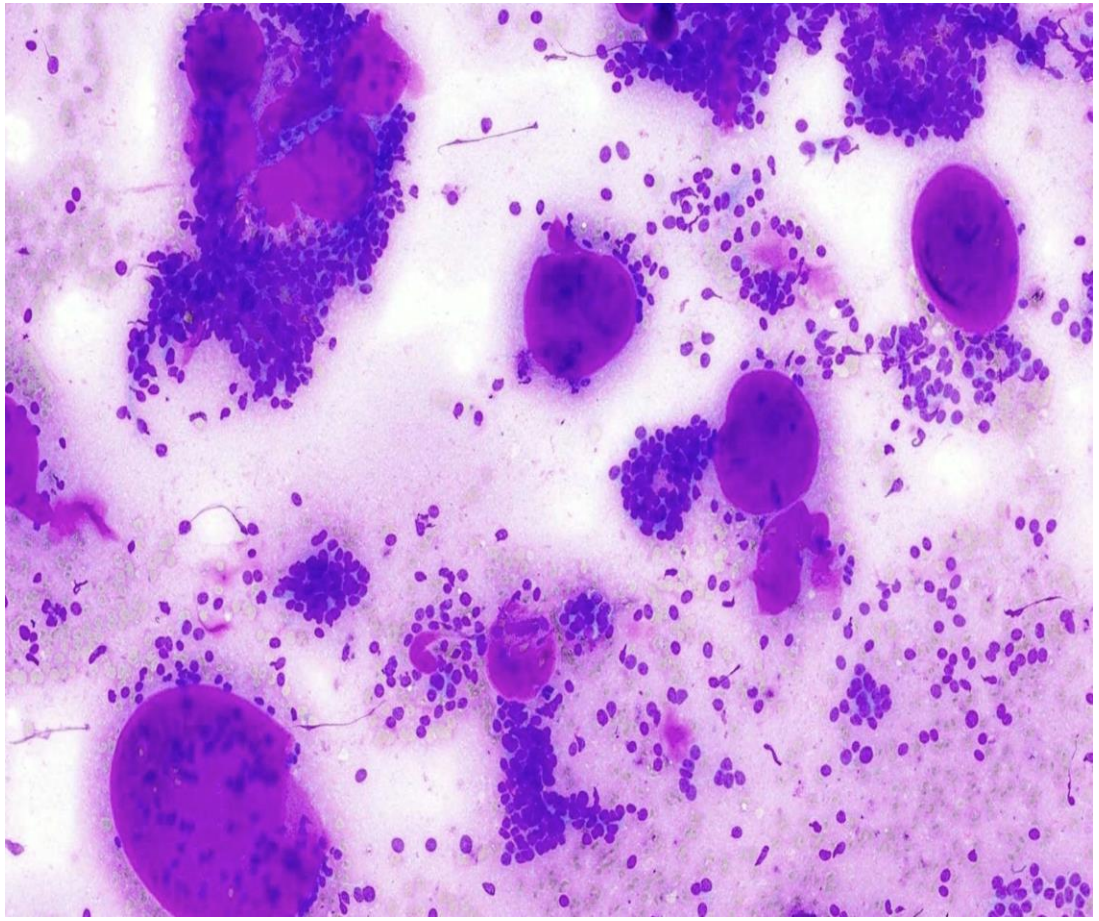
Cribriform AdCC

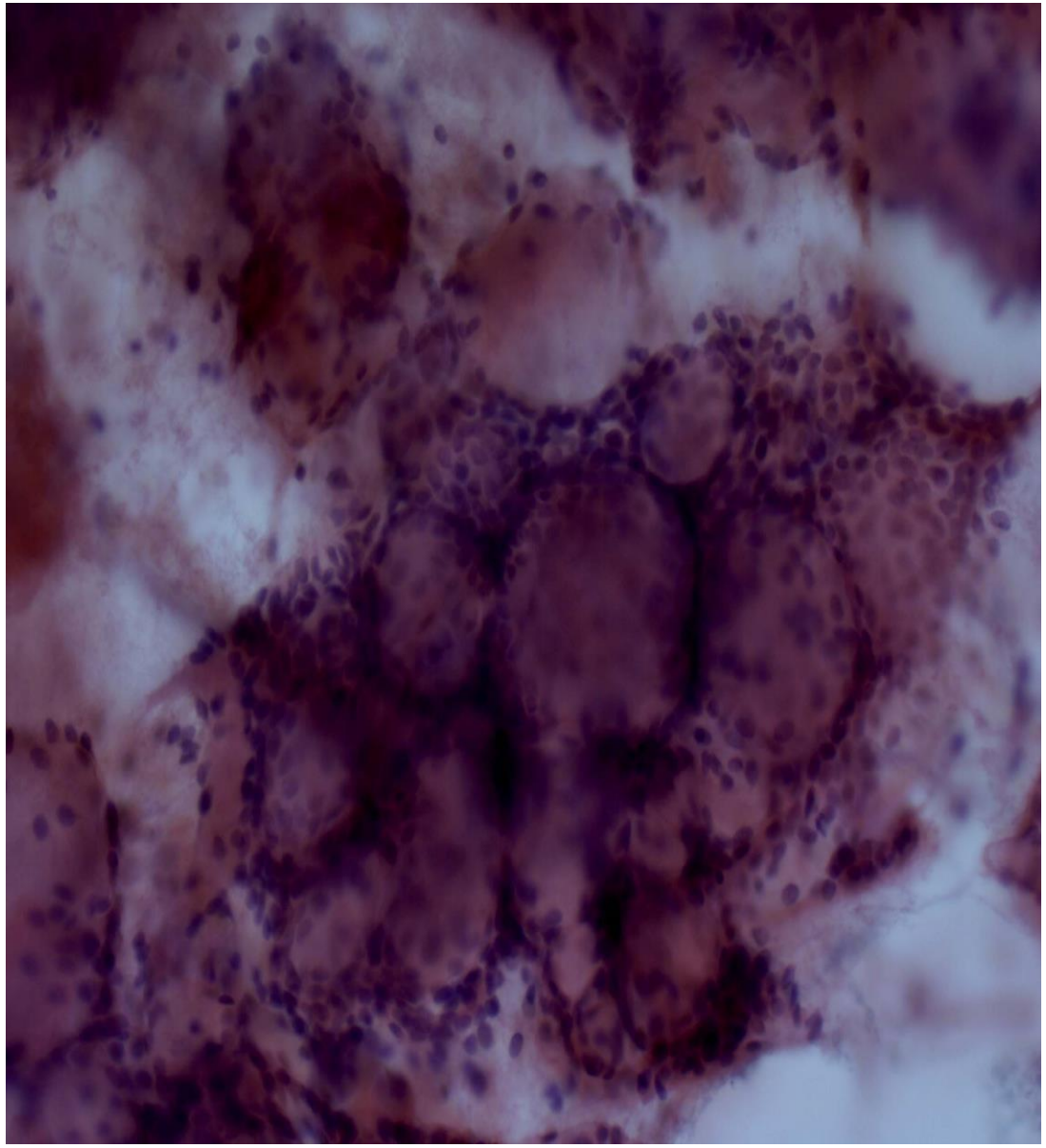
- Acellular globules
- Sharp interface
- Tubules
- Scant cytoplasm
- Round-oval nuclei
- +/- Nucleoli





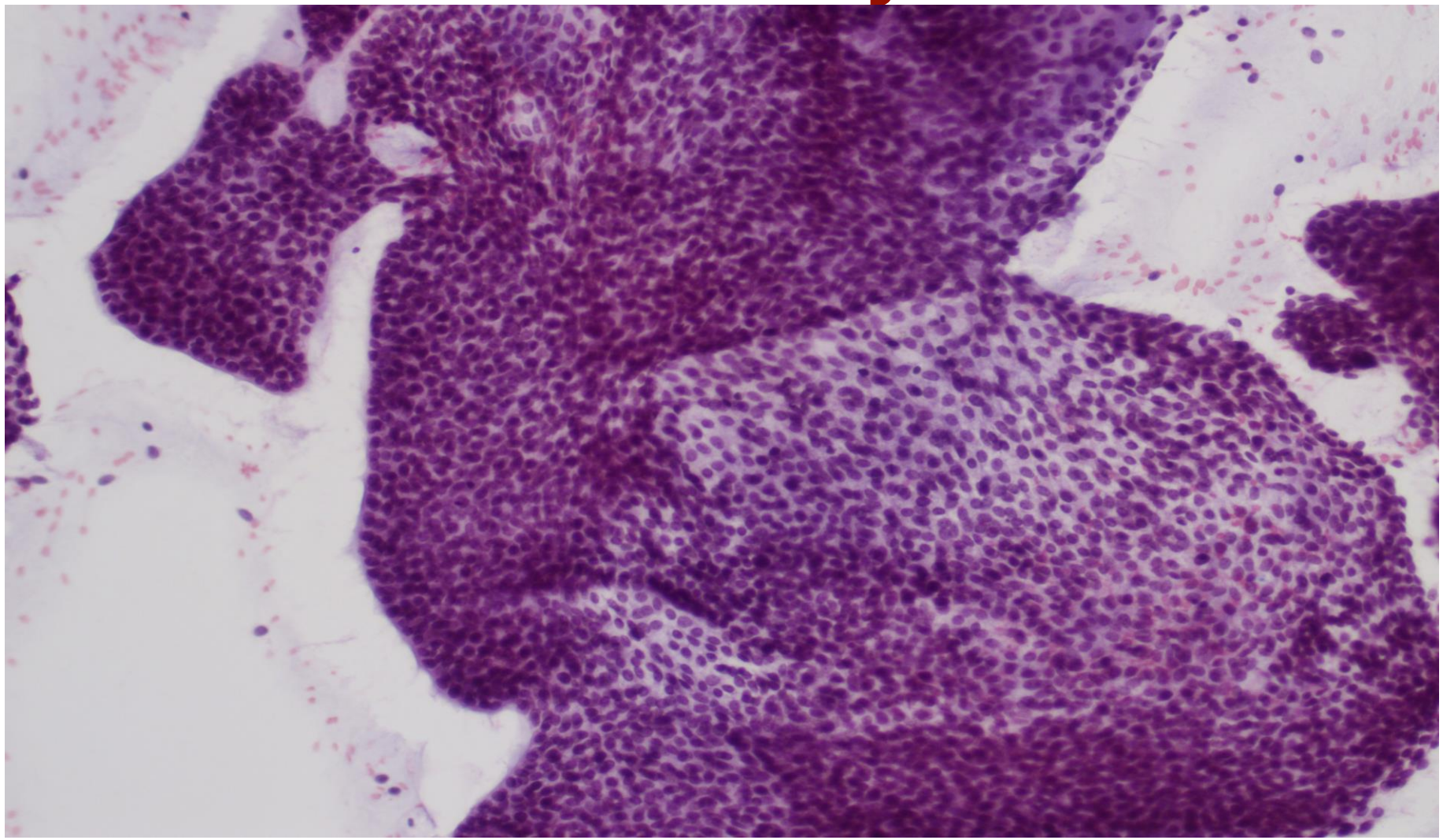
Cribriform AdCC





- Variably sized globules
- Ribbons with branching
- Naked nuclei

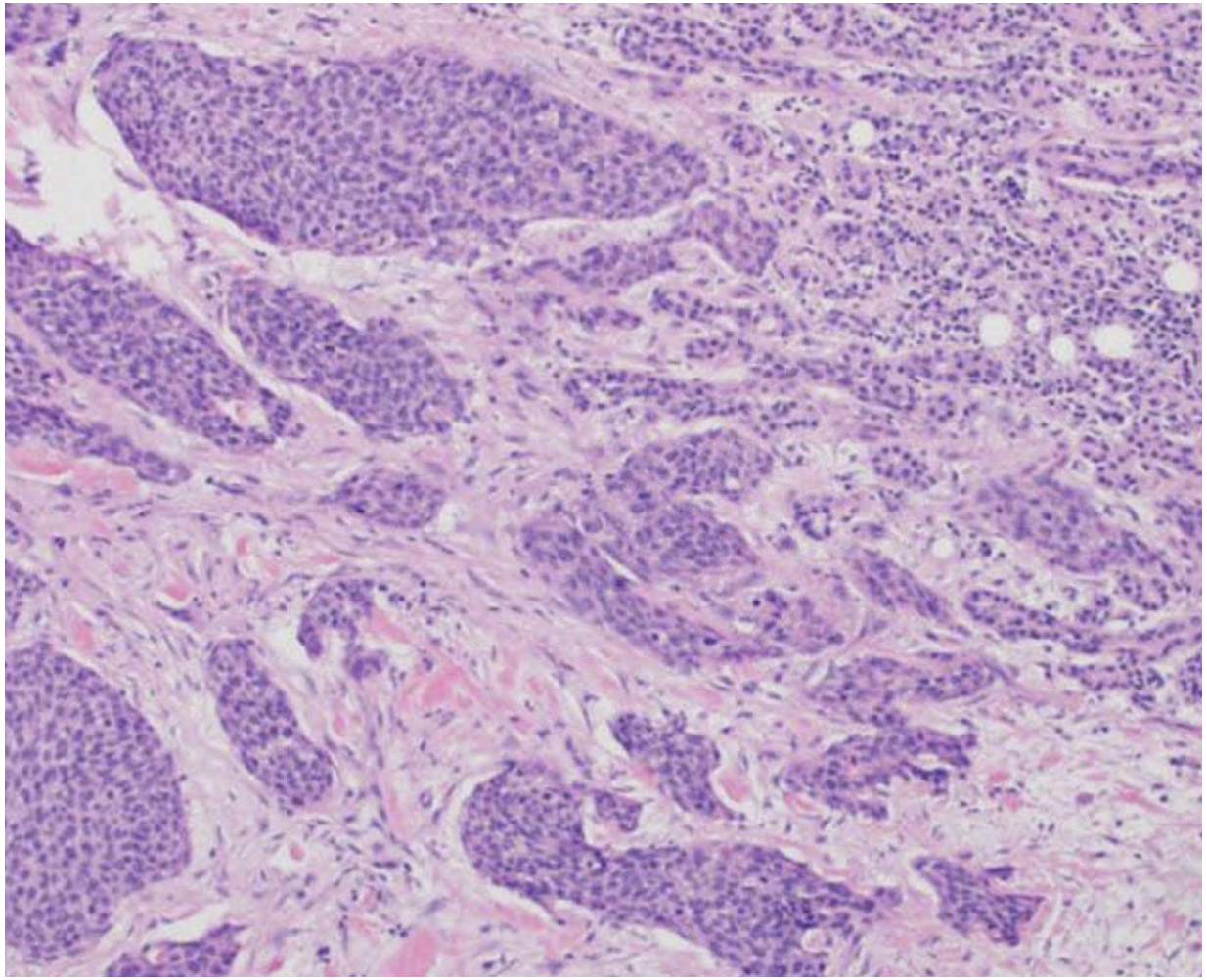
Solid Adenoid Cystic Ca



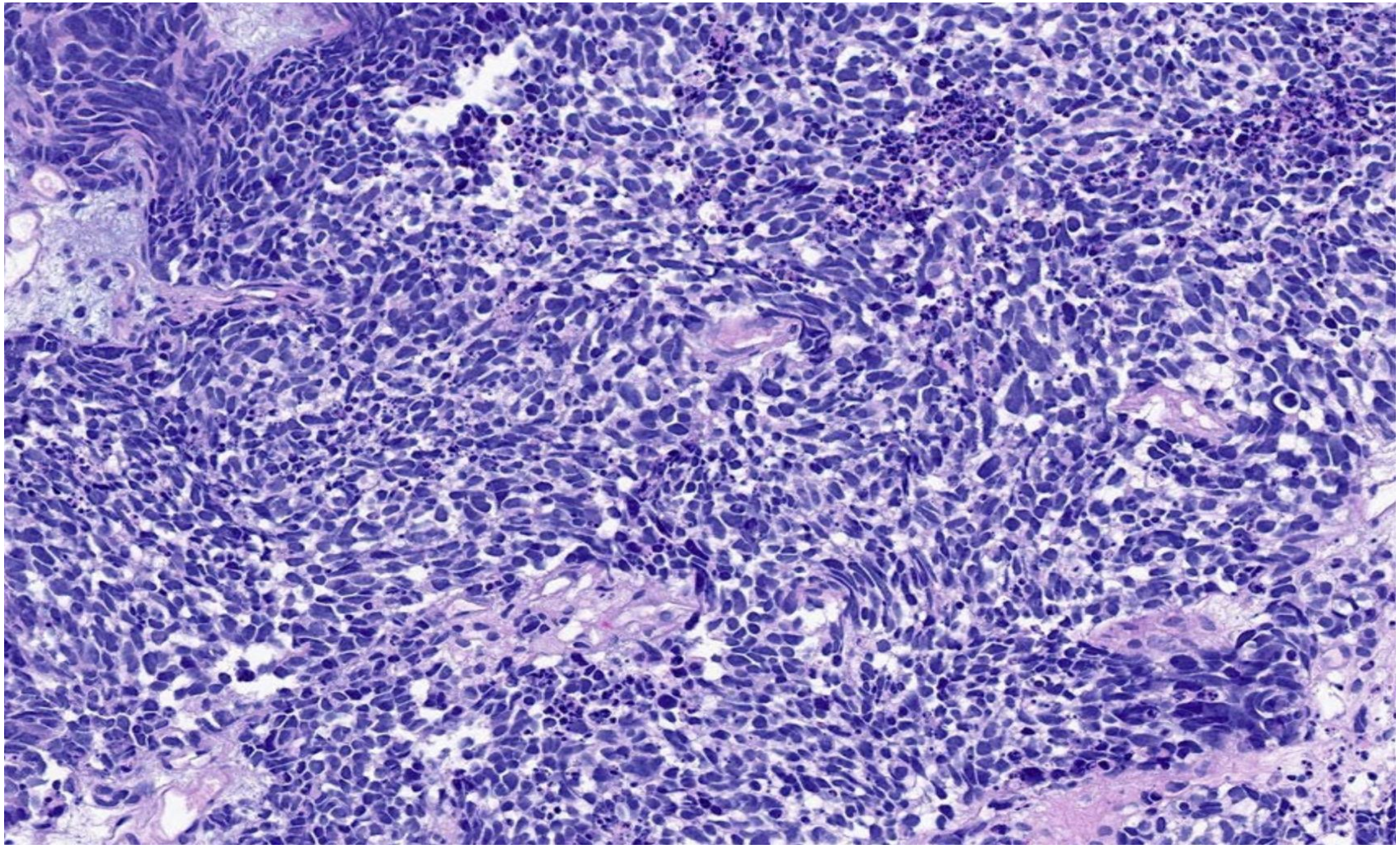
- Lacks globules and cribriforming
- Difficult to distinguish from BCA when significant atypia is absent

Metastatic Basaloid Squamous Cell Carcinoma

- Unusual for a primary salivary basaloid tumor to show pleomorphic nuclear features, so suspect metastasis



Small Cell Neuroendocrine Carcinoma



- 1-2% of all SG tumors
- Pulmonary and cutaneous origins must be excluded

Differential Diagnosis of Basaloid Neoplasms

- **With significant atypia**
  - Poorly differentiated carcinoma, NOS
  - Basaloid squamous cell ca
  - Small cell carcinoma
  - Adenoid cystic carcinoma
  - Basal cell adenocarcinoma
  - Metastatic carcinoma
    - Thyroid, breast, lung
- **Without significant atypia**
  - Cellular PA
  - Basal cell adenoma
  - Adenoid cystic carcinoma
  - Basal cell adenocarcinoma
  - Metastatic carcinoma

*Atypia: moderate-severe pleomorphism and/or large prominent nucleoli

Neoplasms with Intermediate Size Cells and Bland Cytology

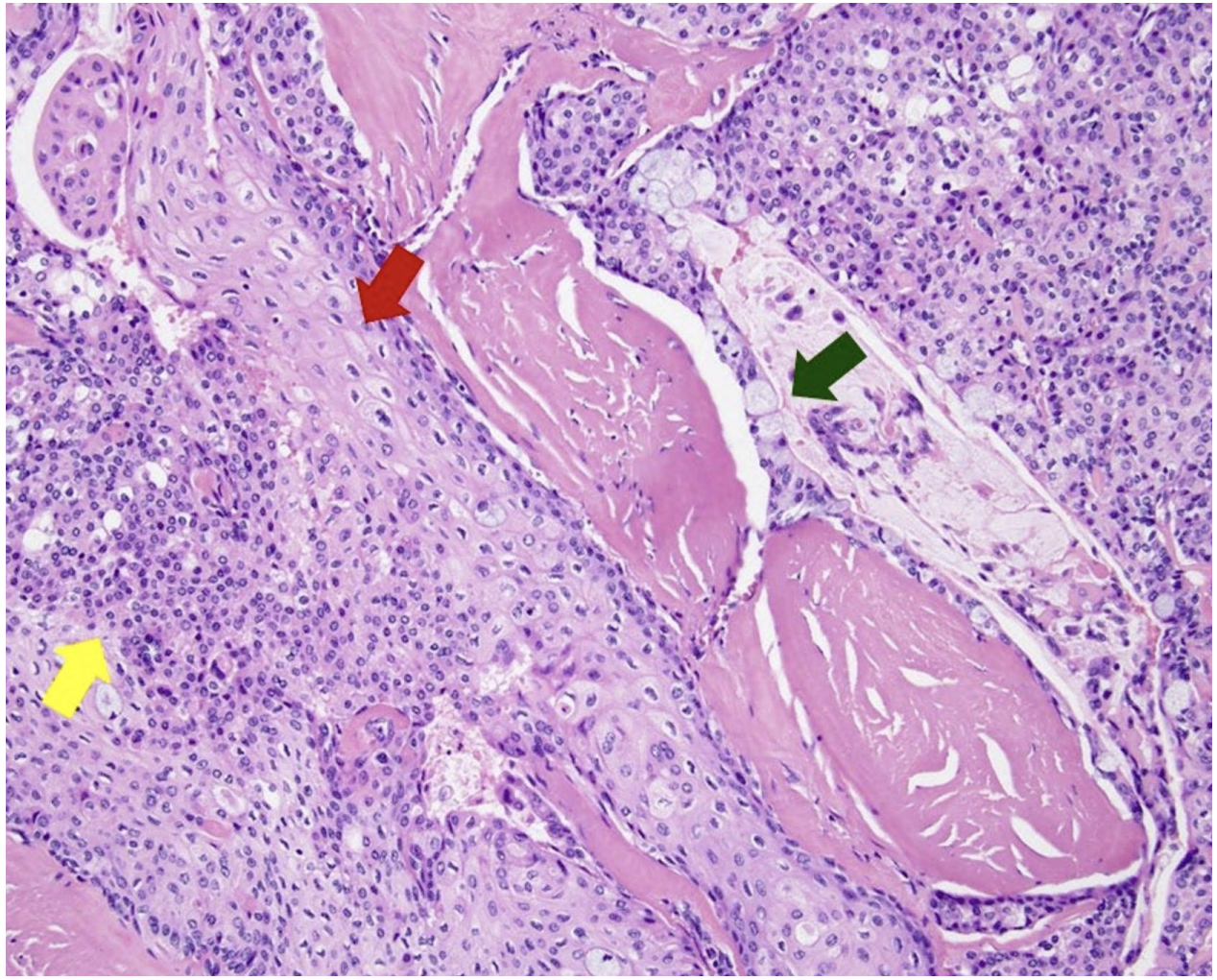
- Moderate amount of Cytoplasm
- No significant atypia
- Include cystic and squamous lesions

Neoplasms with Intermediate Size Cells and Bland Cytology

- Differential diagnosis:
  - Low grade mucoepidermoid carcinoma
  - Pleomorphic adenoma
  - Epithelial-myoepithelial carcinoma
  - Polymorphous adenocarcinoma
  - Chronic sialadenitis with squamous and mucous metaplasia
  - Lymphoepithelial cyst
  - Cystic metastatic squamous cell carcinoma

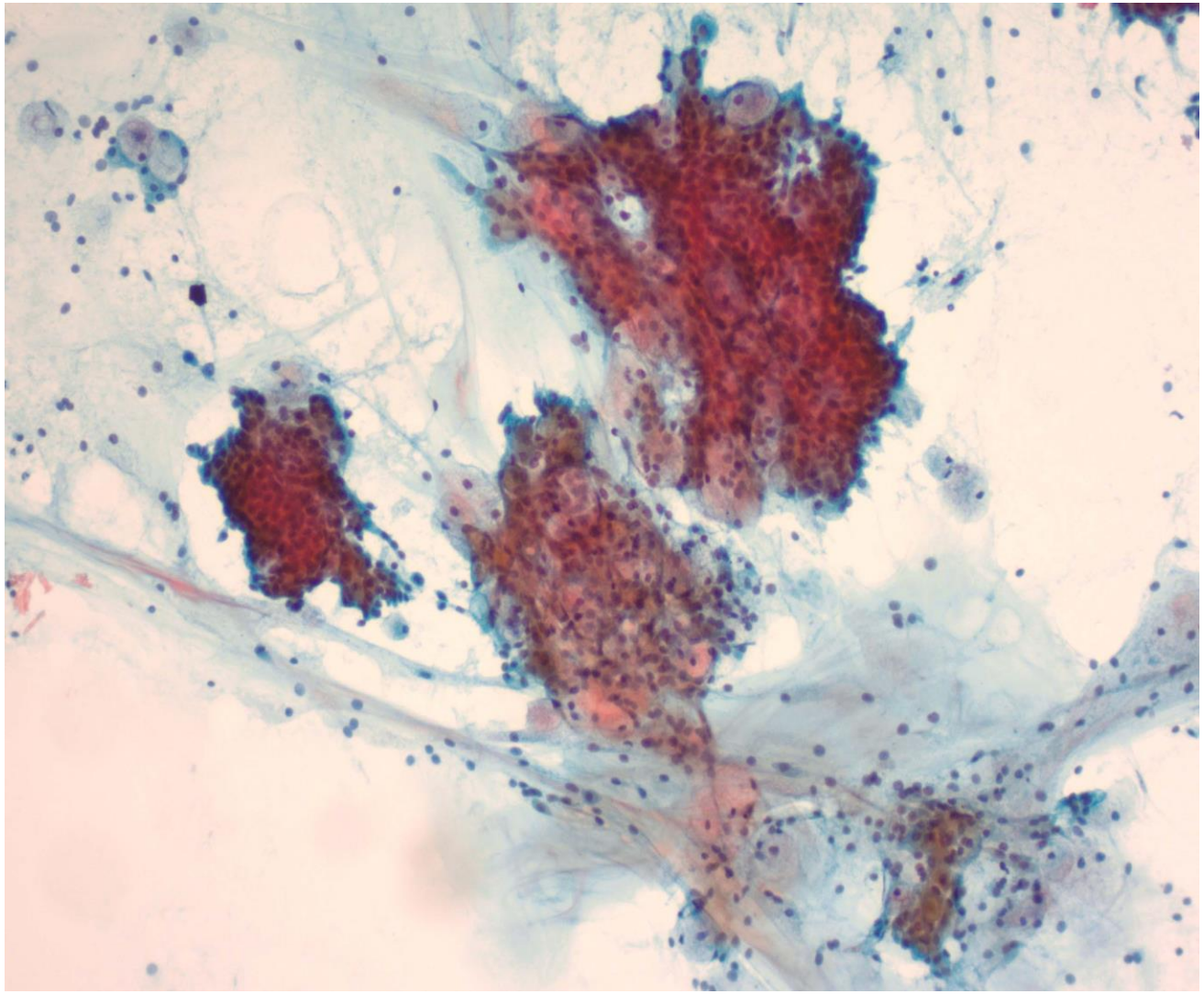
Low grade mucoepidermoid carcinoma

- Most common salivary gland malignancy
- Occurs in all ages, peak=20-40 yrs
- Cytology
  - Mucus producing cells
  - Intermediate cells
  - Squamous cells



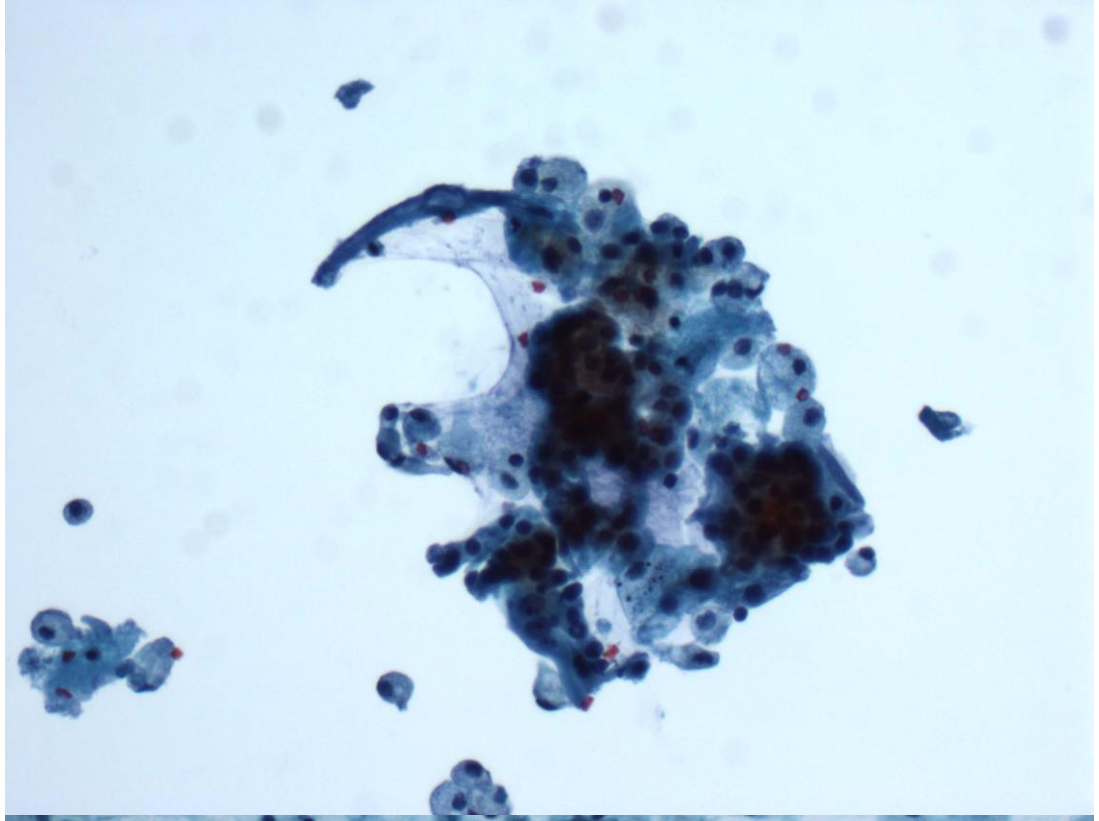
Low grade mucoepidermoid carcinoma

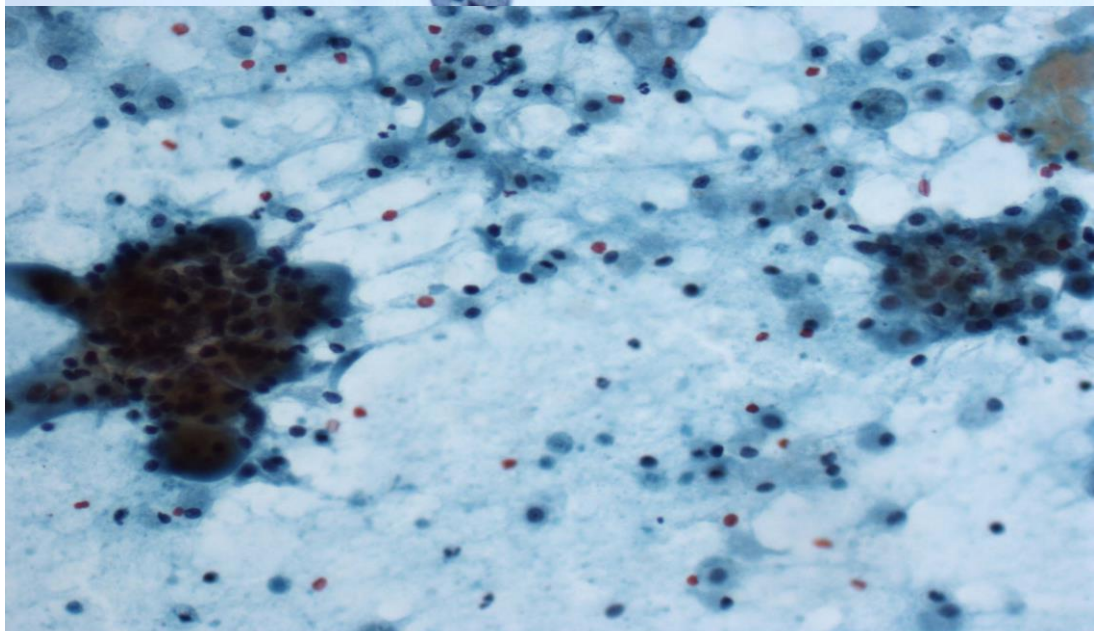
- Flat sheets and 3D clusters
- Variable stringy mucin



Low grade mucoepidermoid carcinoma

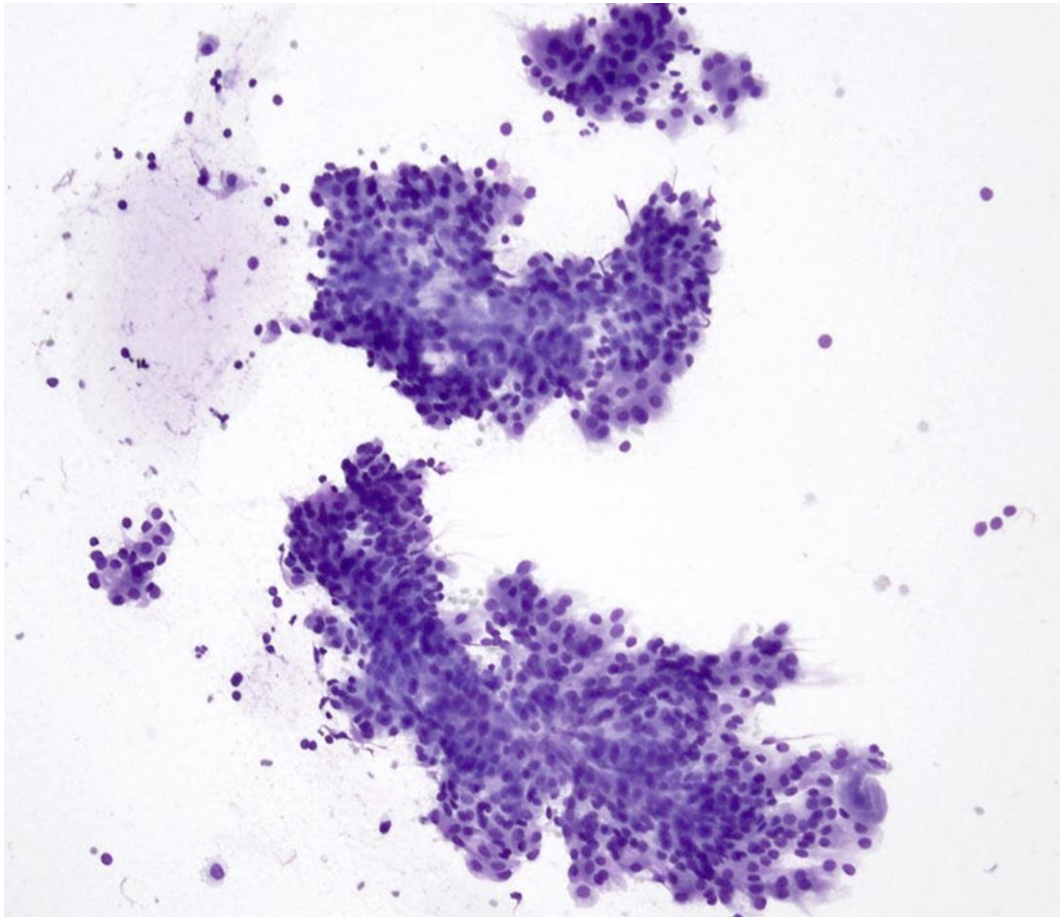
- Mucus cells: resemble macrophages
- Intermediate cells: ductal appearance





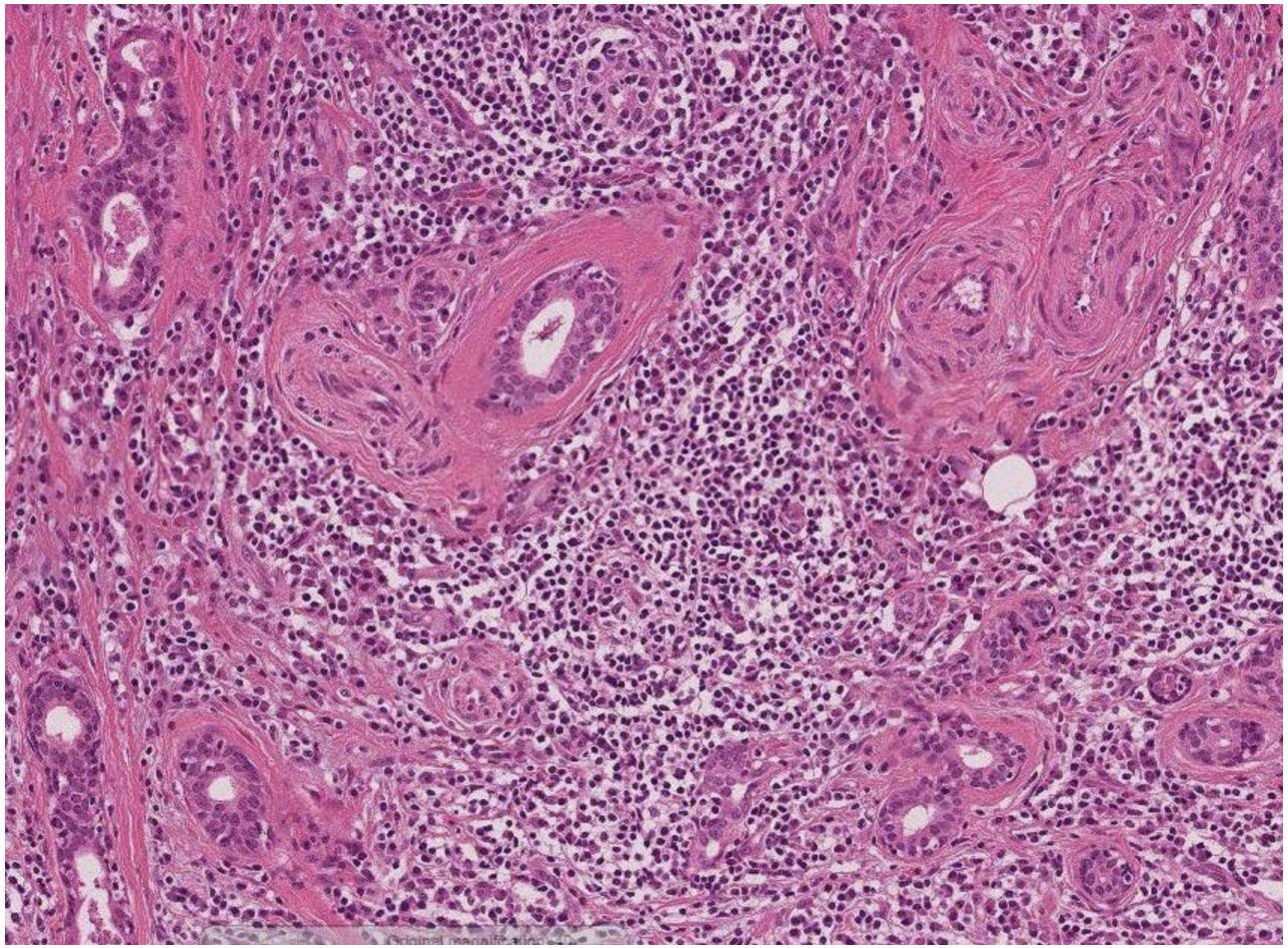
Low grade mucoepidermoid carcinoma

- Squamous cells have a metaplastic appearance
- No fully keratinized epidermoid cells
- Intermediate cells usually predominate – prominent nucleoli



Chronic Sialadenitis

- Usually results from stones or post surgical scarring
- More common in submandibular gland
- Chronic inflammation with fibrosis and acinar atrophy
- Squamous and mucinous metaplasia

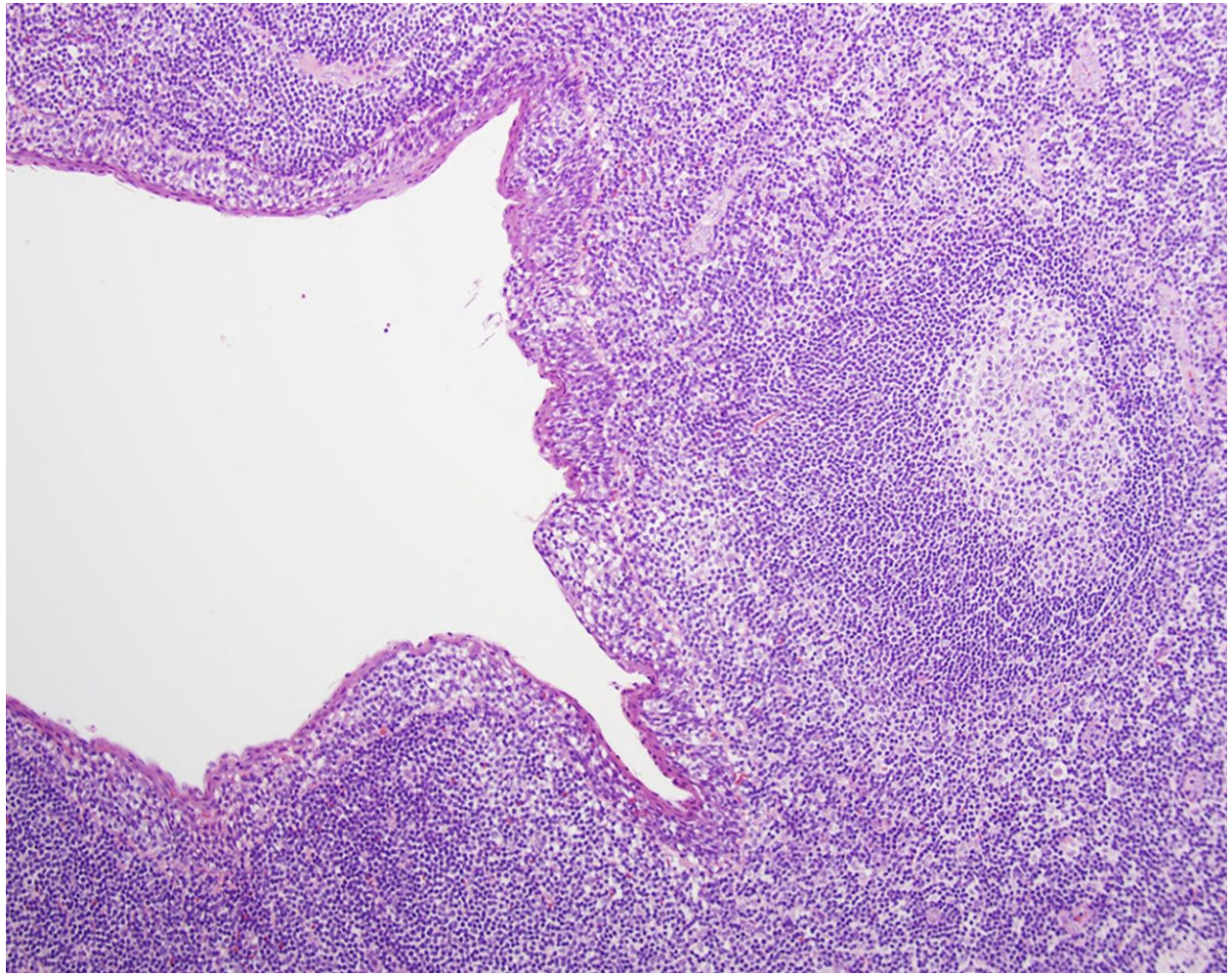


Chronic Sialadenitis

- Ductular proliferation in CS can be confused with basaloid neoplasm
- Basaloid neoplasm: higher cellularity and many naked nuclei
- Sialadenitis: low cellularity and inflammatory cells

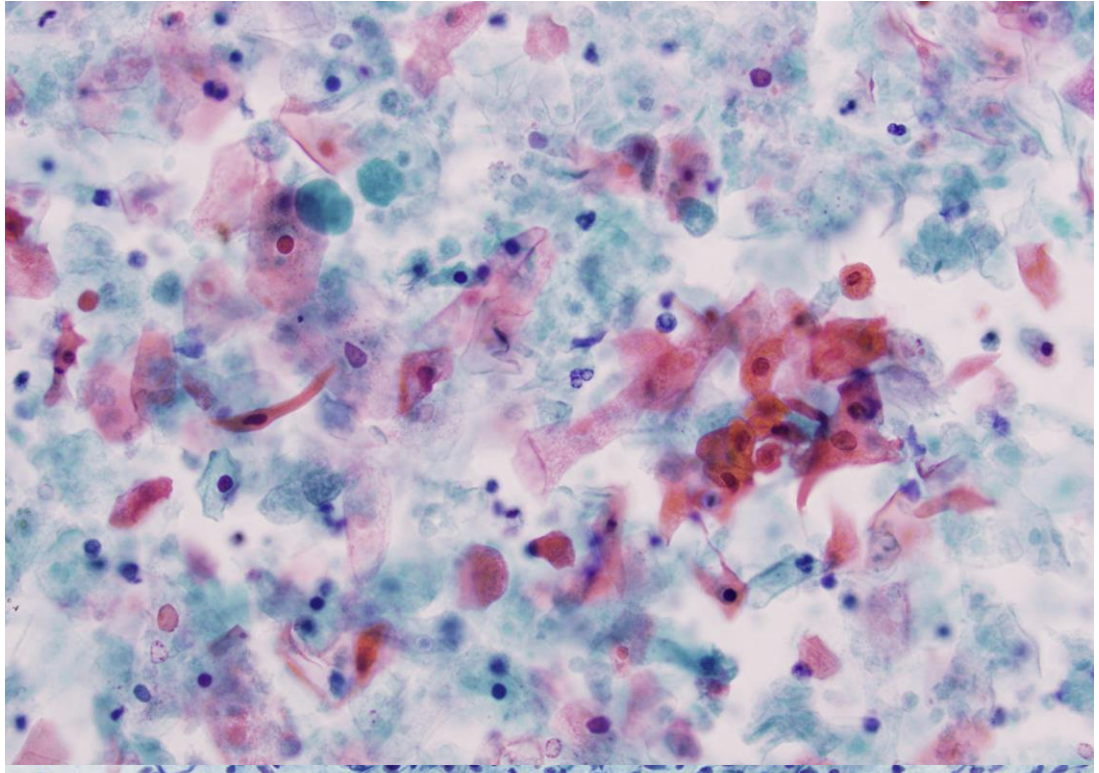
Lymphoepithelial cyst

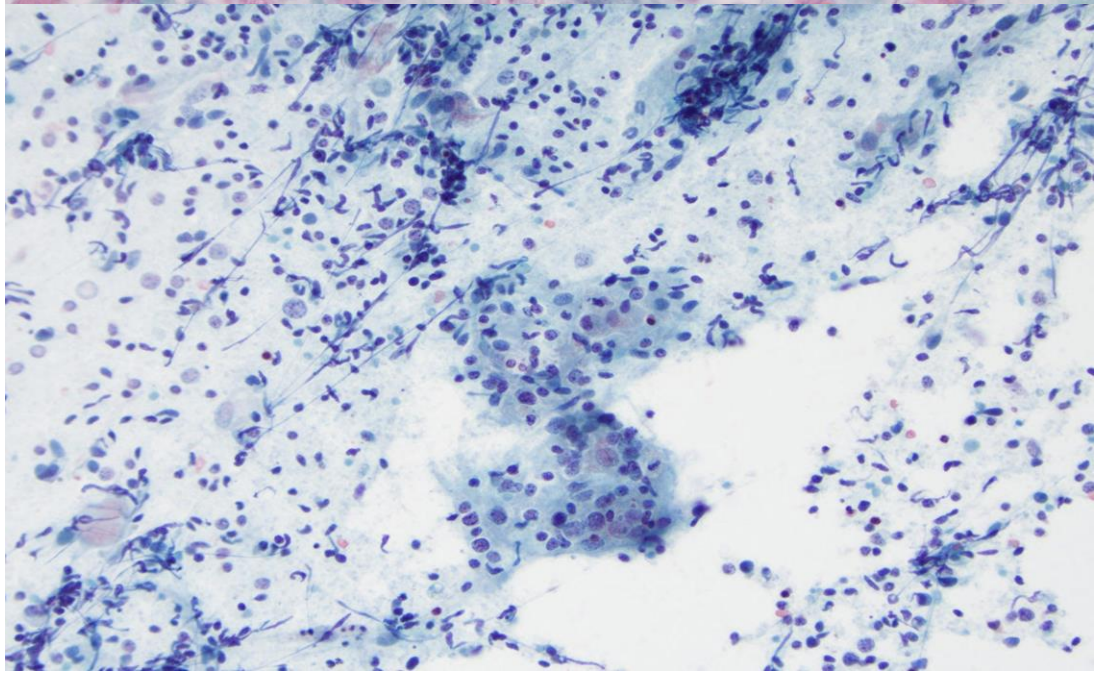
- Most common cystic lesion of lateral neck
  - Anterior border of sternocleidomastoid
- Occasionally in and around parotid gland
- Any age, more common in early adult life



Lymphoepithelial cyst

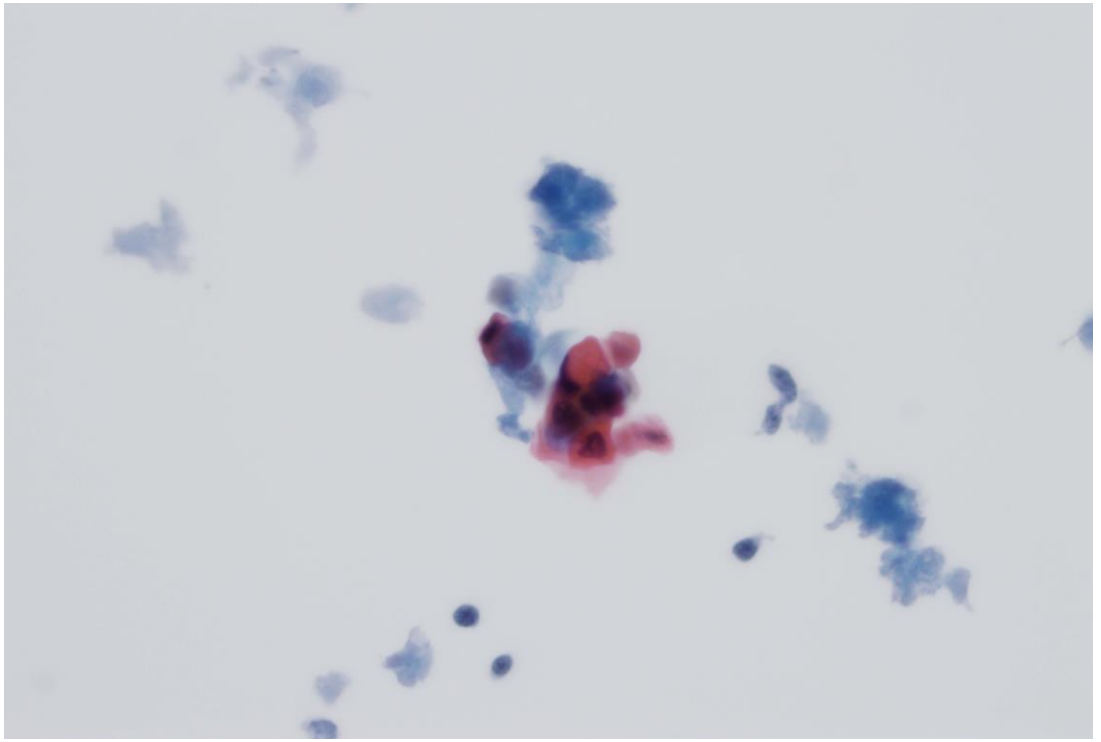
- Anucleated and nucleated squamous cells and neutrophils
- Lymphocytes not commonly seen
- Degenerative atypia

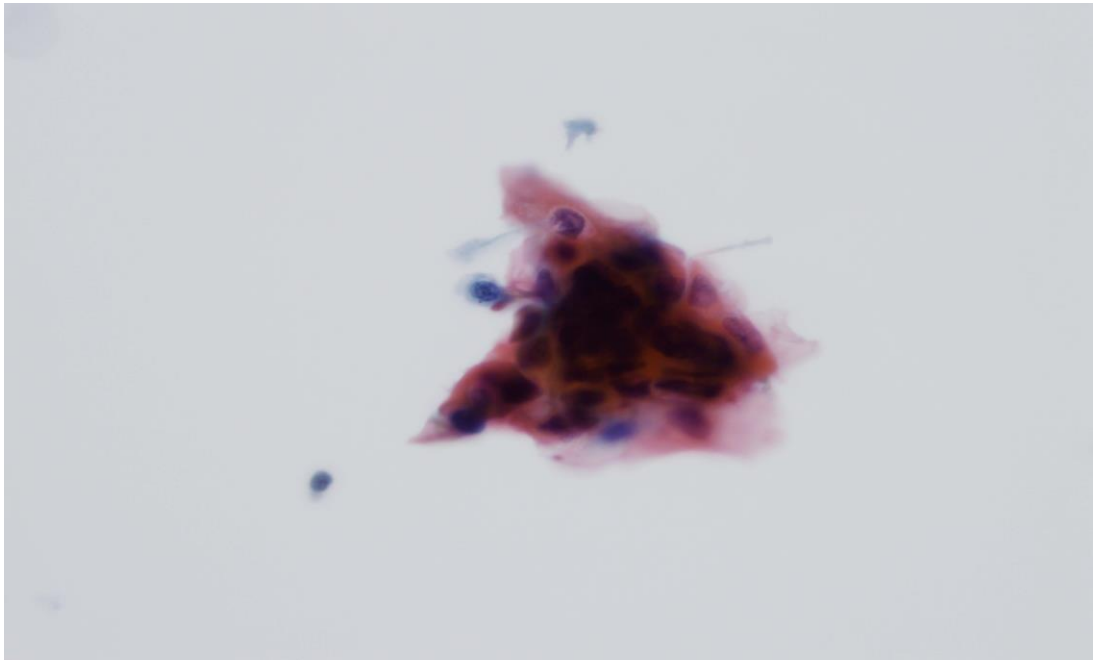




Cystic Metastatic Squamous cell Carcinoma

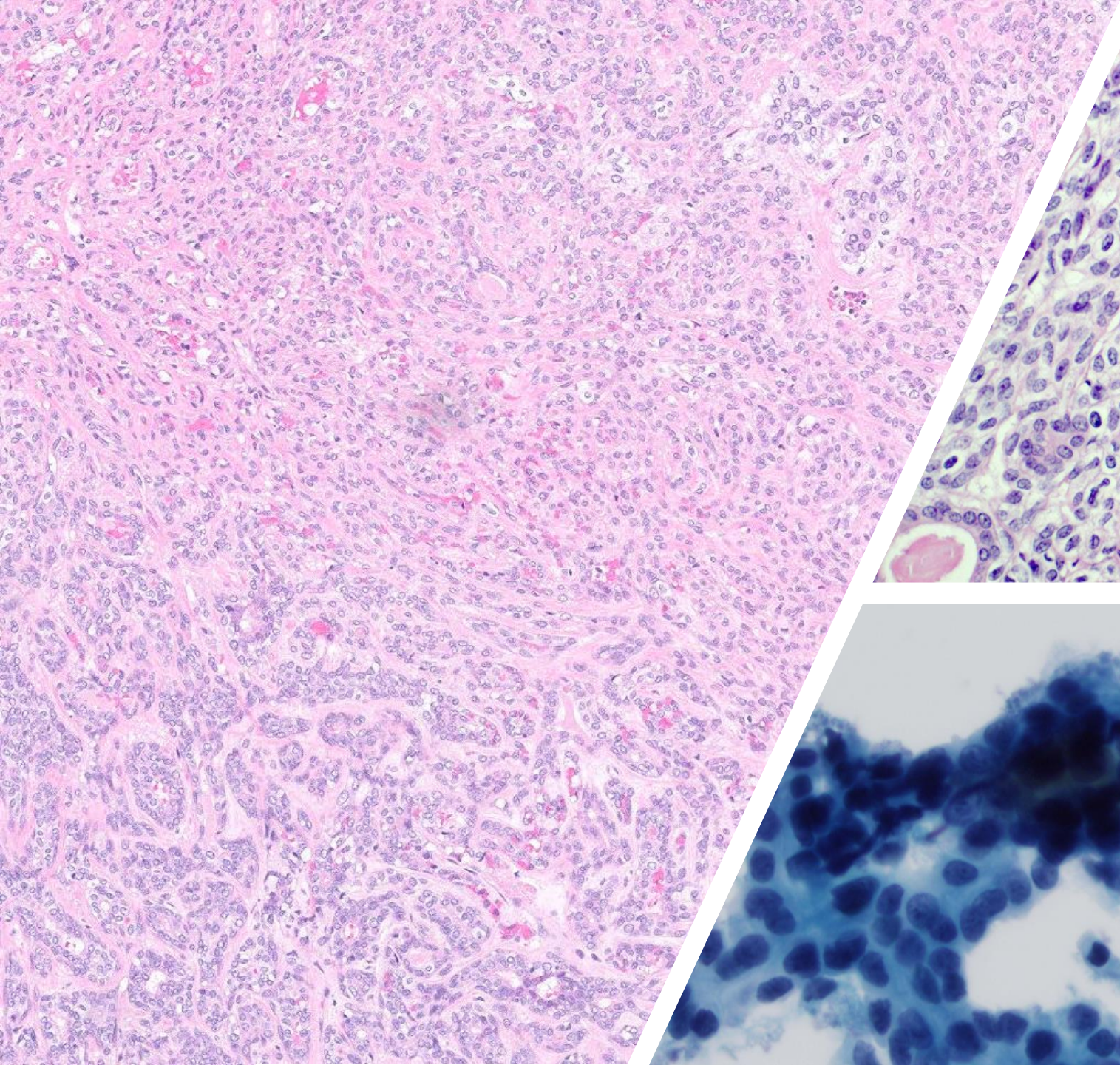
- Markedly atypical squamous cells
  - Clusters and/or single cells
- Potential primary sites: tonsils, base of tongue, nasopharynx
- Ancillary studies: p16, HR-HPV CISH

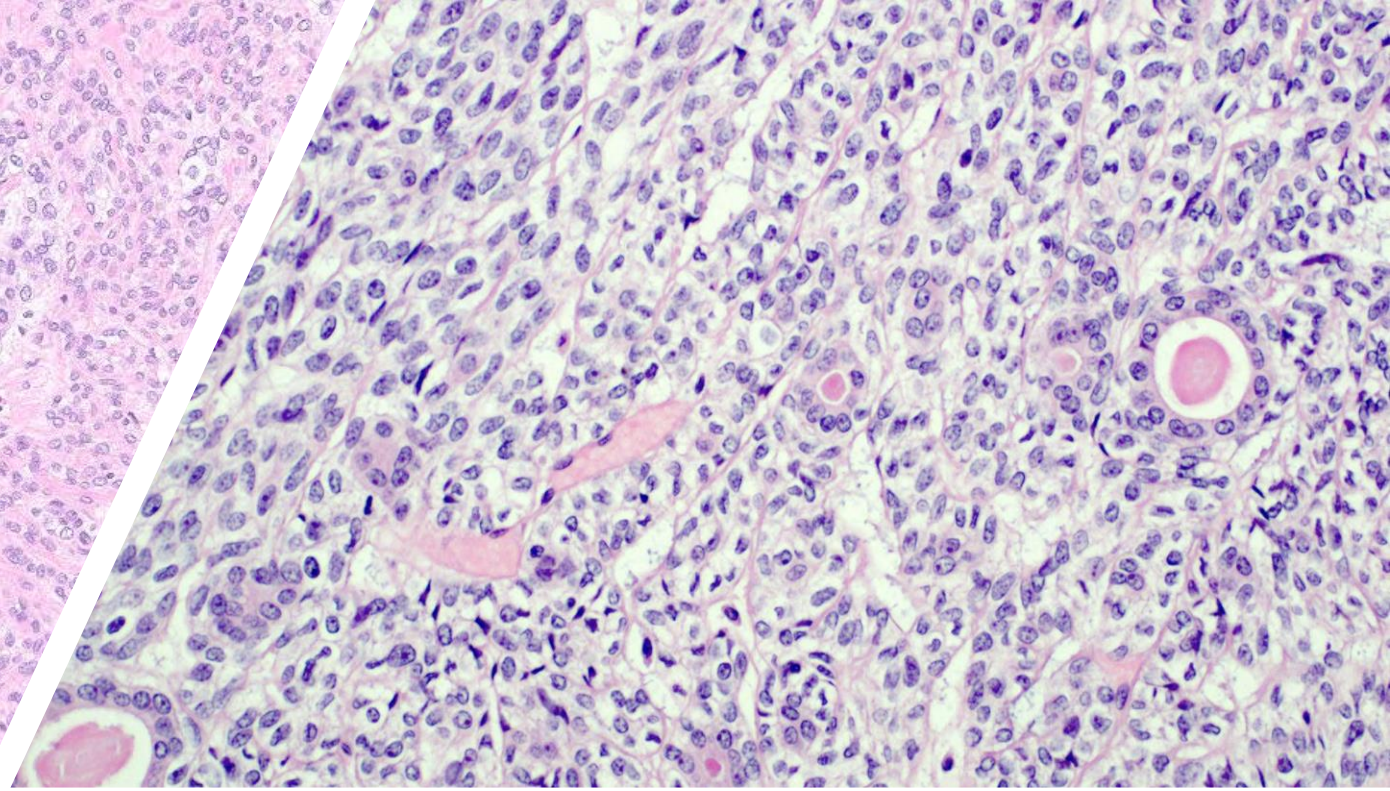


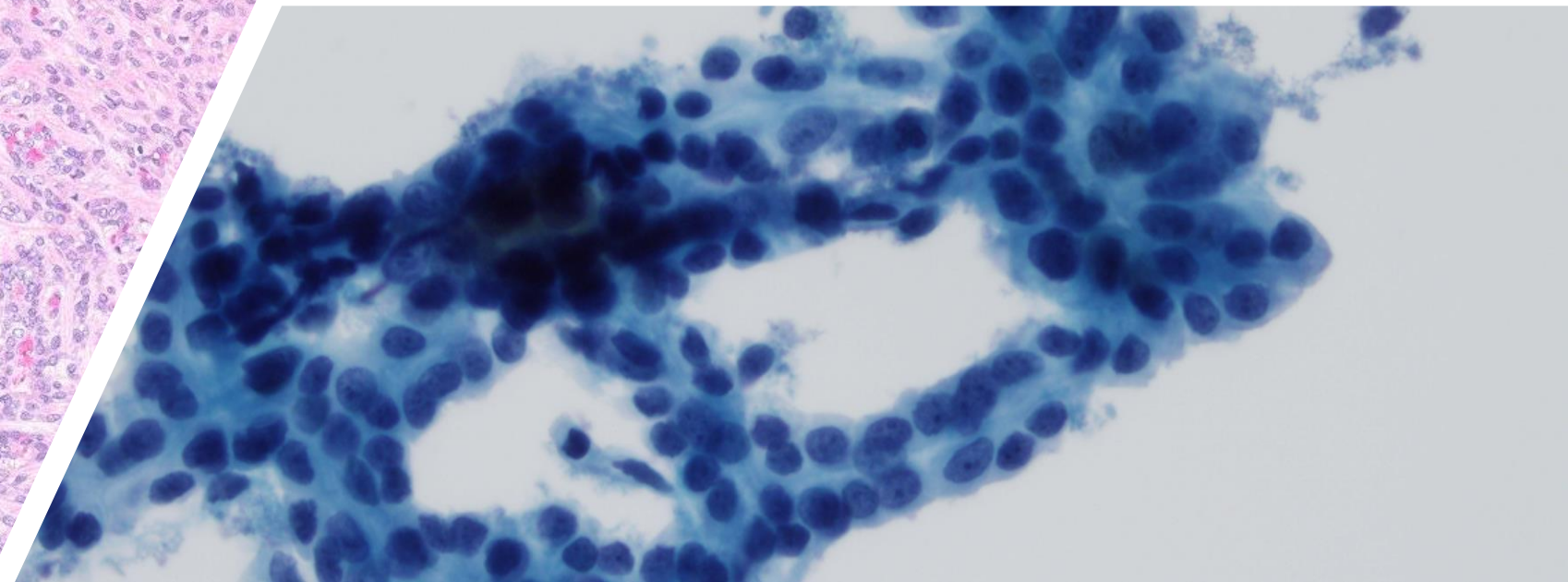


Epithelial-Myoepithelial Carcinoma

- Two distinct cell populations
  - Ductal: tightly cohesive basaloid cells
  - Myoepithelial: less cohesive large pale (clear) cells
- Occasionally predominance of clear or basaloid cells
- Variable acellular mesenchymal component
- Background mixed naked nuclei or basaloid cells

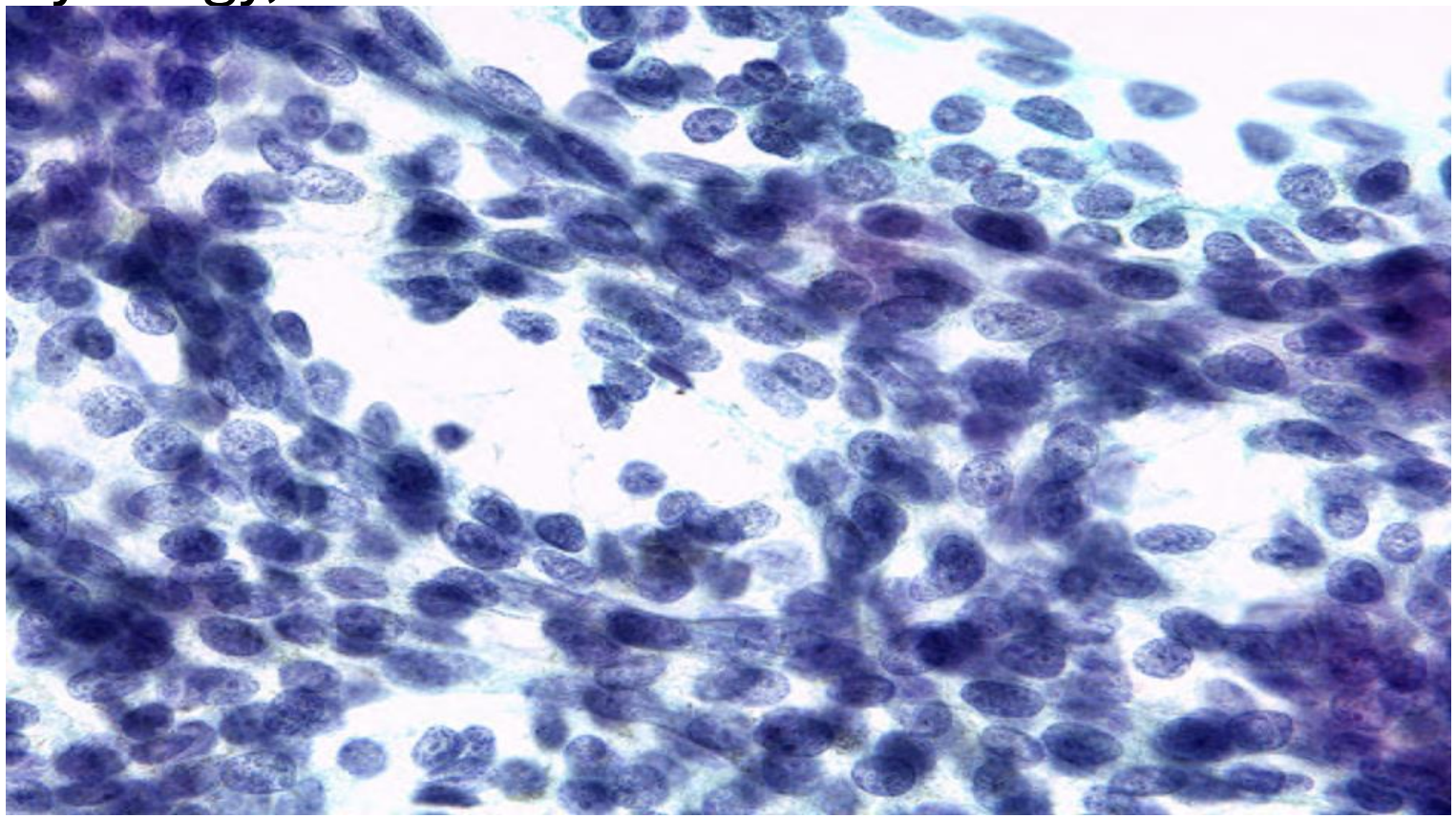






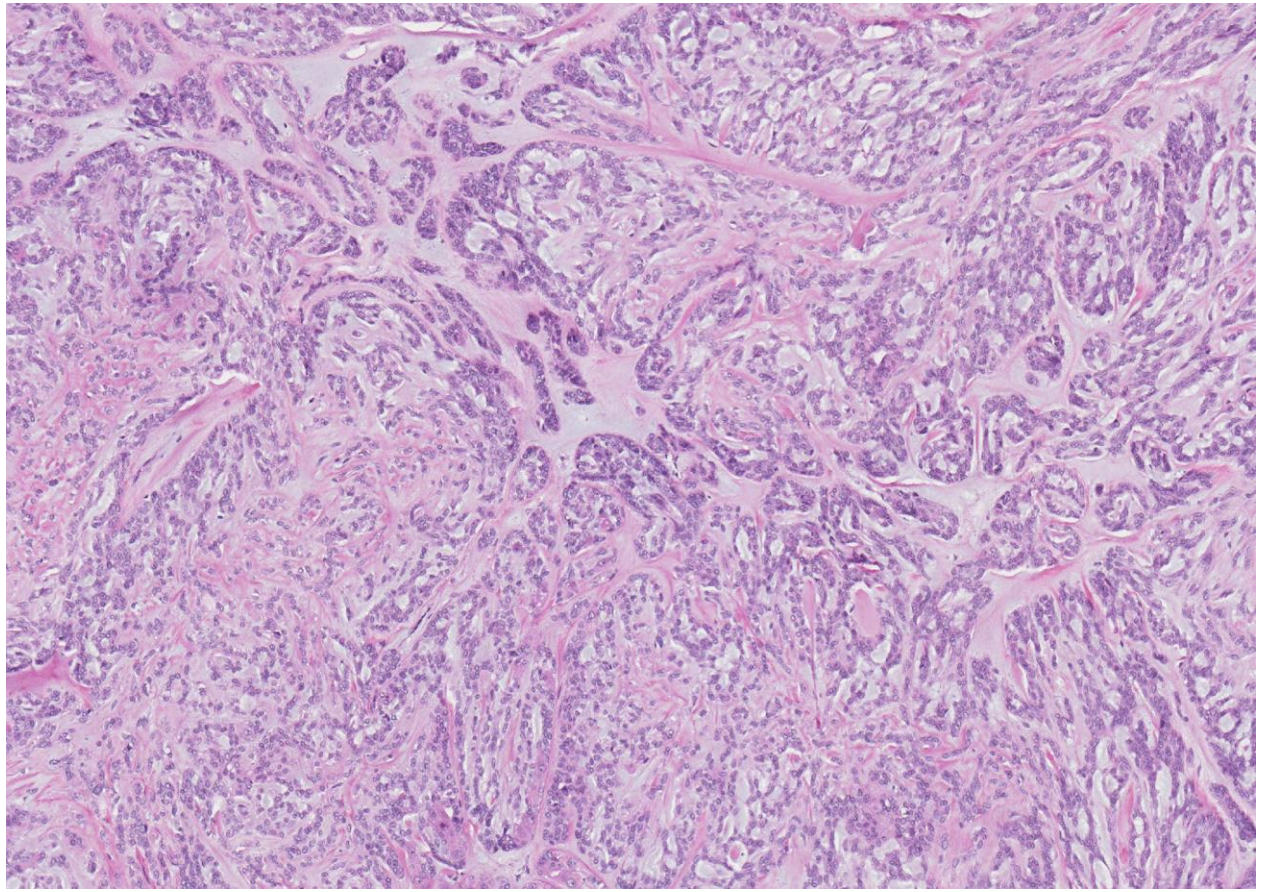
Polymorphous low-grade Adenocarcinoma

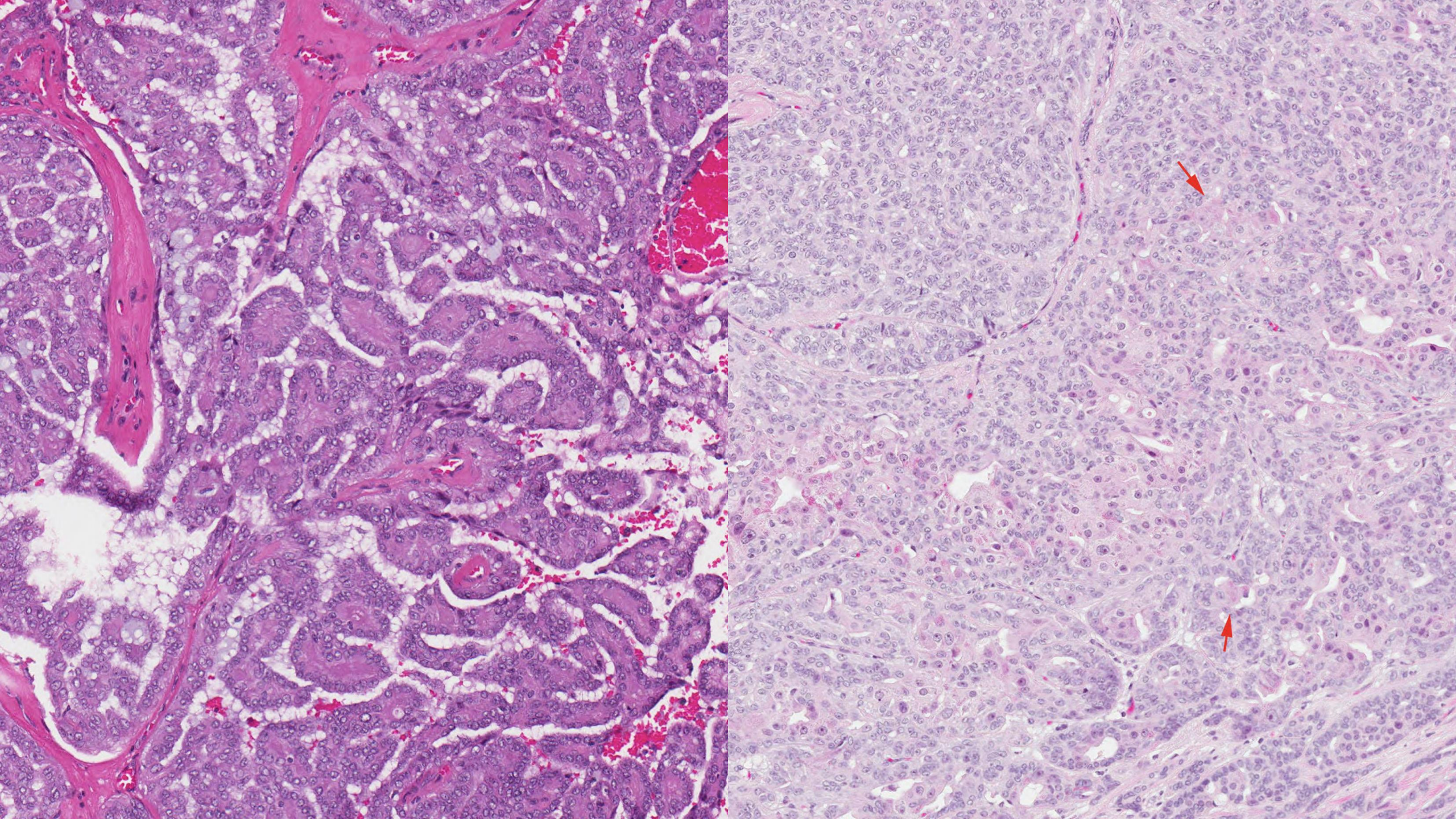
- Sheets – papillary and tubular architecture
- Bland cytology, intermediate sized cells



Polymorphous low-grade Adenocarcinoma

- Moderate delicate cytoplasm
- Uniform nuclei, small distinct nucleoli
- Occasional acellular stromal spheres in background
- May be impossible to distinguish from LG MEC
  - Almost exclusively in minor salivary glands, especially palate





Salivary Gland Neoplasms with Abundant Cytoplasm

- Large cells
- Include oncocytic and clear cell lesions
- Without significant atypia (Benign vs. Malignant)
- With significant atypia (Malignant NOS)

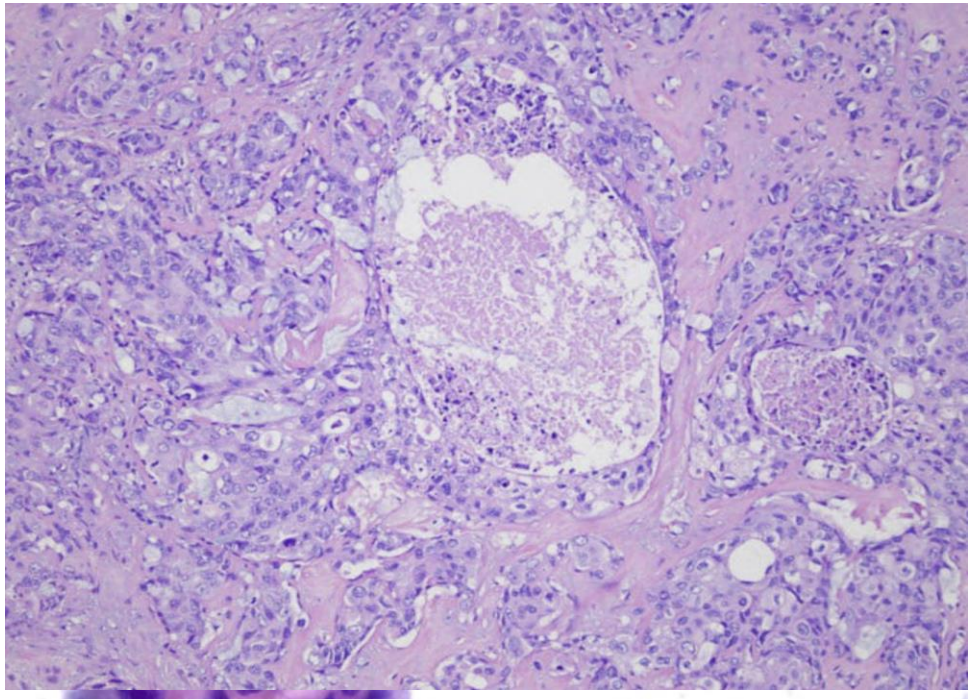
Salivary Gland Neoplasms with Abundant Cytoplasm

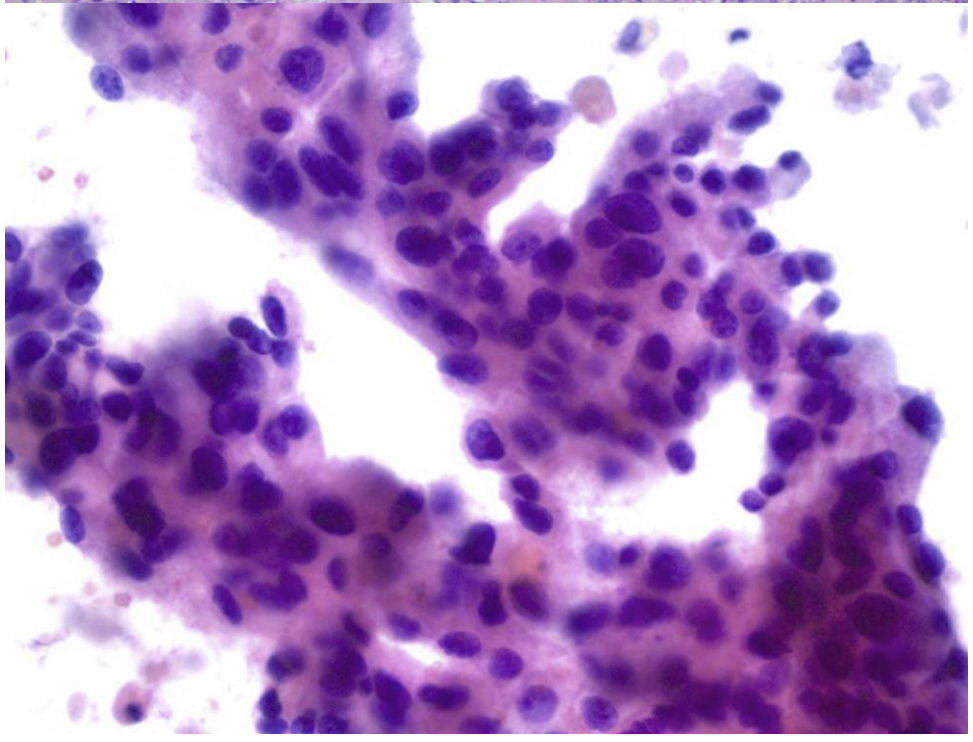
- **With atypia**
  - Salivary duct carcinoma
  - High grade mucoepidermoid carcinoma
  - Poorly differentiated carcinoma, NOS
  - Metastatic carcinoma
- **Without atypia**
  - Warthin tumor
  - Oncocytoma
  - Acinic cell carcinoma
  - Secretory carcinoma

Salivary Duct Carcinoma

- High grade malignancy
- Poor prognosis
- Most aggressive
- Parotid
- Males

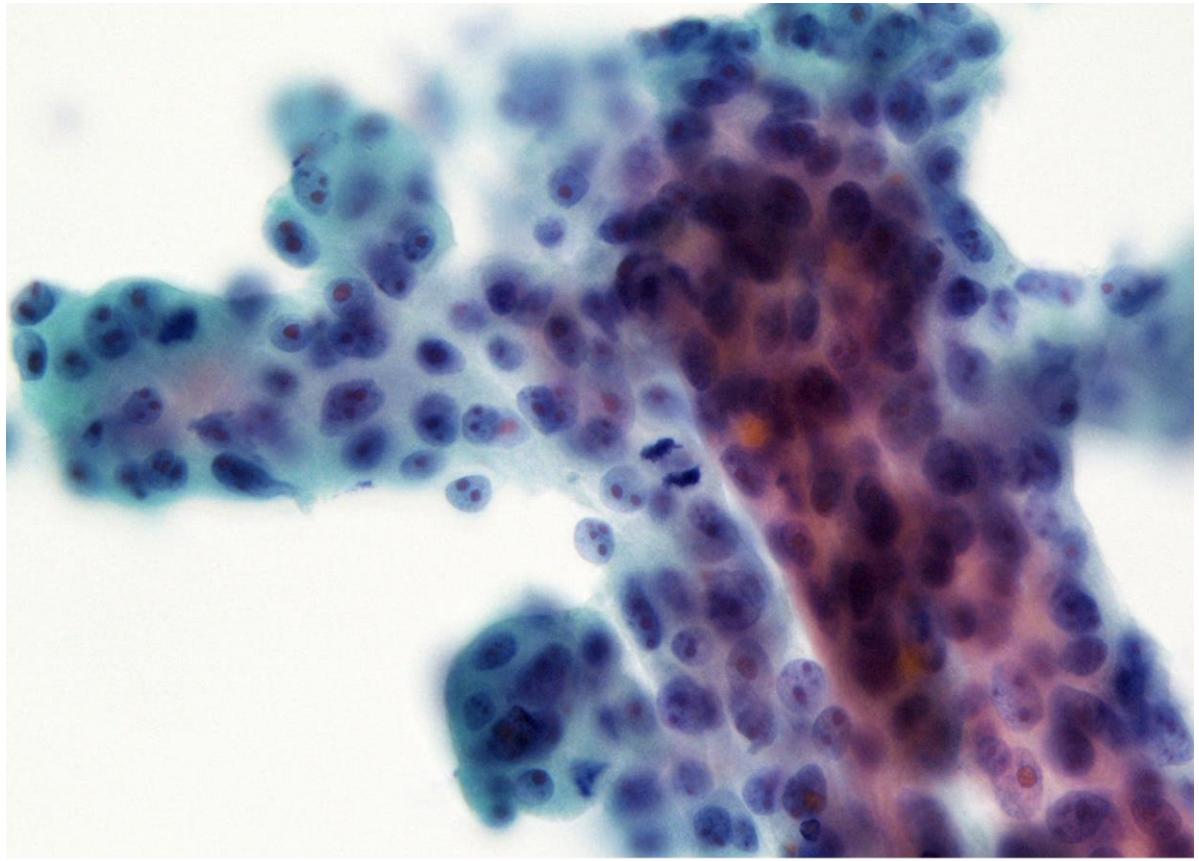
- Resembles DCIS and invasive breast carcinoma
  - Cribriform, micropapillary, comedo





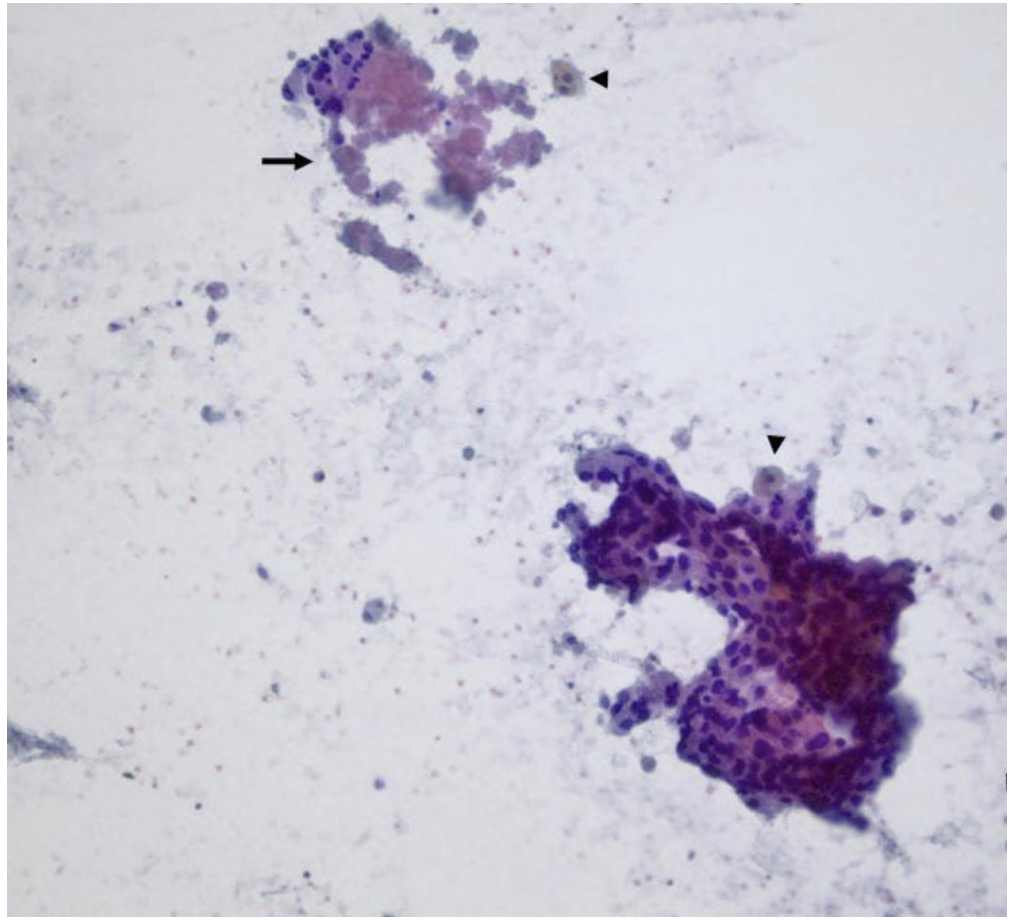
Salivary duct carcinoma

- Large cells, abundant cytoplasm – Oncocytic appearance
- Nuclear pleomorphism
- Large prominent nucleoli



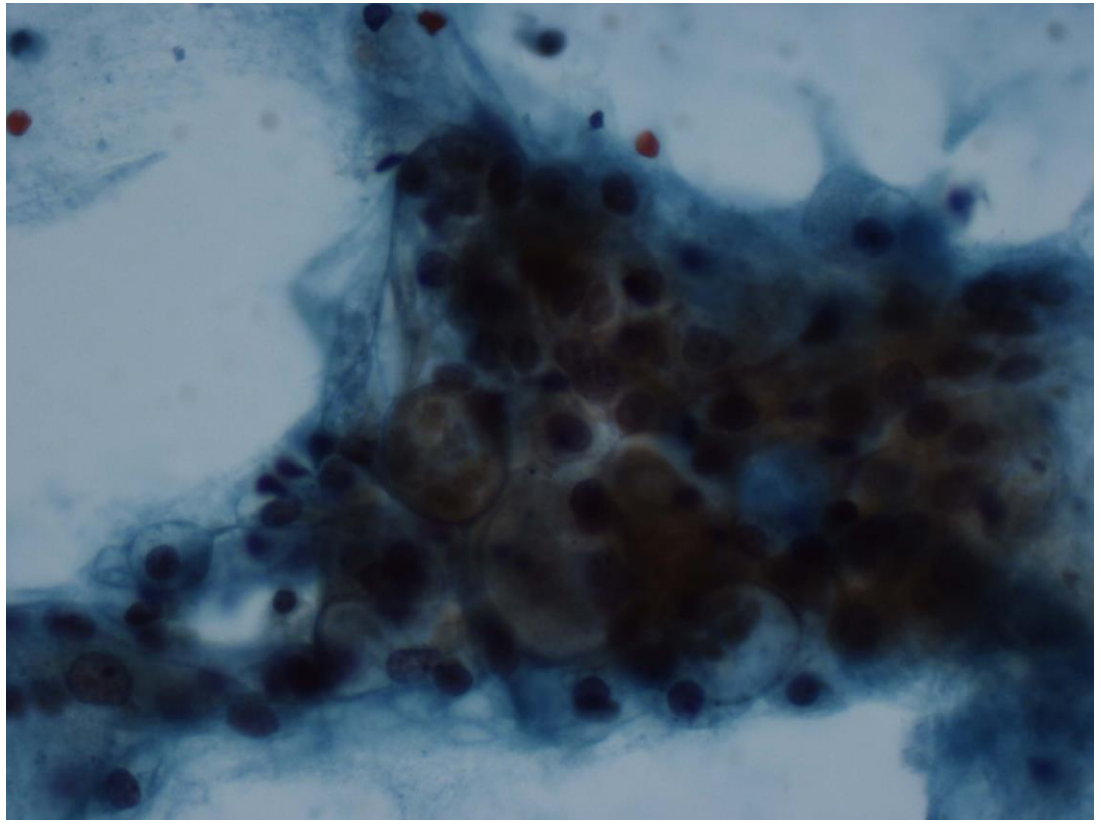
Salivary duct carcinoma

- SDC can show a deceptively bland appearance
- Abundant necrosis + oncocytic appearance



High-Grade Mucoepidermoid Carcinoma

- Large pleomorphic cells with epidermoid or undifferentiated features
- Admixture of glandular and squamous components
- Glandular cells difficult to identify

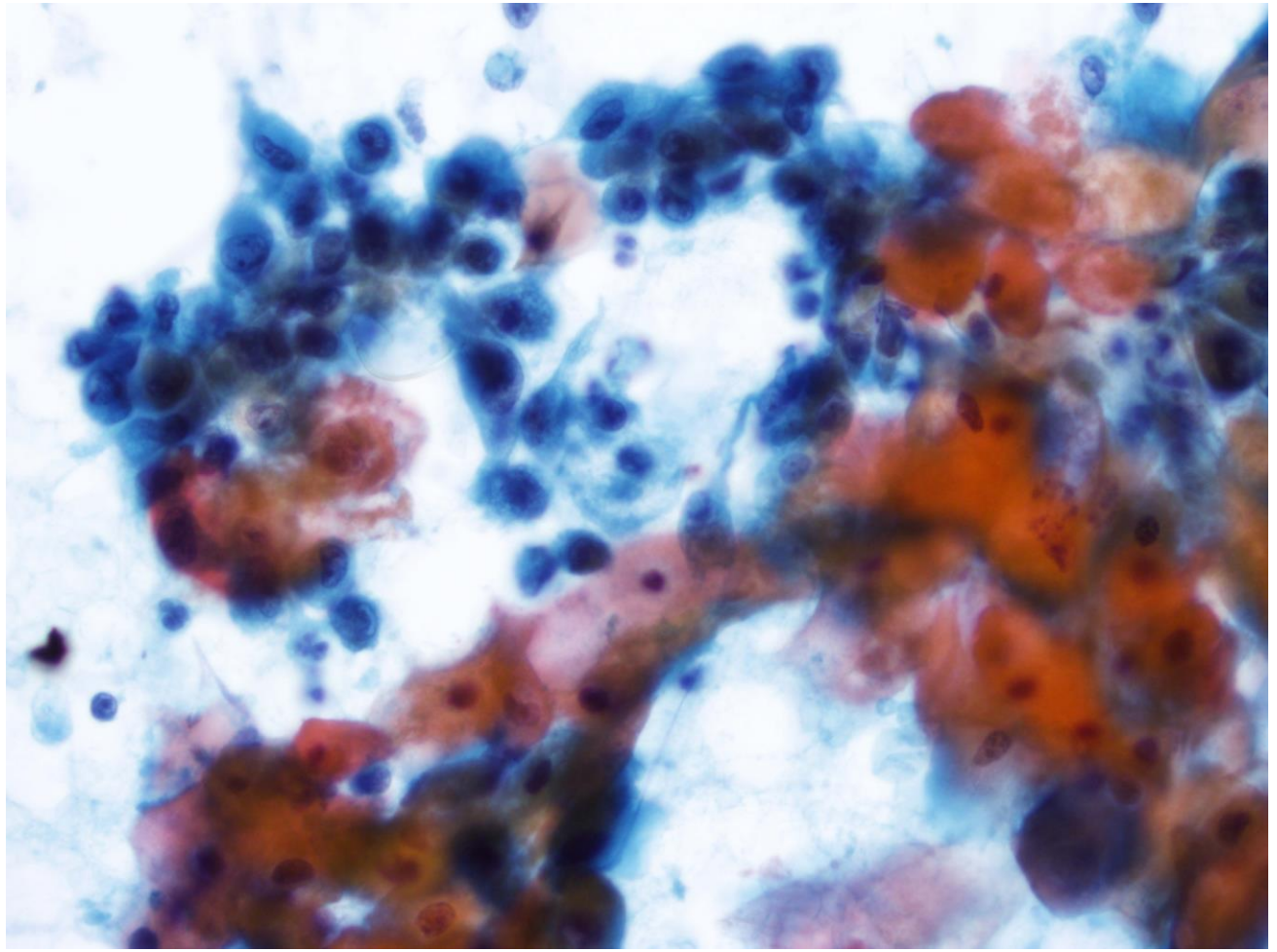


High grade adenocarcinoma, NOS

- May be difficult to differentiate from other high-grade carcinomas

Squamous cell carcinoma

- Primary squamous cell carcinoma
  - Very rare, <1%)
- Metastatic squamous cell carcinoma
- Management:
  - Radiation therapy (mets) vs. radical excision & nodes dissection (primary)



Metastatic malignancies

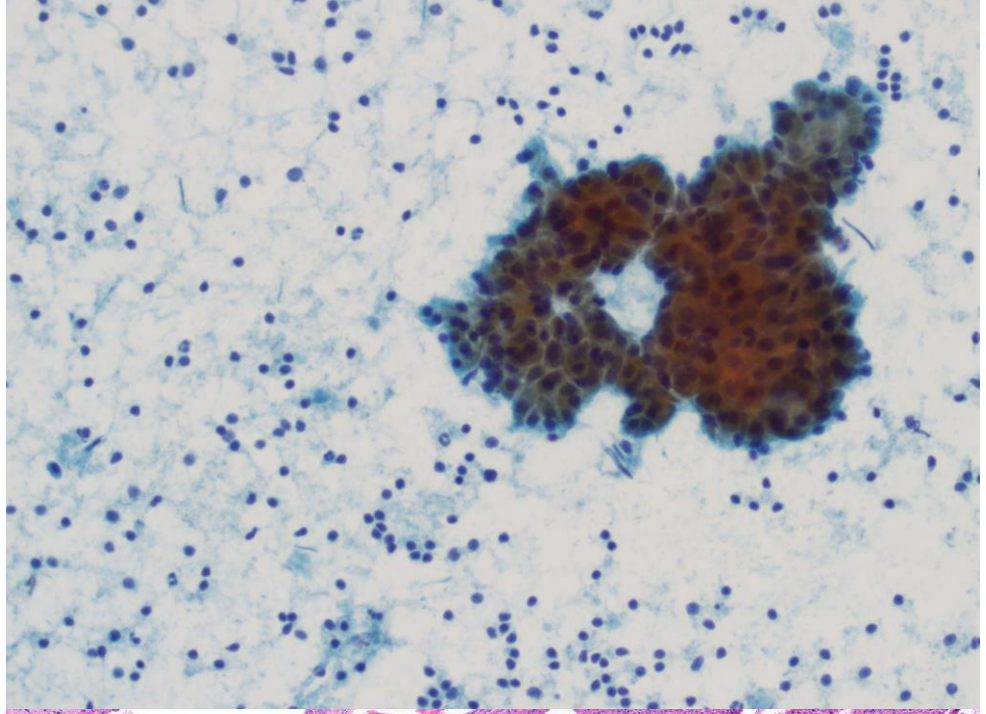
- Intra/peri-parotid LNs
- Supra-clavicular primaries
  - Most common primary sites: Scalp, face, ENT
  - Most common histology: Squamous cell carcinoma, melanoma
- Infra-clavicular primaries
  - Lung, kidney, breast are most common
- Must exclude kidney if prominent clear cell features

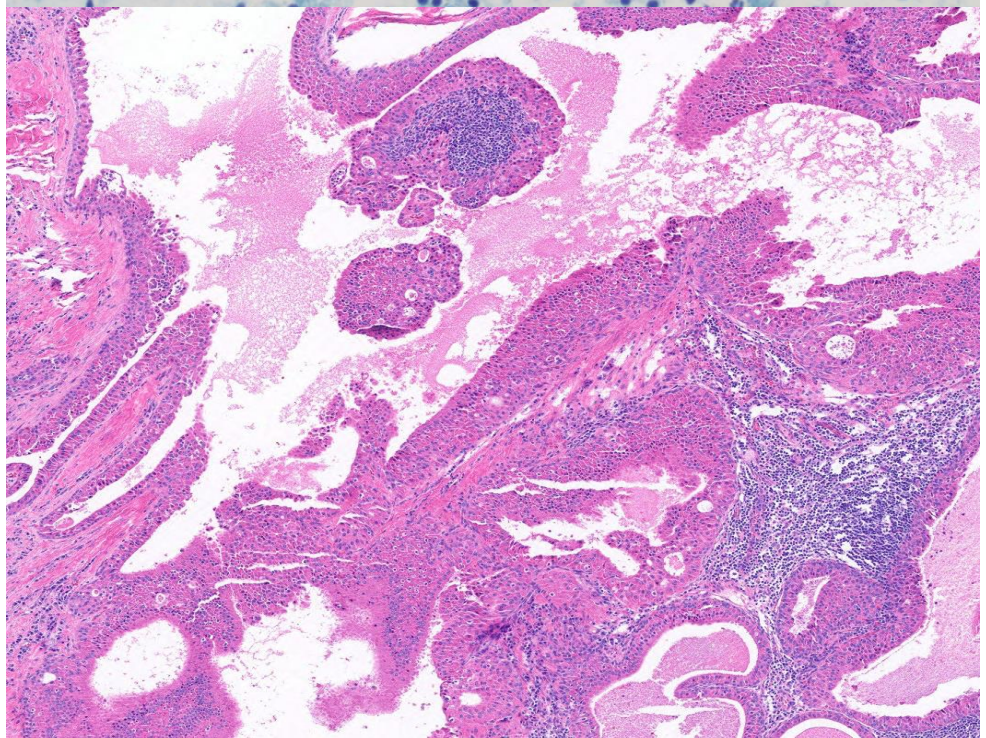
Salivary Gland Neoplasms with Abundant Cytoplasm

- With atypia
  - Salivary duct carcinoma
  - High grade mucoepidermoid carcinoma
  - Poorly differentiated carcinoma, NOS
  - Metastatic carcinoma
- **Without atypia**
  - Warthin tumor
  - Oncocytoma
  - Acinic cell carcinoma
  - Secretory carcinoma

Warthin Tumor

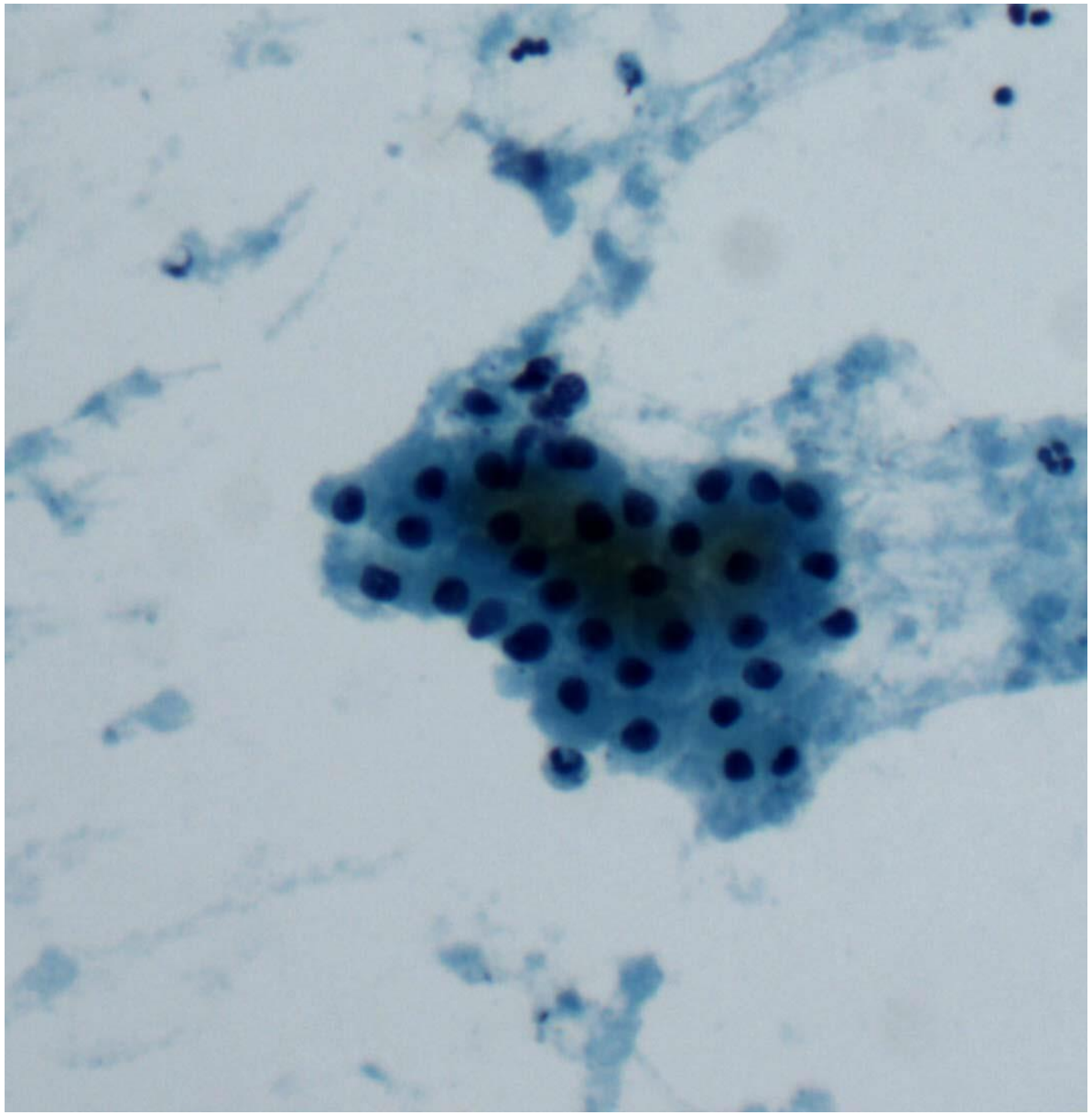
- 5-10% of parotid tumors
- Almost always arises in parotid gland
- Male, average age=60 years, smoking history
- Thin watery mucoid aspirate



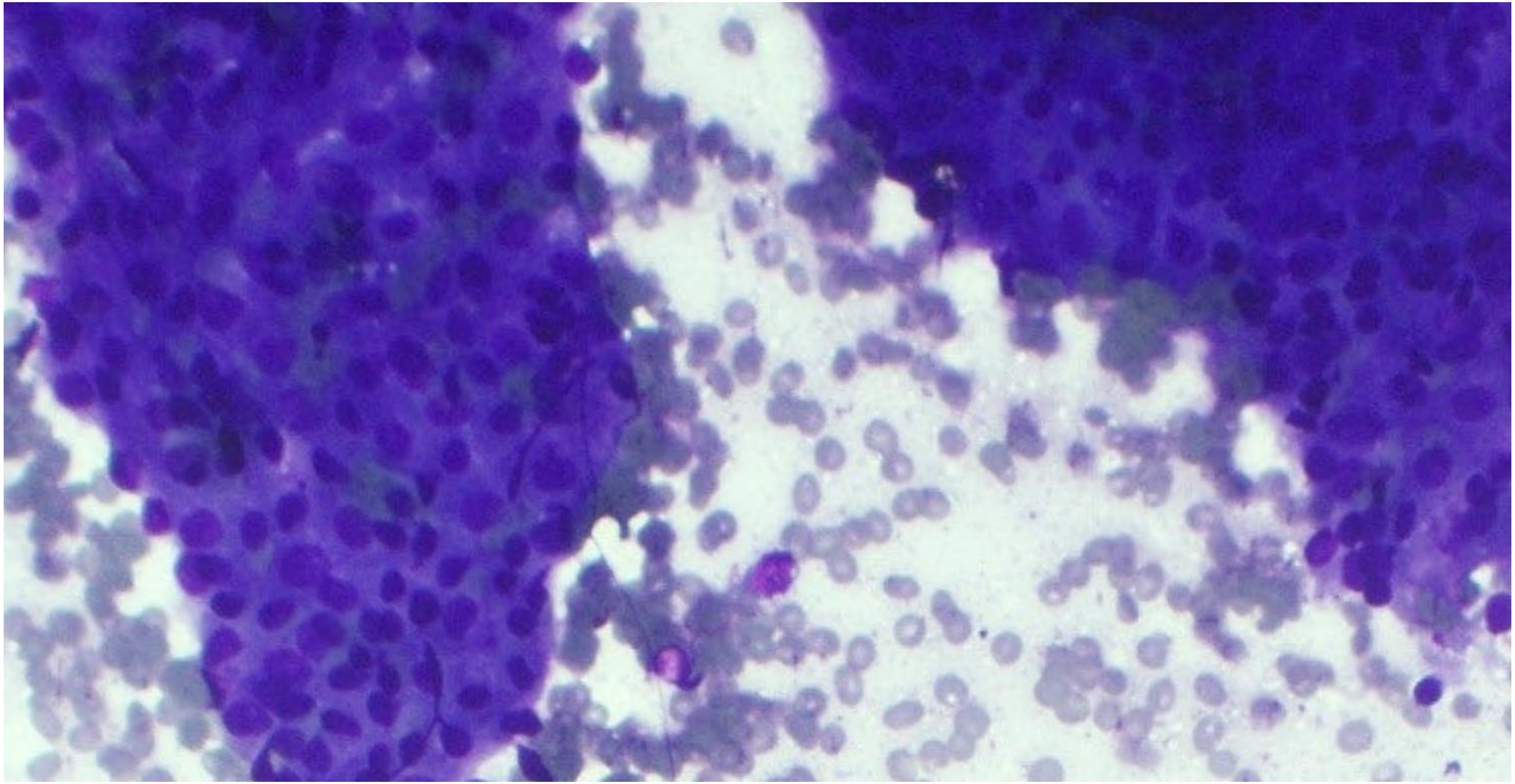


Warthin Tumor

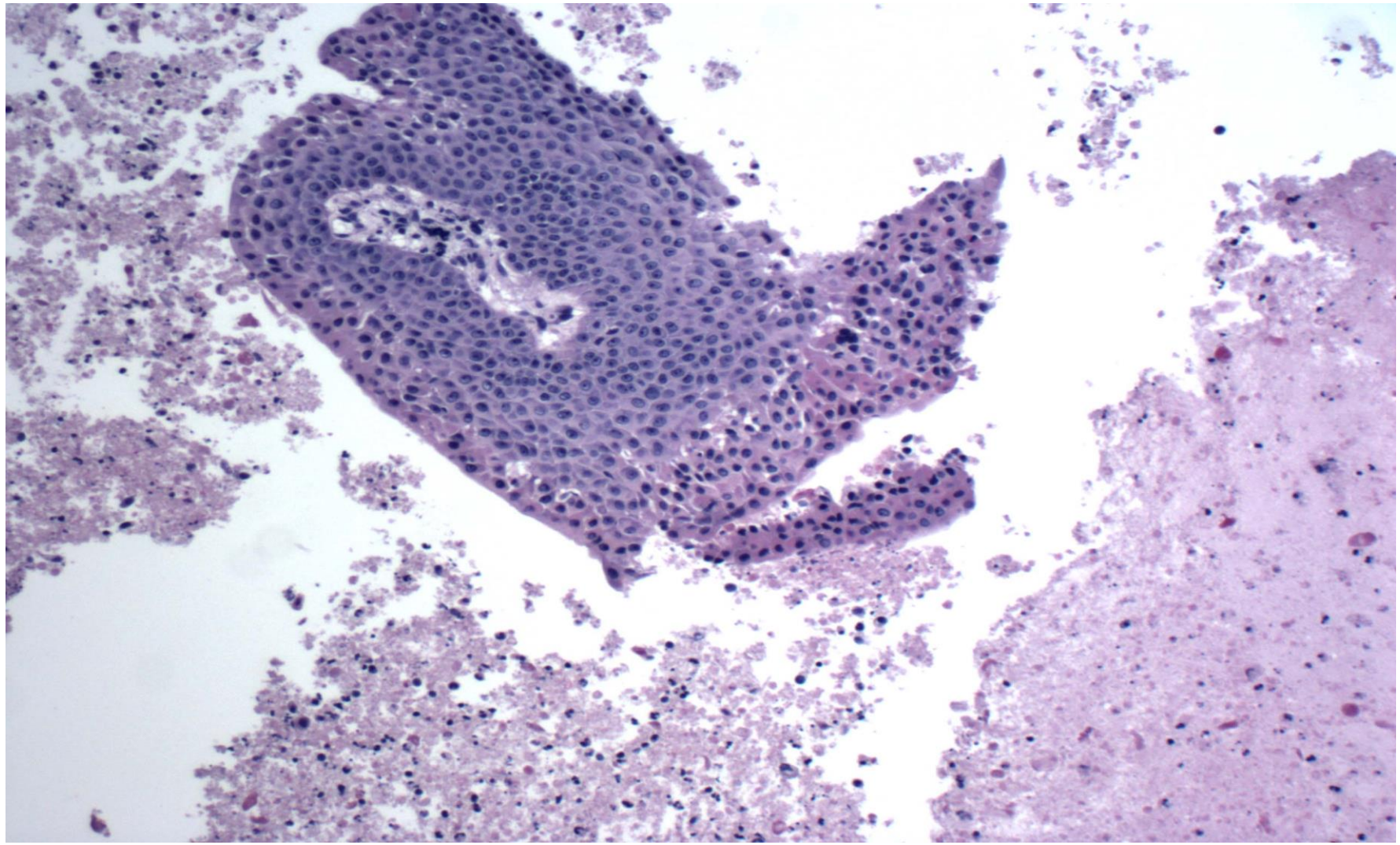
- Oncocytes
  - Abundant dense cytoplasm
  - Centrally placed nuclei
  - Large prominent nucleoli



Oncocytic-rich Warthin tumor/Oncocytoma

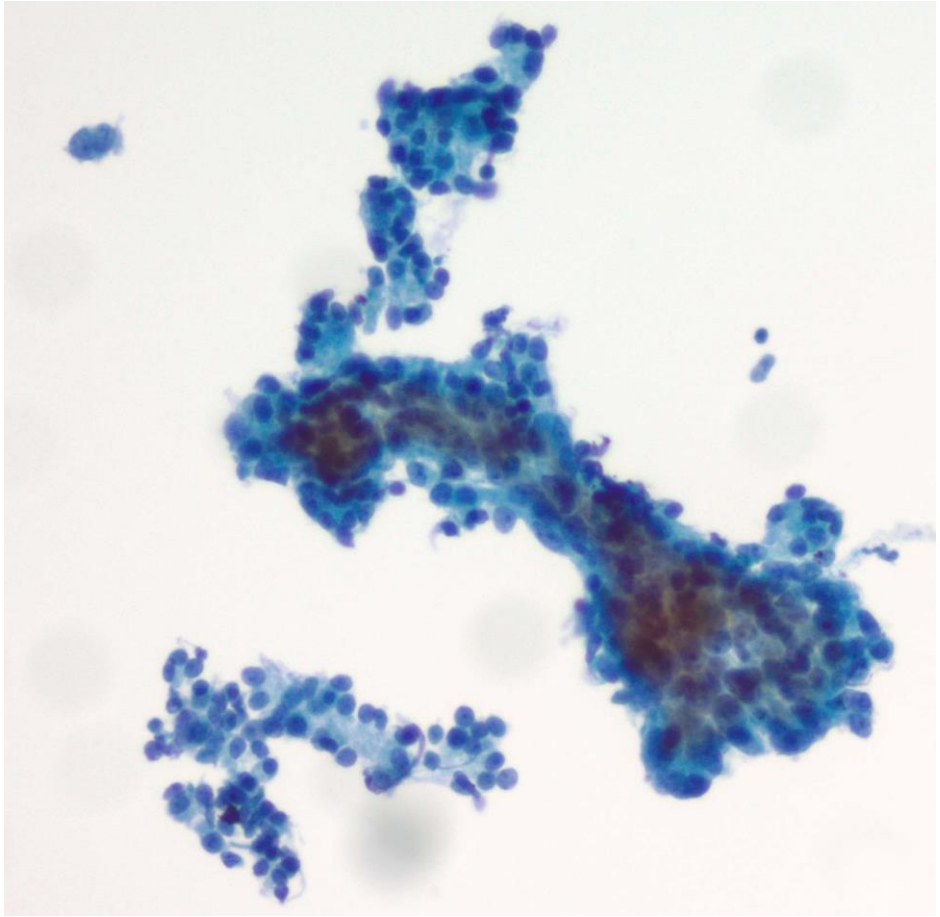


Warthin tumor with Squamous Metaplasia



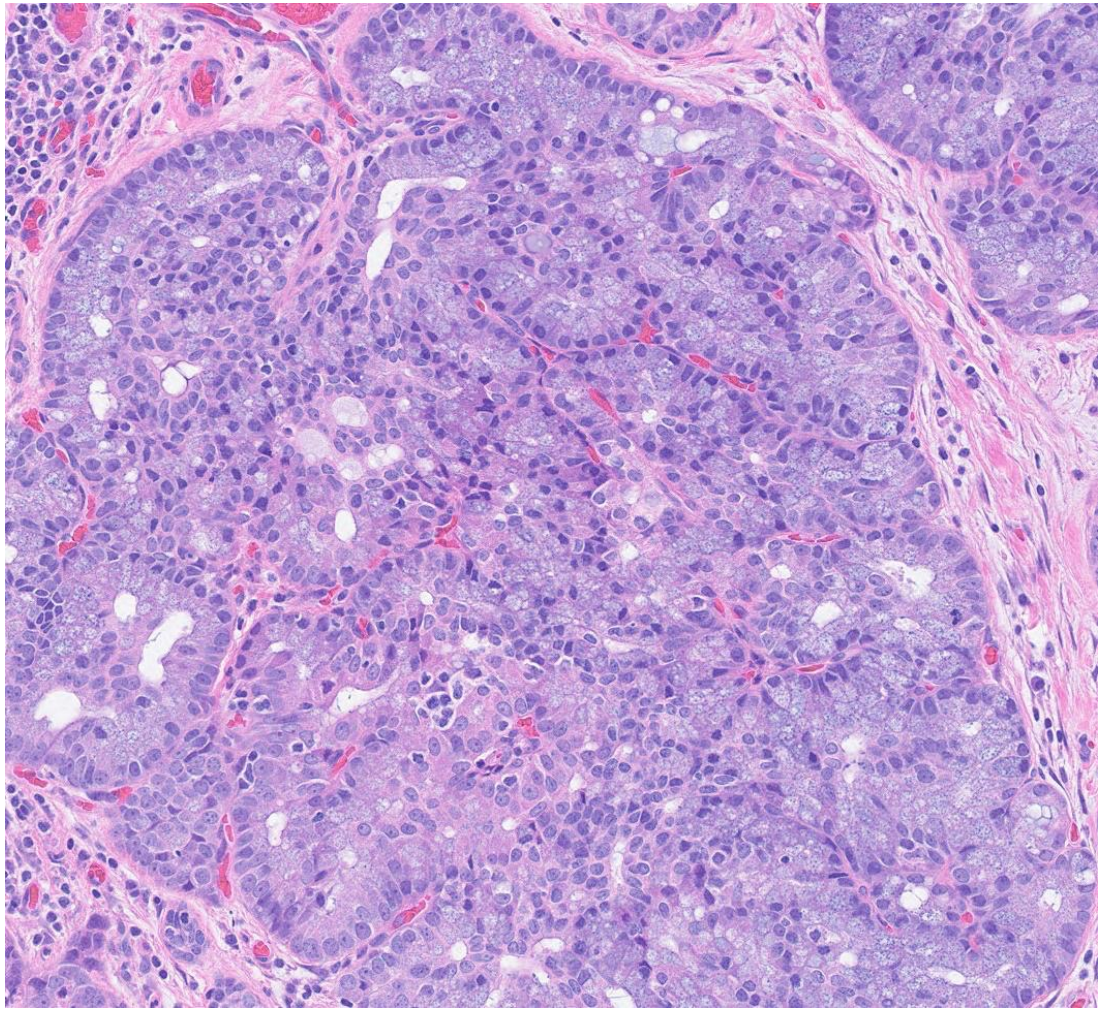
Acinic Cell Carcinoma

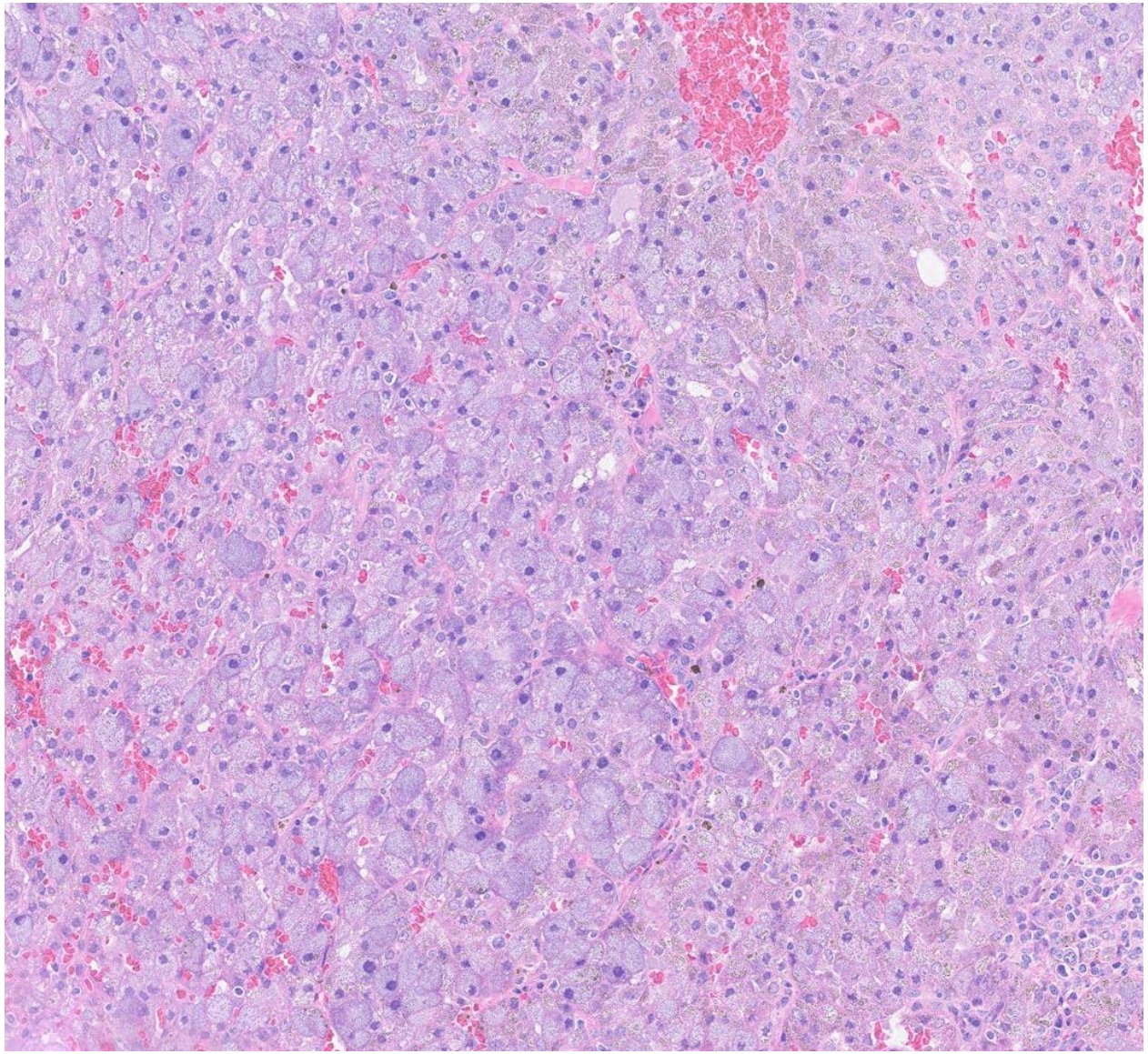
- Low-grade malignancy
- Parotid, F>M
- Any age – Peak 60-70 yrs
- Cytology:
  - Flat sheets
  - Large cells
  - Papillary/acinar formation
  - Thin capillaries
  - Many naked nuclei

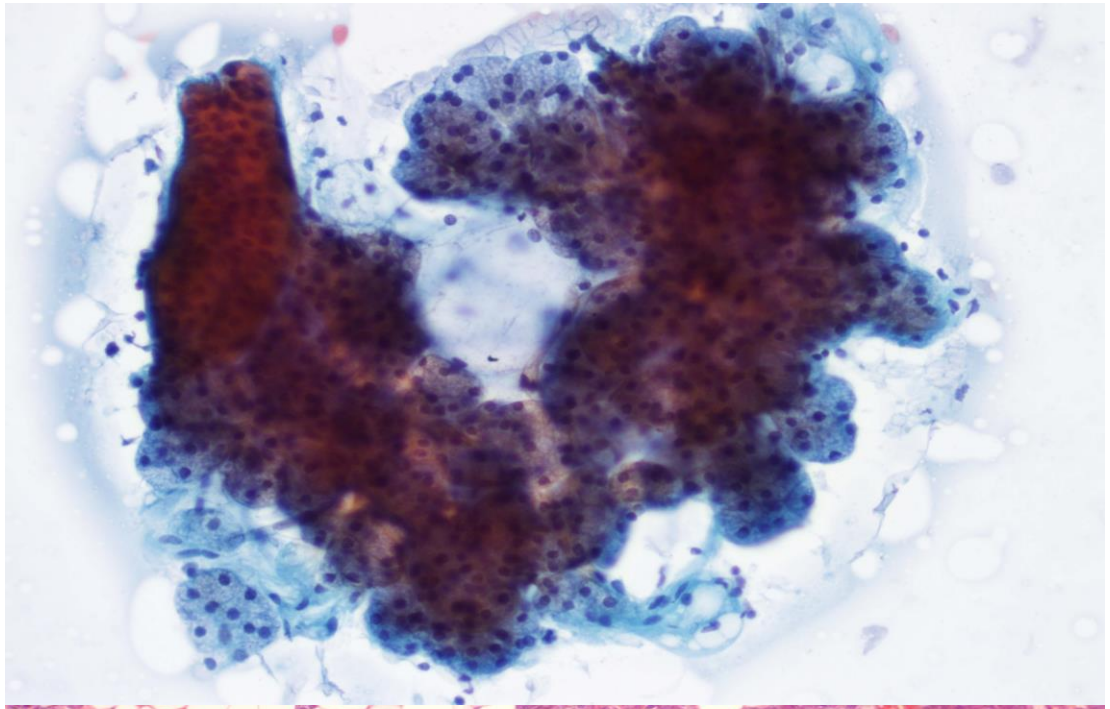


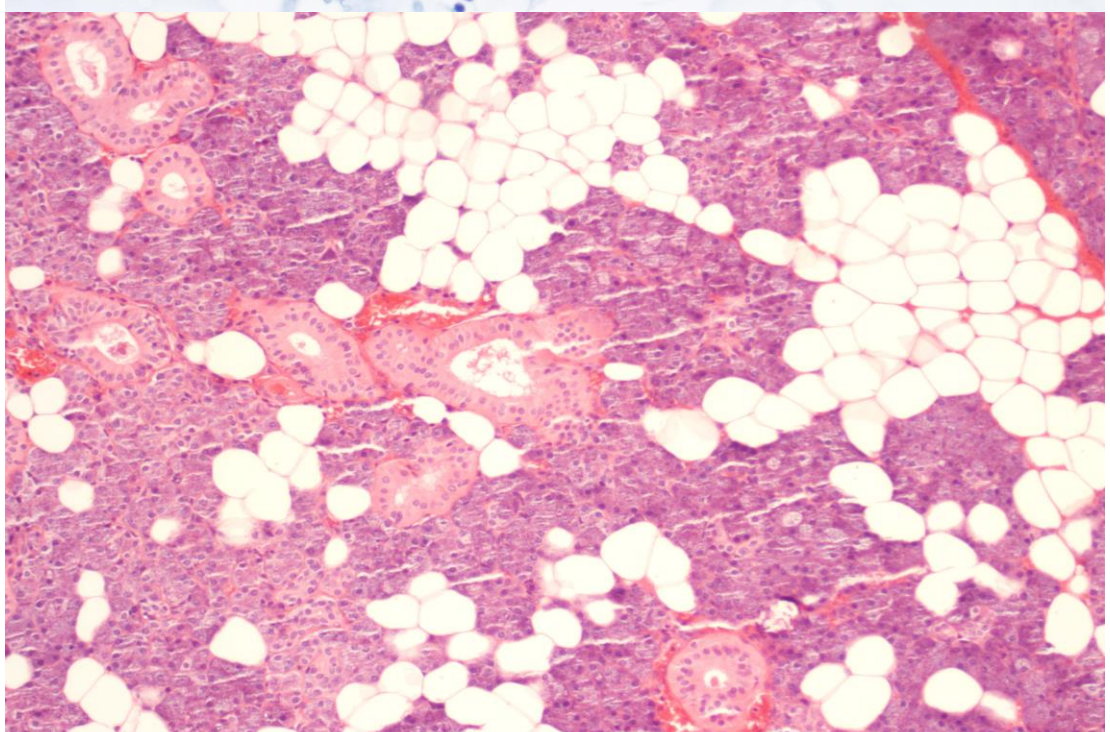
Acinic Cell Carcinoma

- Abundant delicate vacuolated cytoplasm
- Eccentric nuclei, distinct nucleoli
- Zymogen granules
- DOG1-positive





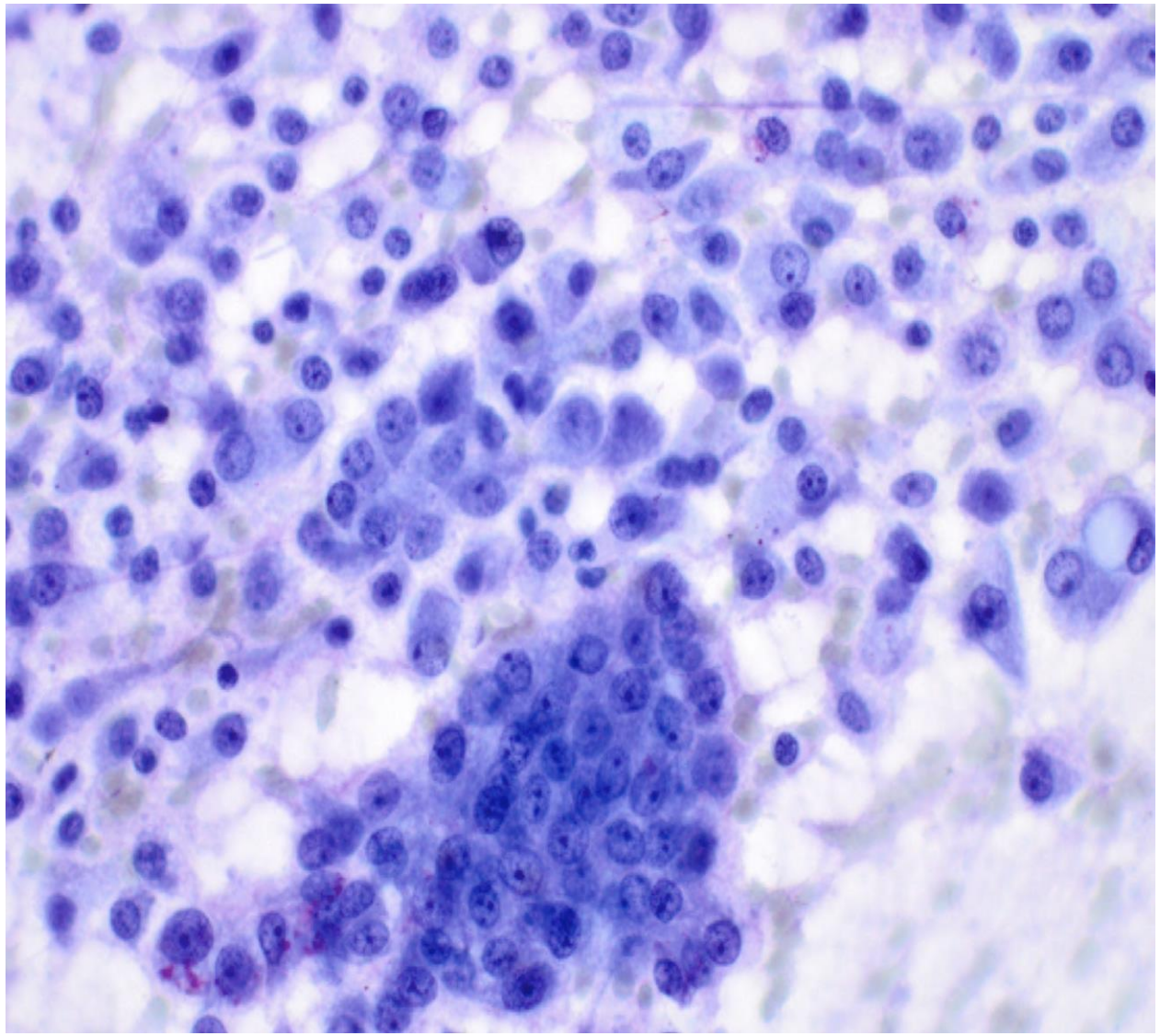




DDX: Benign acinar tissue

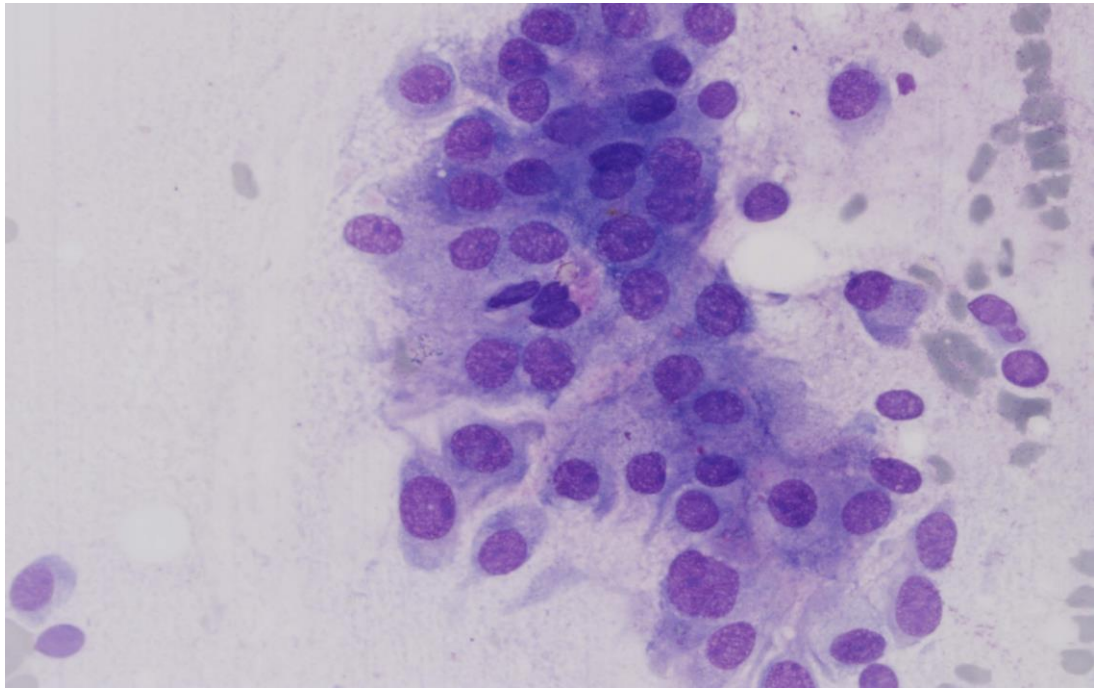
Secretory Carcinoma

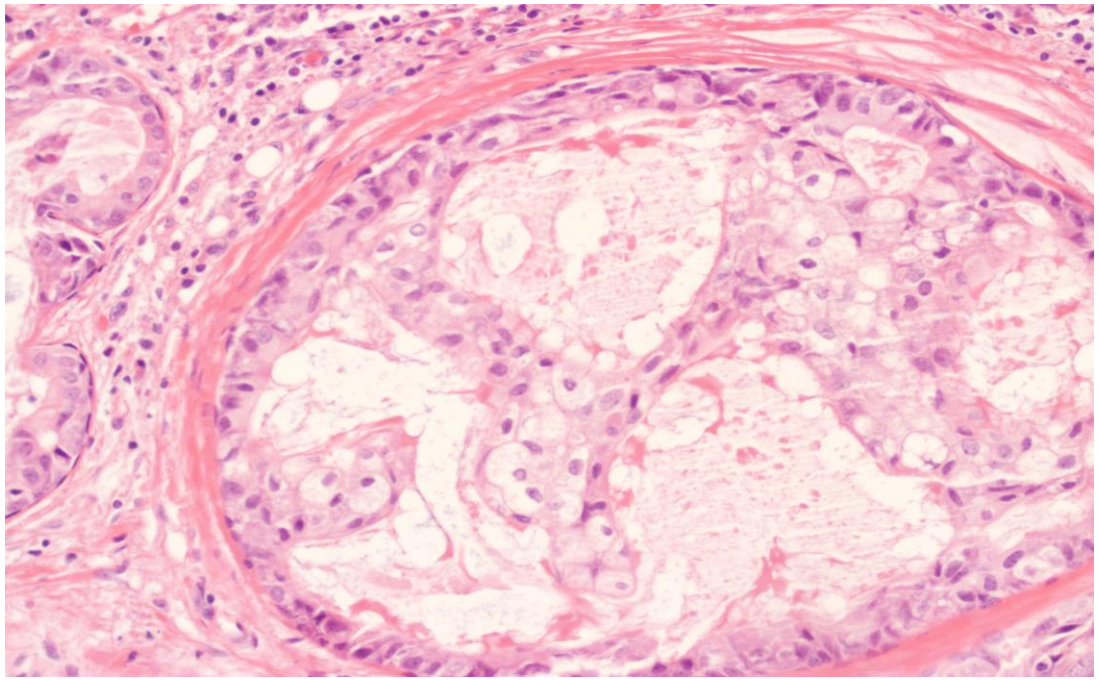
- Loosely cohesive clusters, papillary fragments
- Large cells with well defined cytoplasmic borders – bland cytology
- Oncocytic appearance
- Single intact cells in background



Secretory Carcinoma

- Microvacuolated cytoplasm (soap bubble)
- Occasionally large solitary vacuoles
- No cytoplasmic granules
- Uniform round-oval nuclei – no significant atypia
- Finely granular chromatin and small nucleoli



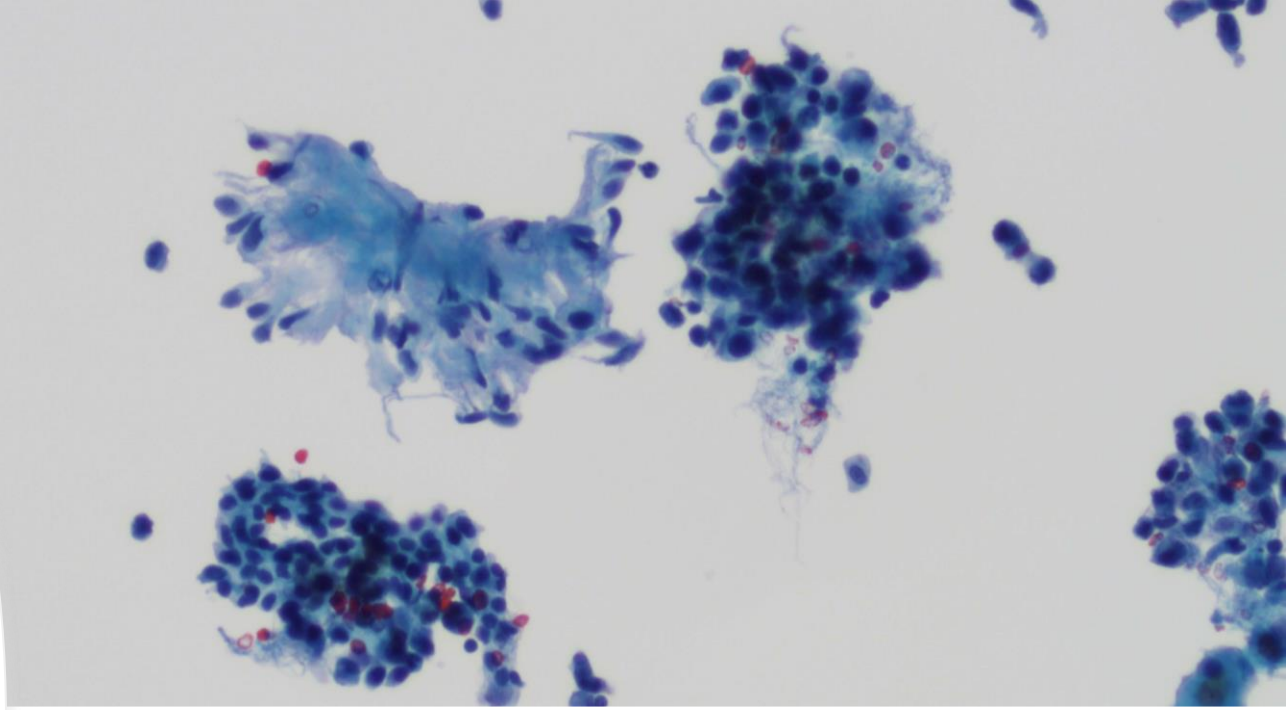


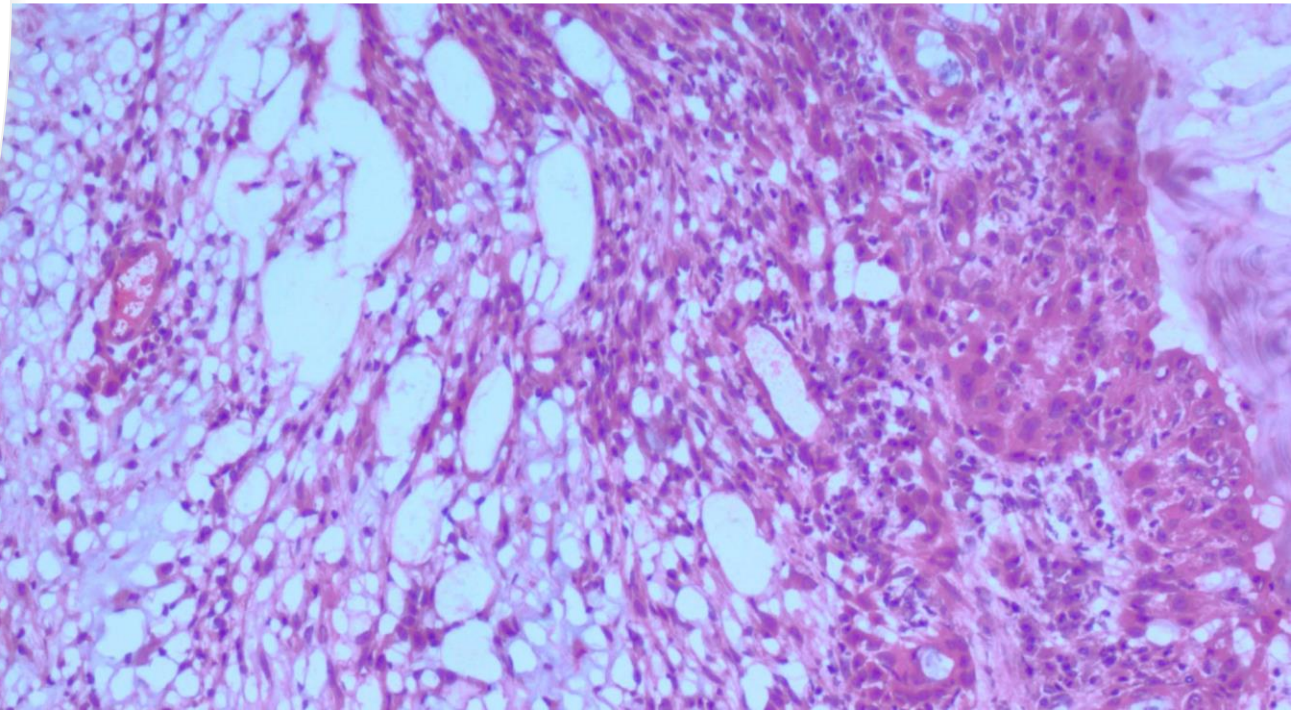
The Many Cytologic Faces of Pleomorphic Adenoma

"The Great Pretender"

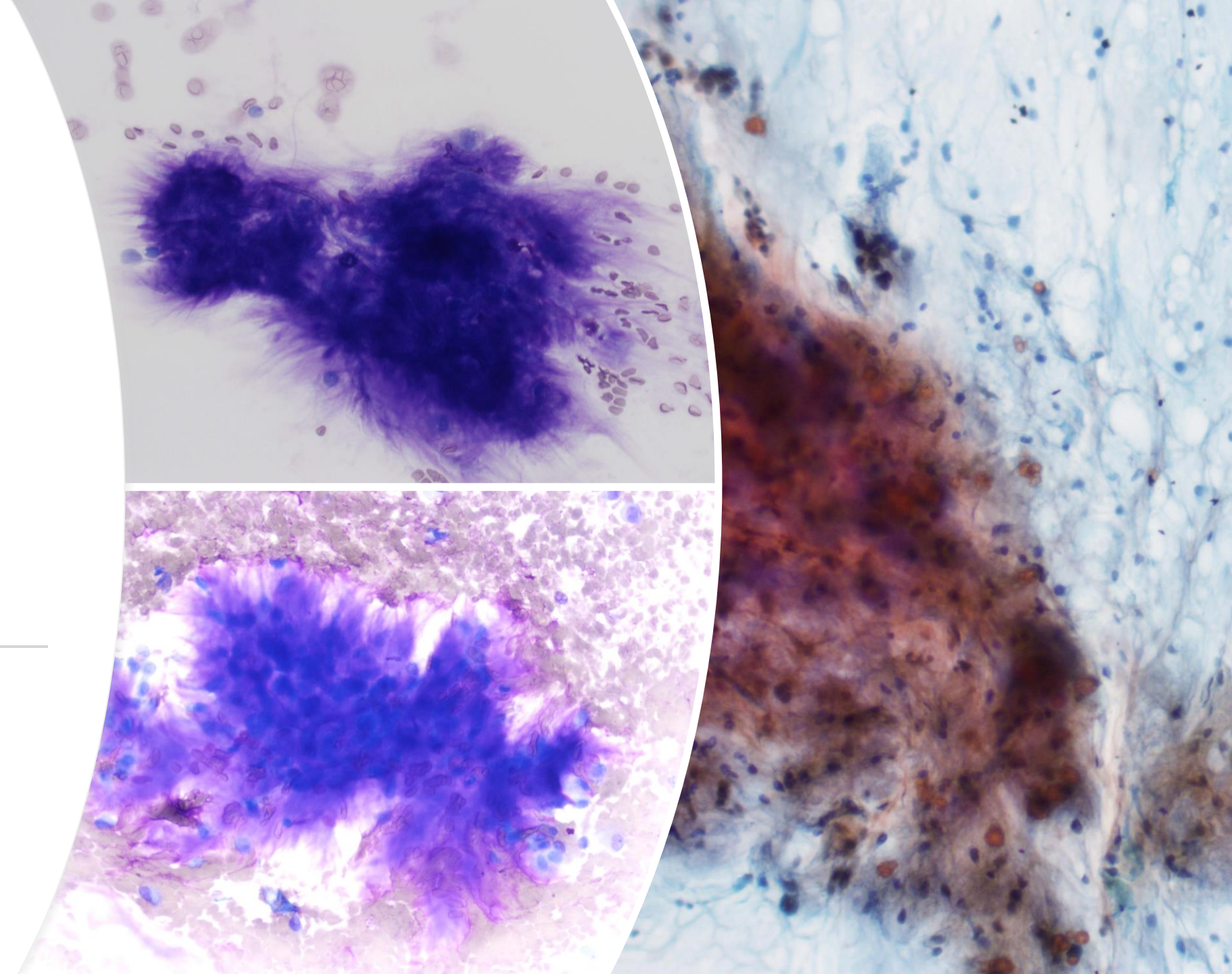
Pleomorphic adenoma

Mixture of epithelial (ductal), myoepithelial & mesenchymal elements

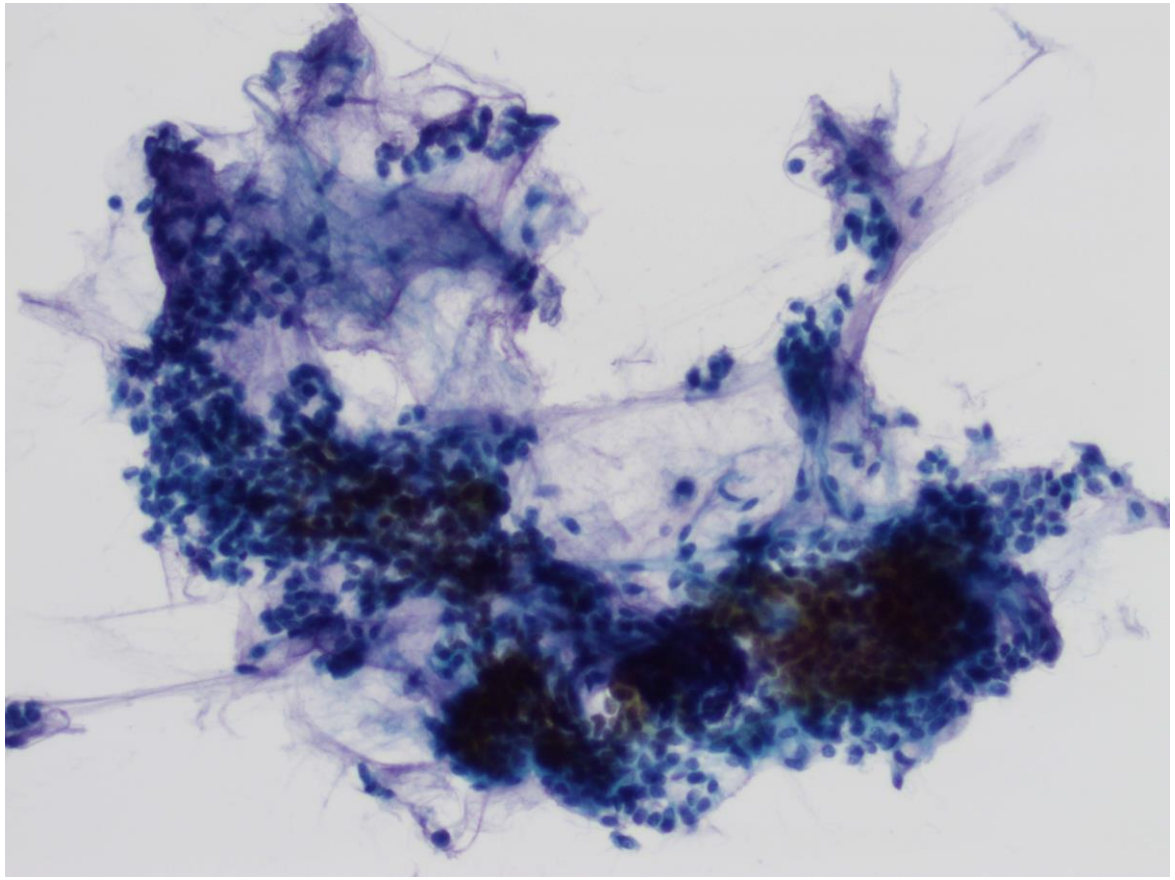


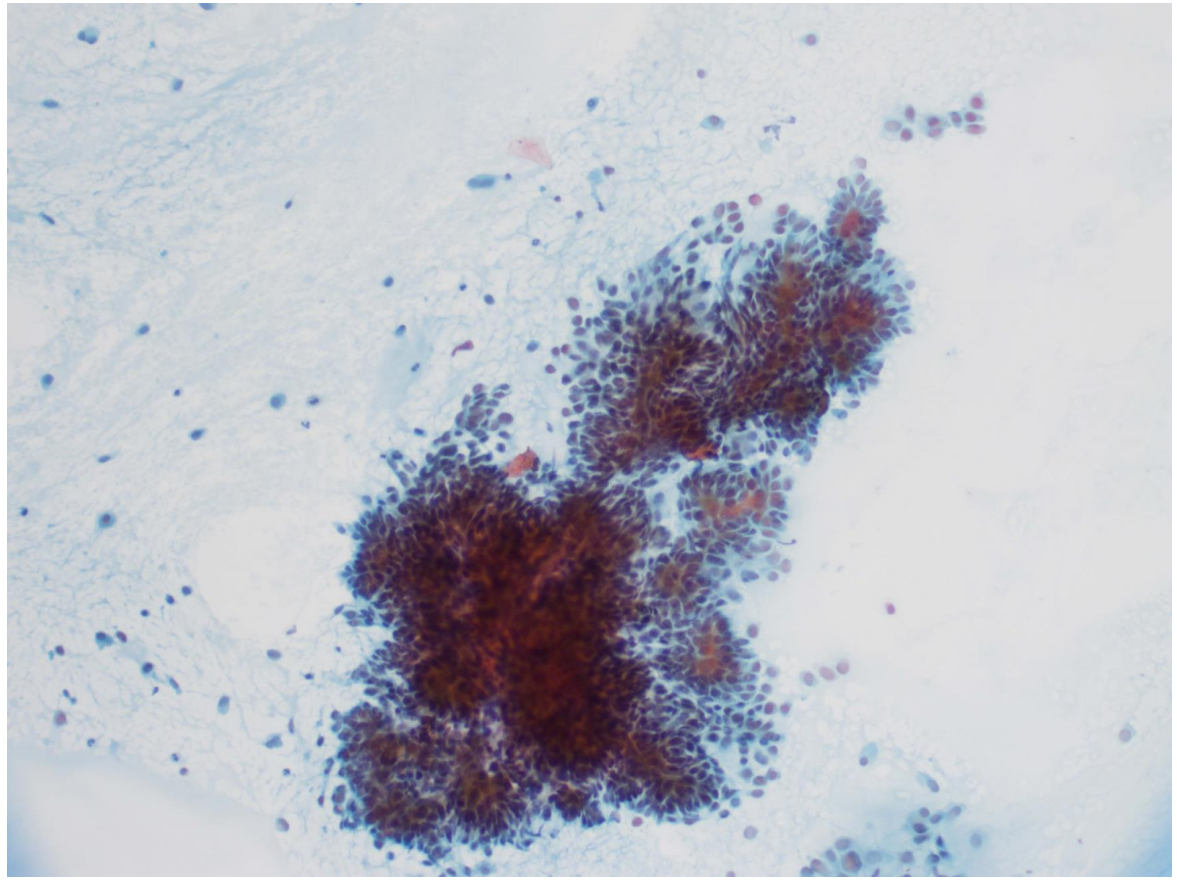


Stroma of PA



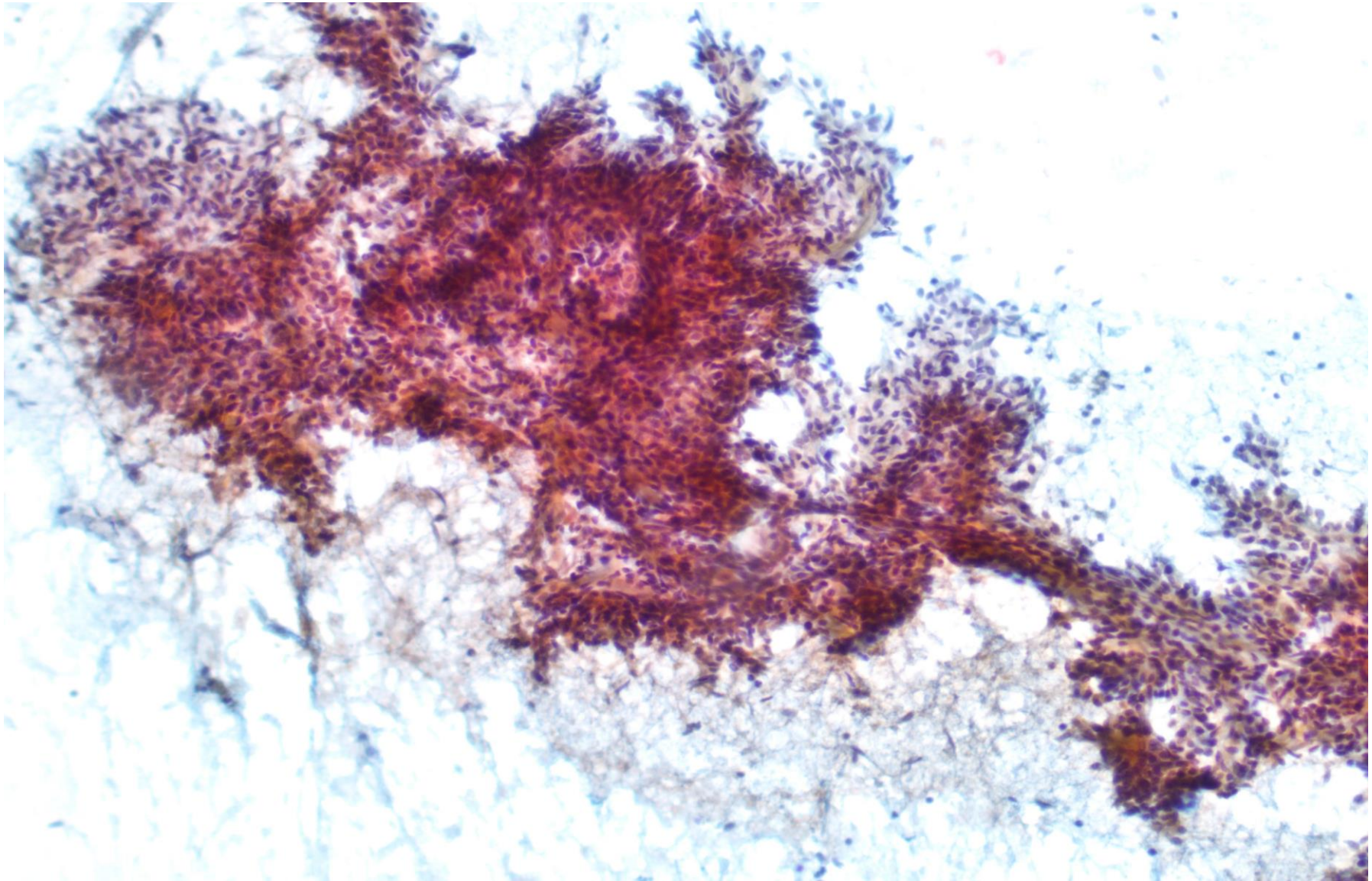
Cellular component of PA



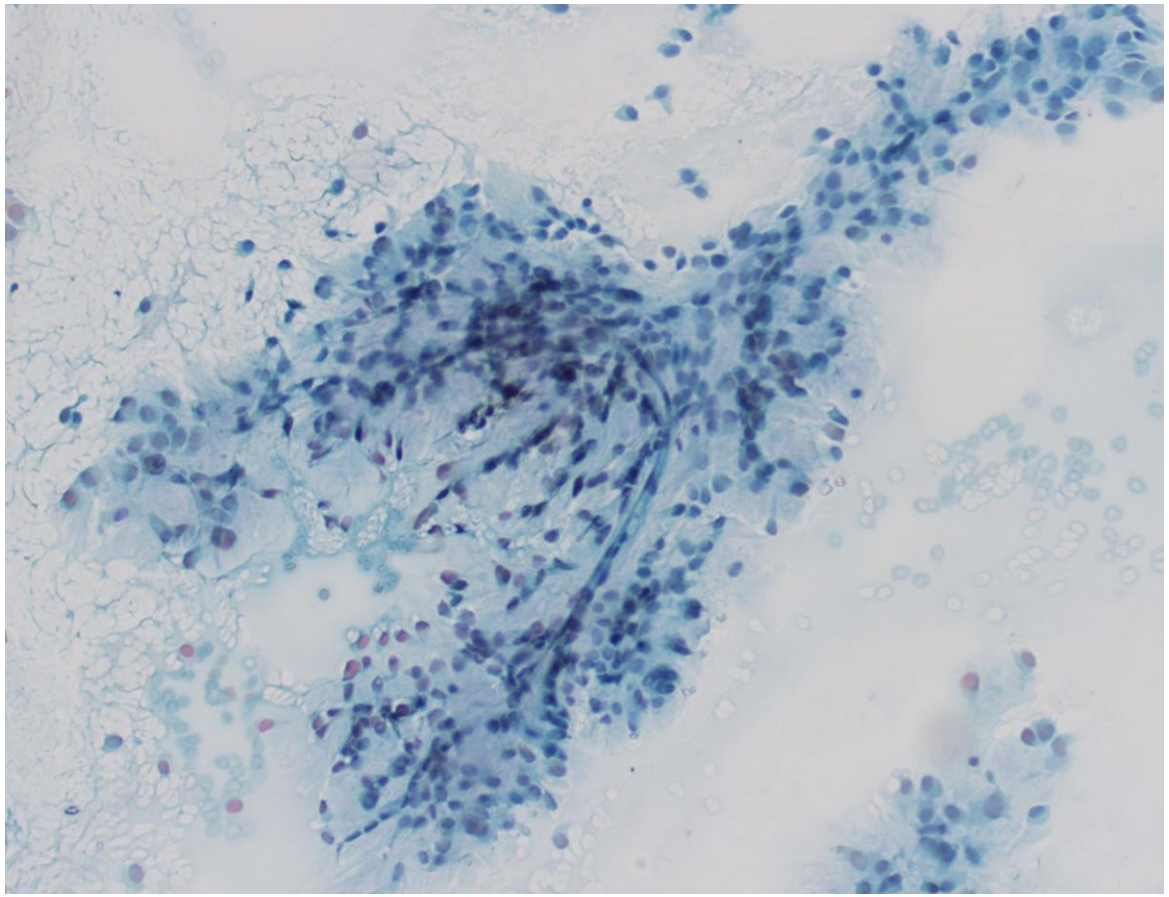


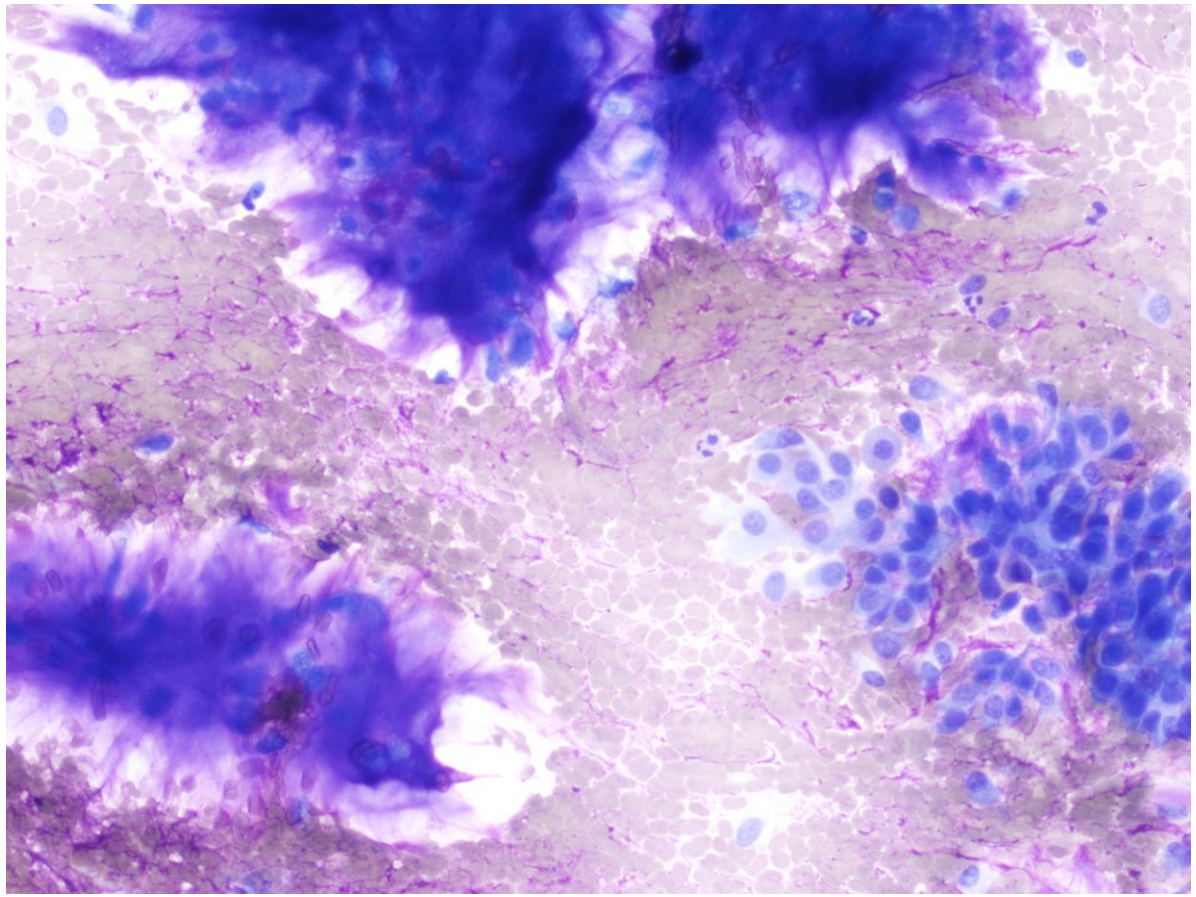
- Cohesive clusters with indistinct community borders
- Haphazard nuclear arrangement
- Round-oval nuclei, occasional spindling
- Moderate cytoplasm, inconspicuous or absent nucleoli

Cellular PA/Myoepithelioma

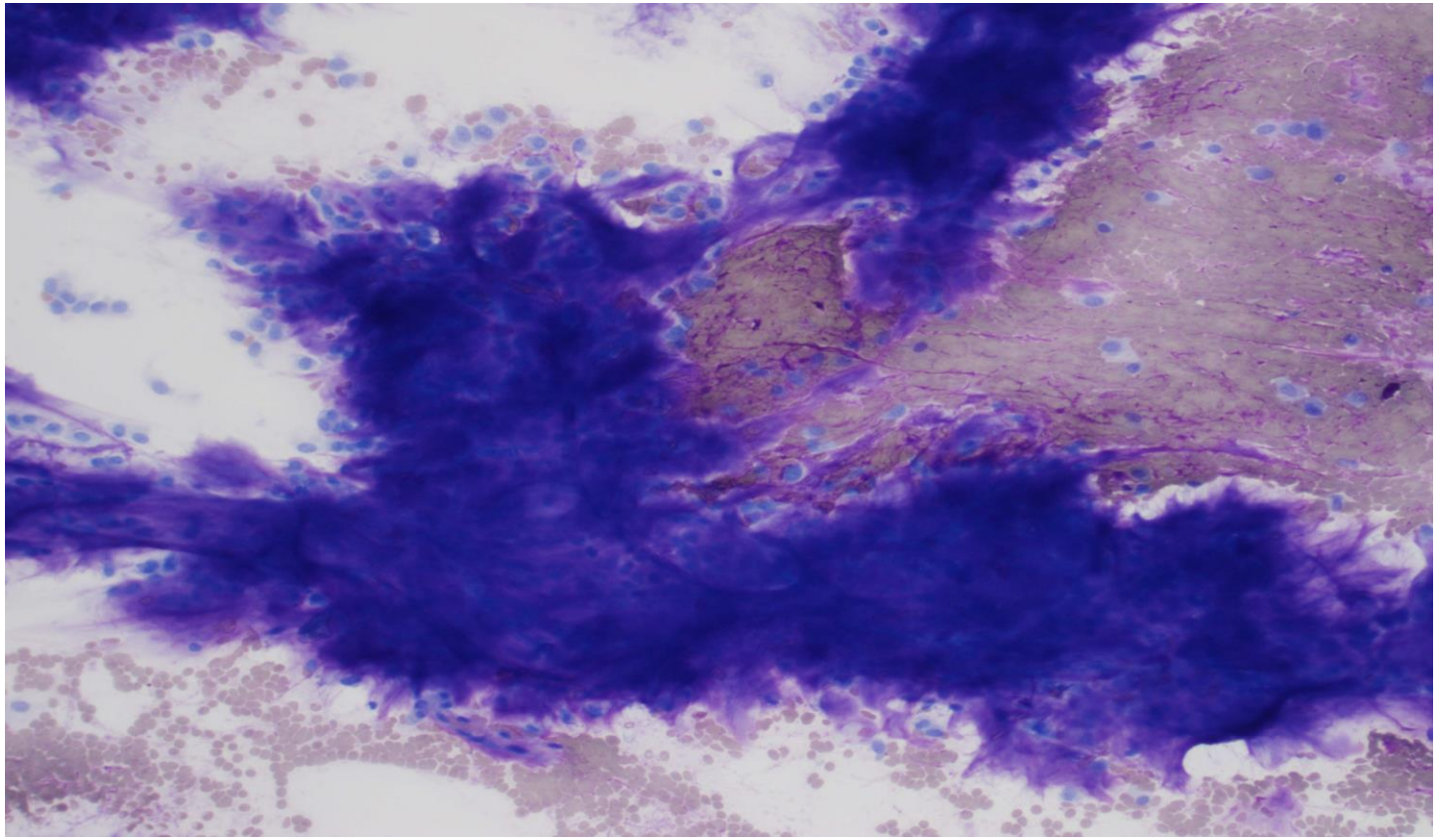


Myoepithelial cells in background of PA





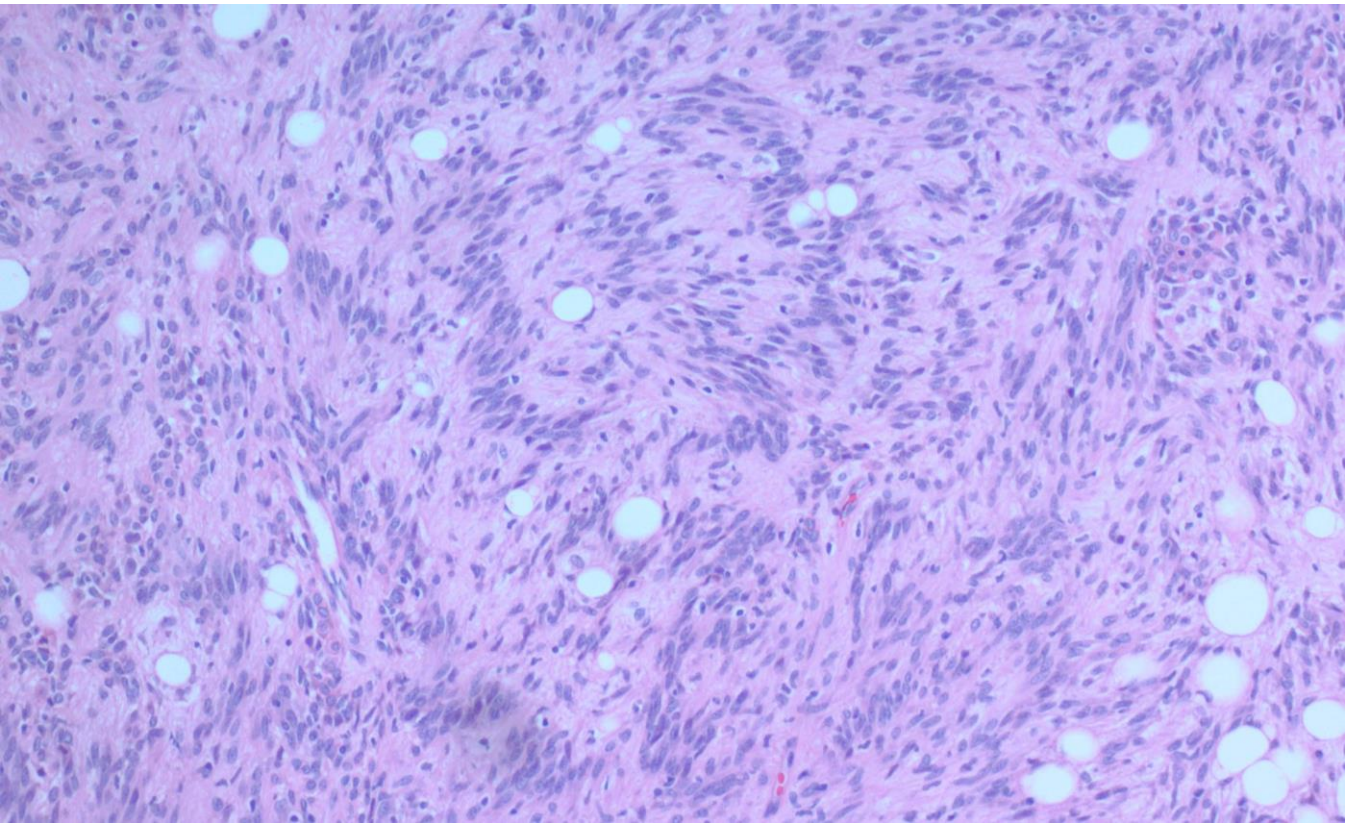
Hyaline PA vs. Myoepithelioma

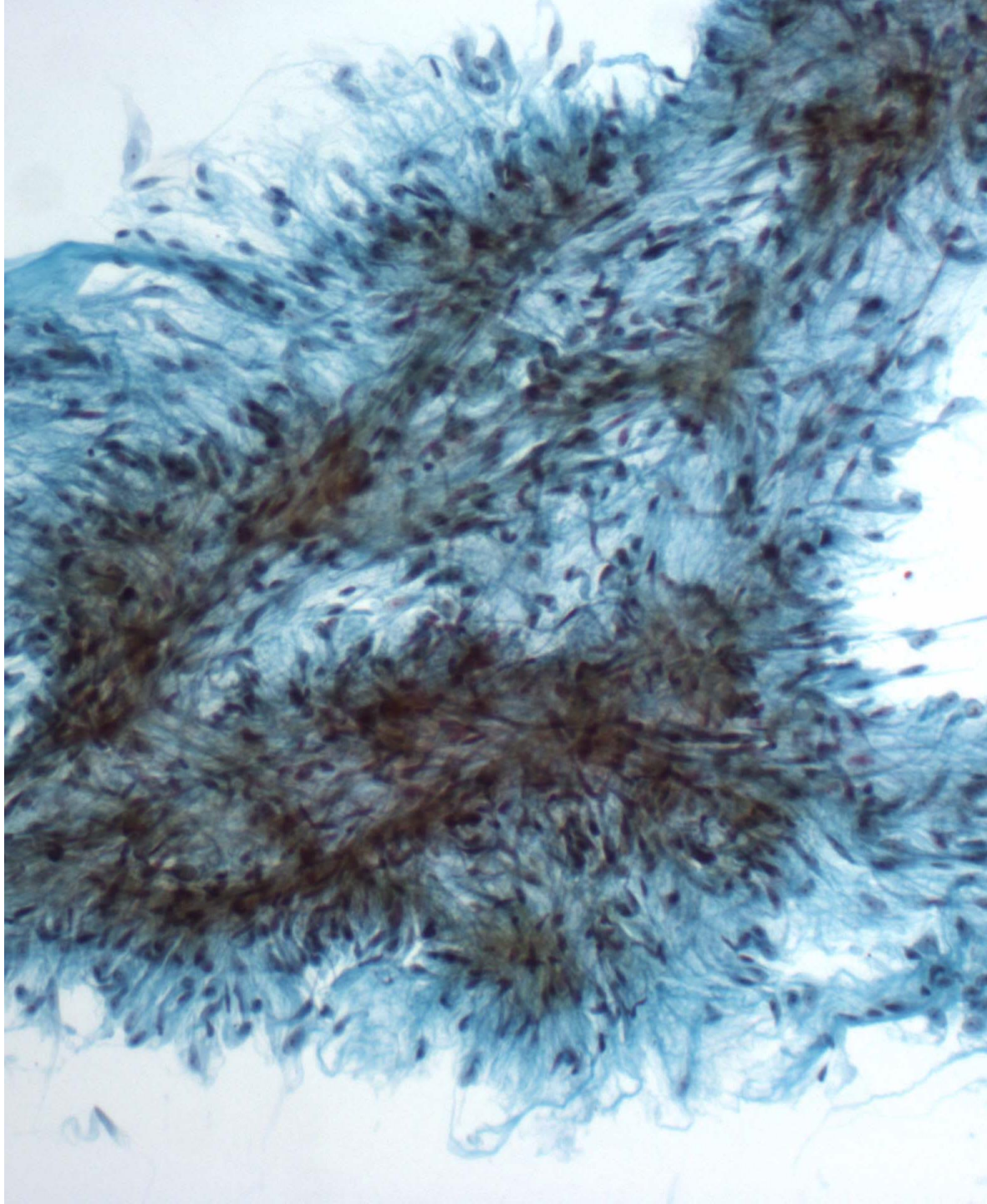


- Plasmacytoid cells
- Abundant dense cytoplasm and eccentric nuclei
- Mostly in minor salivary glands

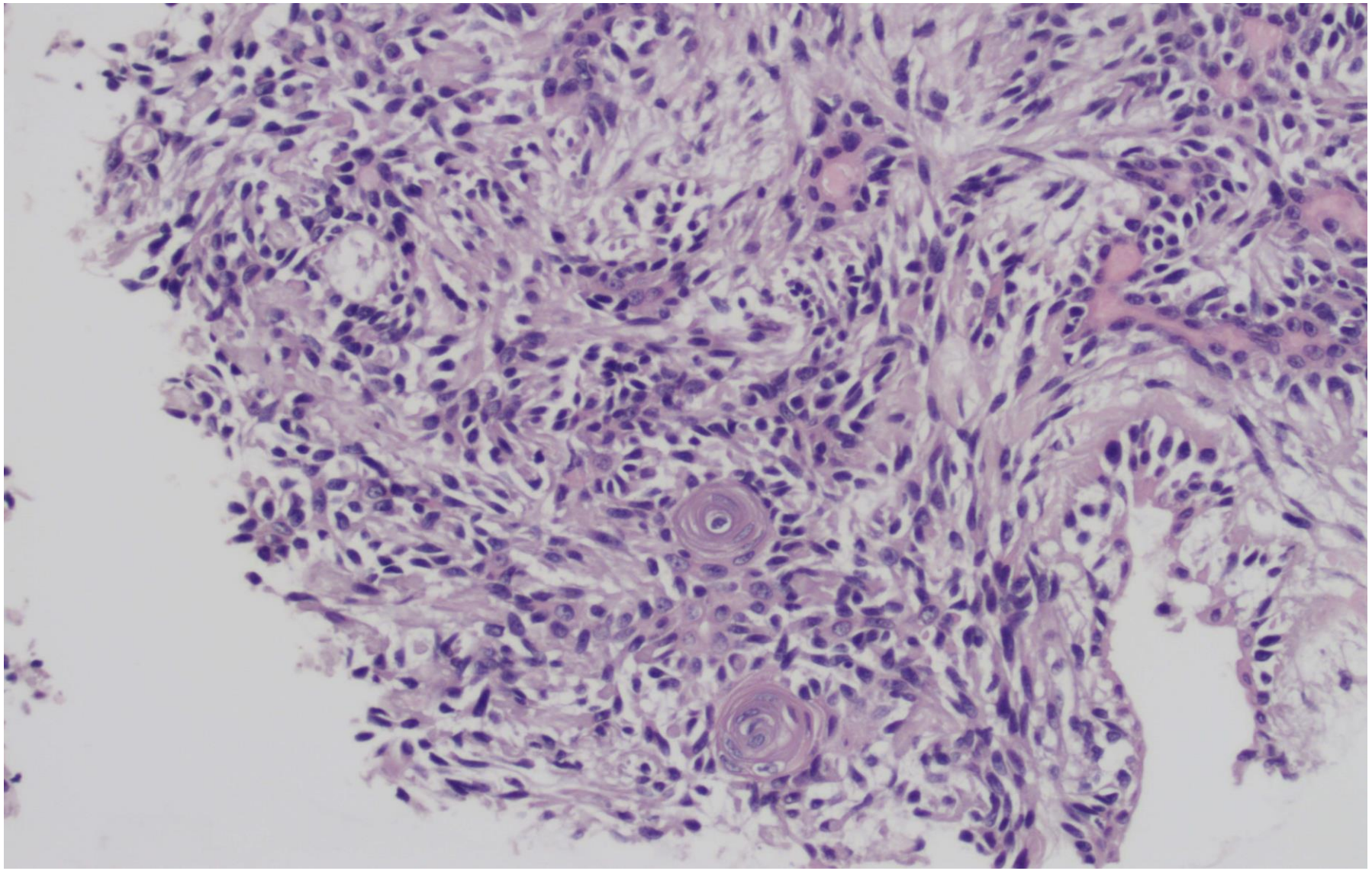
Spindle PA

- DDX:
  - Nerve sheath tumor
  - Leiomyoma
  - LG Sarcoma
  - Myoepithelioma





PA with Squamous Metaplasia



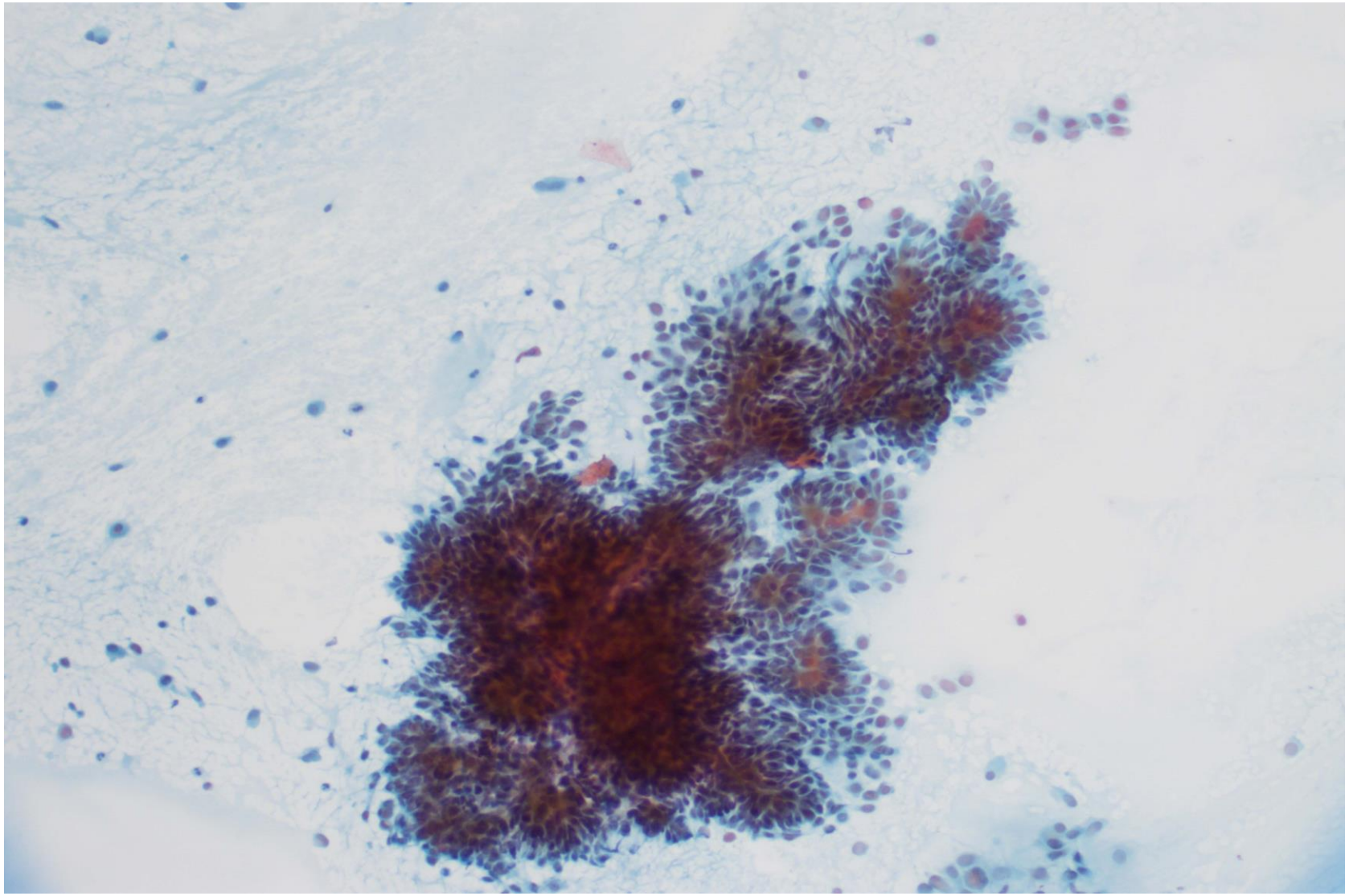
Diagnostic Challenges in PA

Epithelial/Myoepithelioma
If predominates, may suspect basaloid neoplasms e.g. BCA, AdCC, or LG Carcinoma

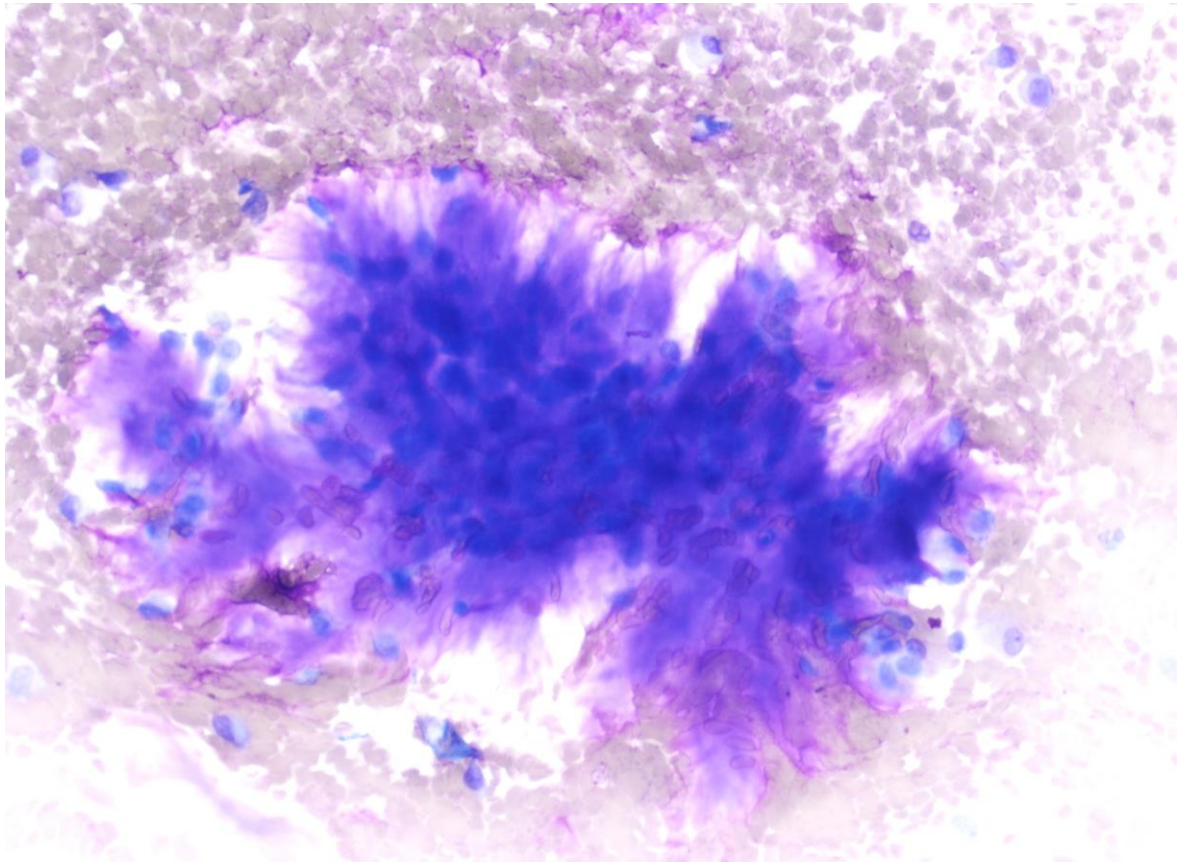
Biphasic

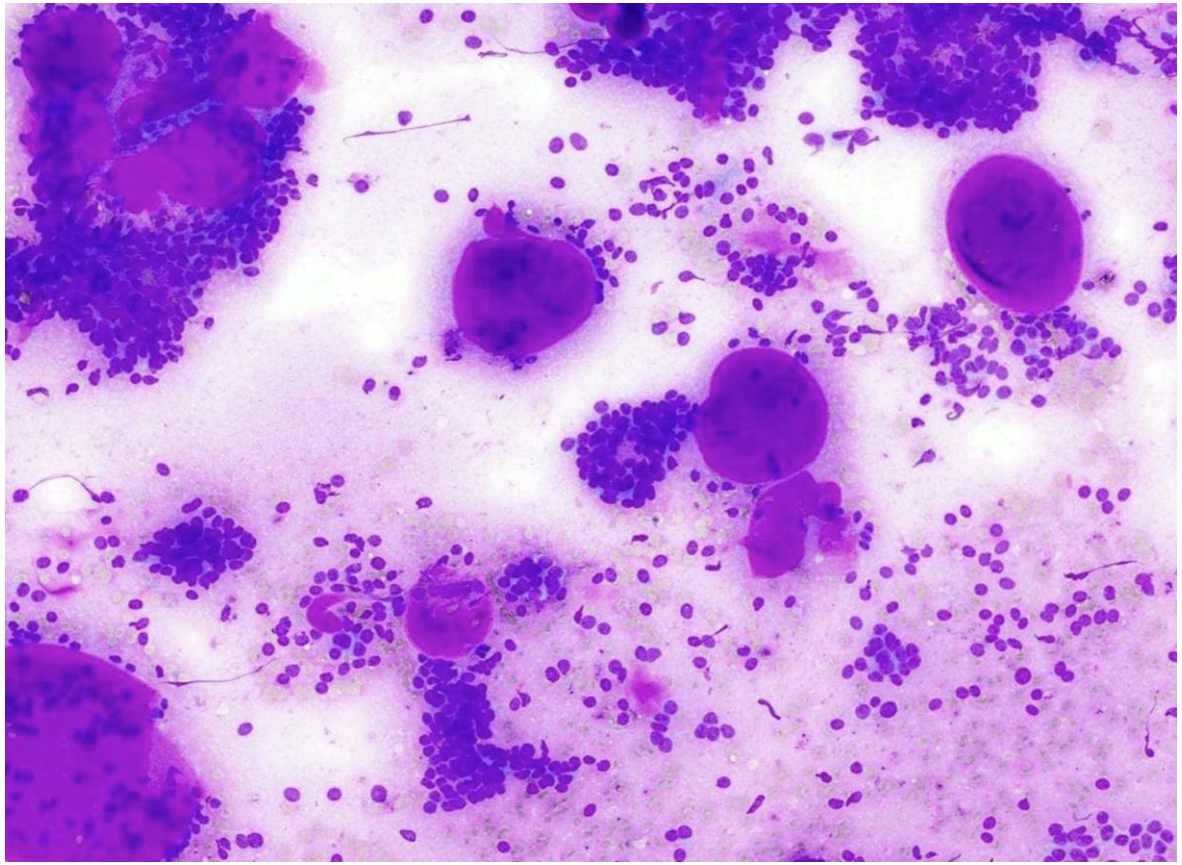
Mesenchymal
Myxoid component may be confused with mucin in LGMEC

Cellular PA vs. BCA or AdCC

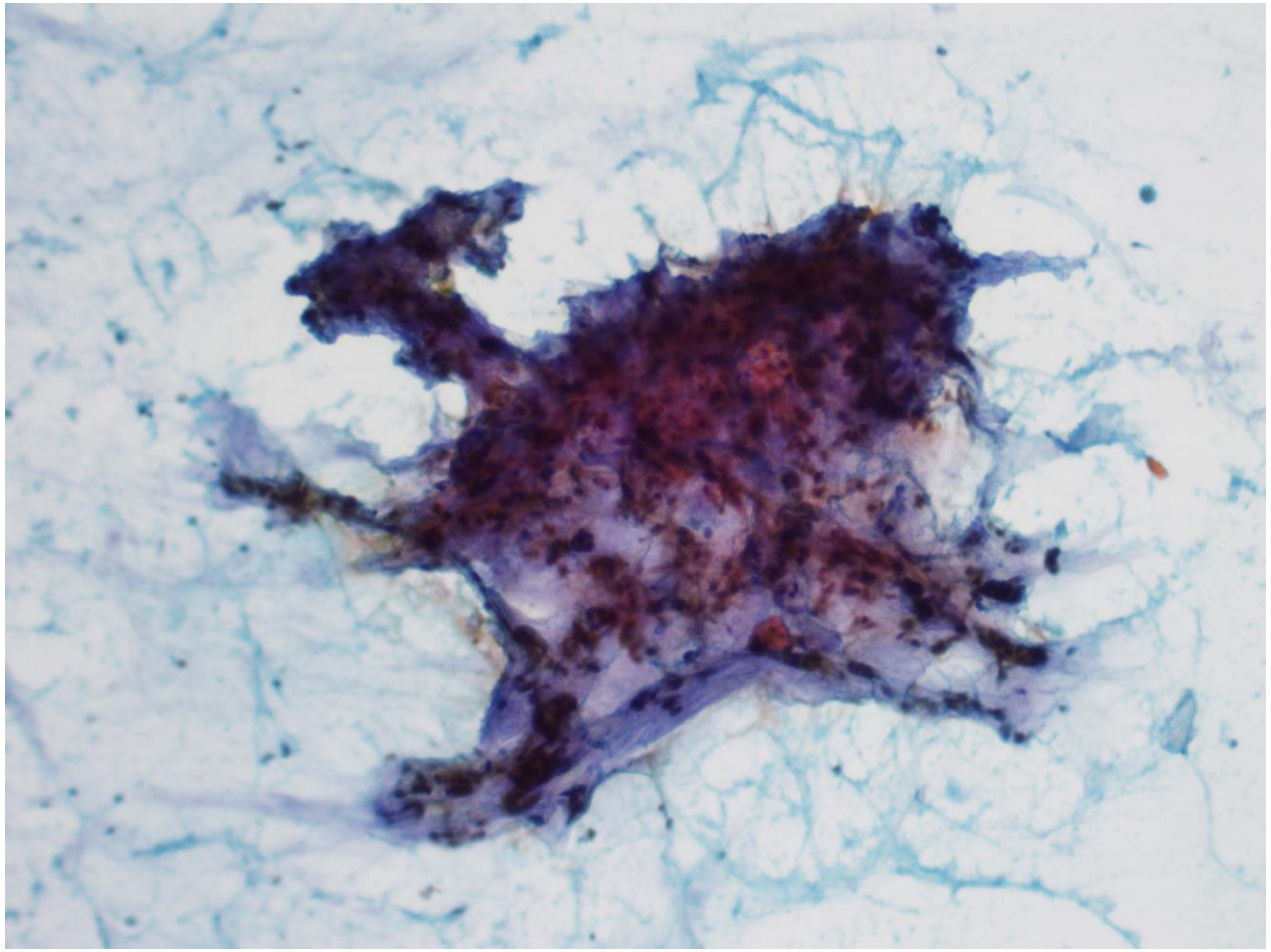


PA vs. BCA or AdCC

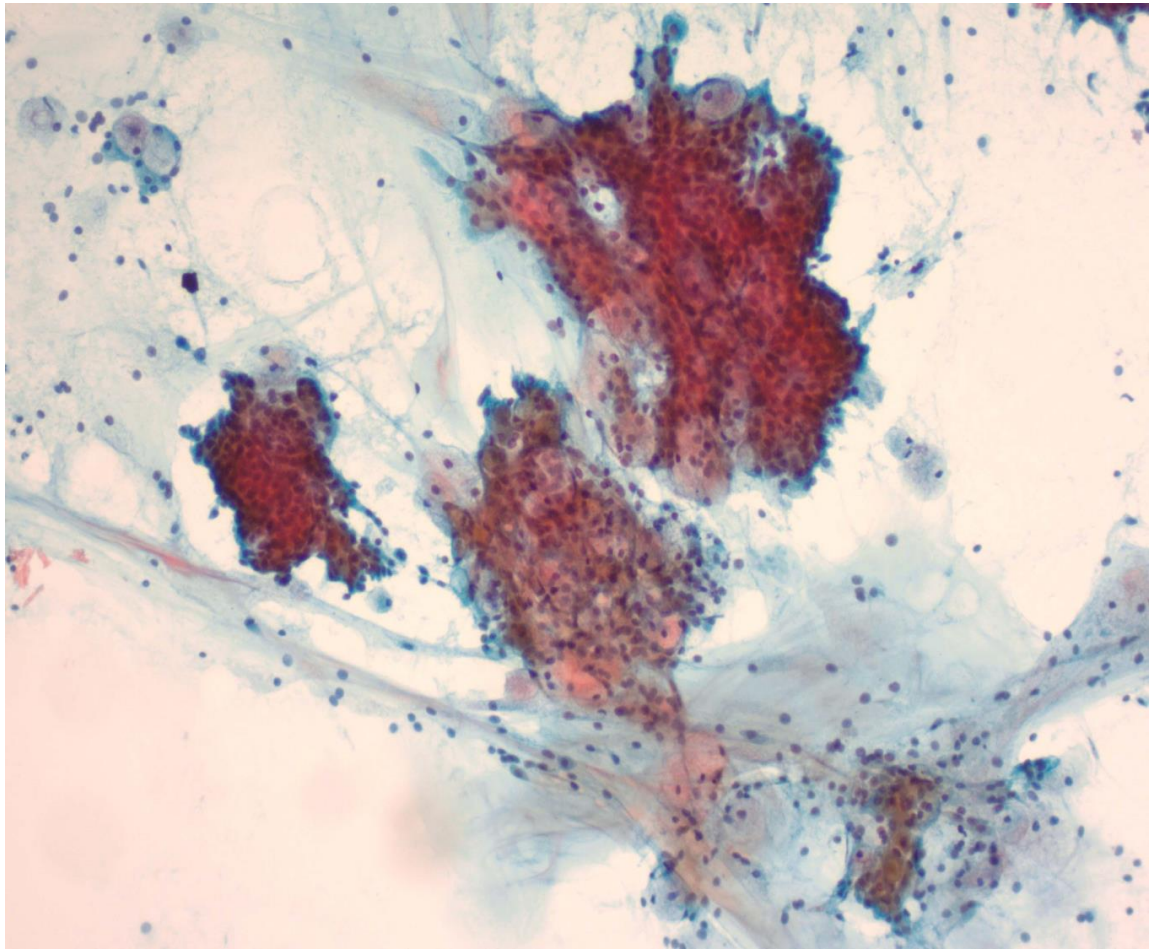




Stroma - PA

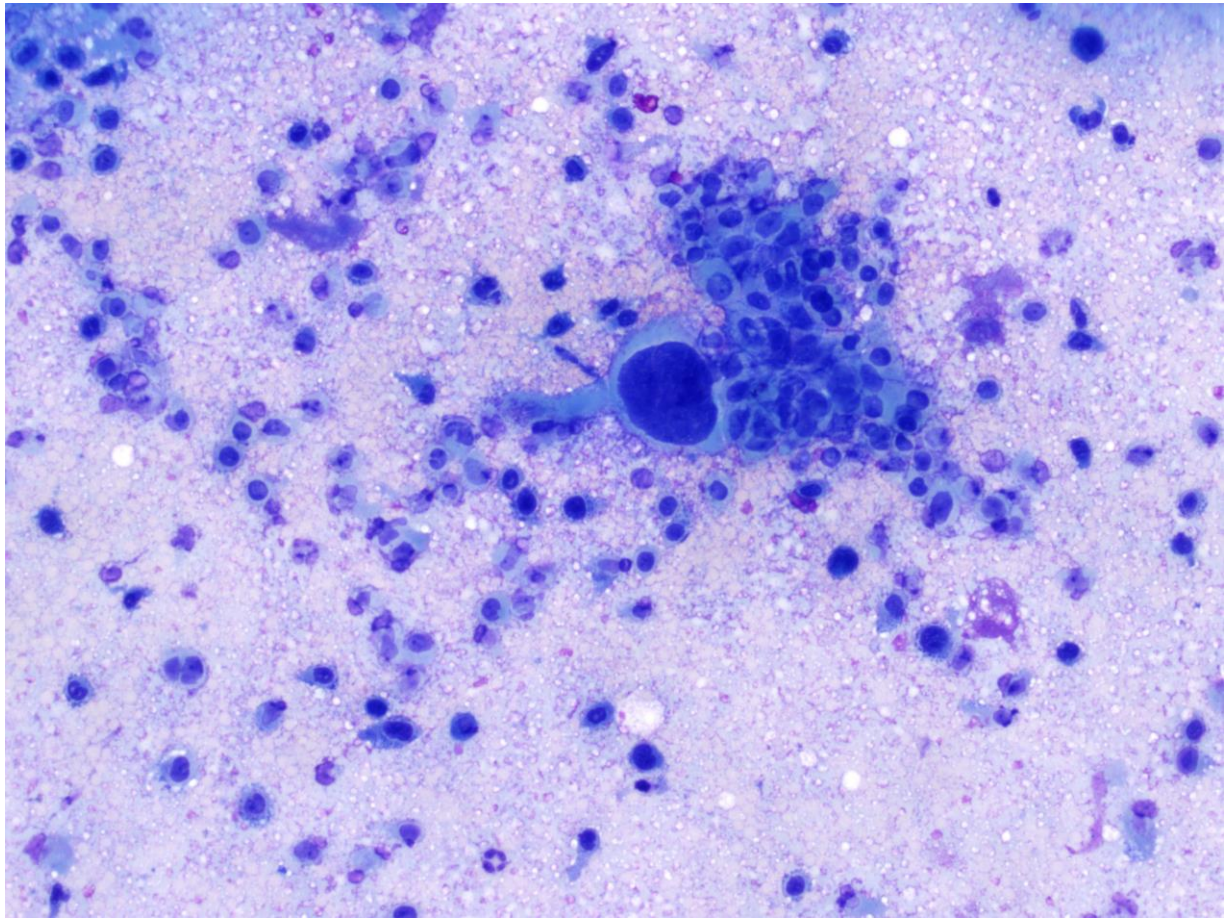


Mucin – LG MEC



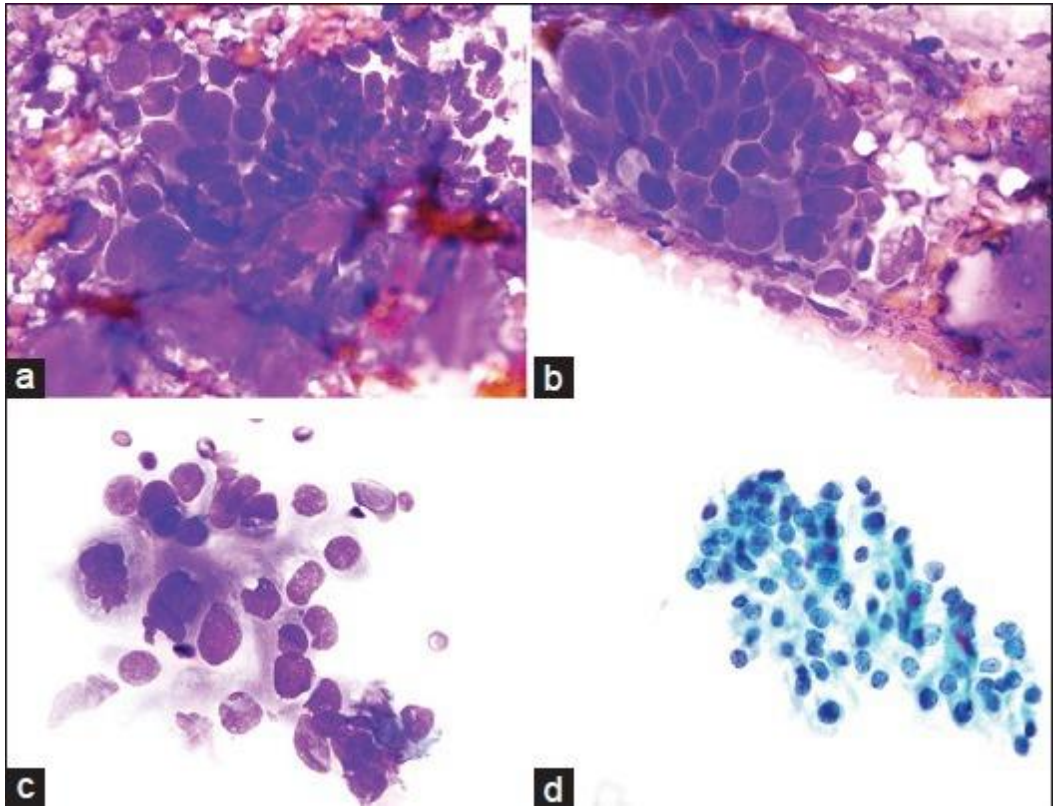
Cytologic atypia in PA

- Up to 20% of PA
- Scattered large cells with round-oval nuclei and prominent nucleoli
- Otherwise, classic PA cytology
- Accounts for many false-positive diagnoses
  - Most cases turn out to be benign on surgical follow-up
- Occasionally, atypia is more diffuse



Cytologic atypia in PA vs. CAXPA

- Typically, CAXPA is associated with high-grade carcinoma
  - FNA almost always contains anaplastic malignant cells without evidence of PA
  - Most common: Adeno NOS & SDC followed by MCA and EMCA
- A definitive diagnosis of CAXPA should not be rendered in the absence of overt malignant cytologic features



Cytojournal. 2015 Apr 29;12:7.

Ancillary studies to improve the FNA diagnosis

- Immunocytochemistry
  - LBP
  - Smears
  - Cell block (FFPE)
- FISH
- RT-PCR
- Next-gen sequencing

Useful IHC Stains

Tumor Type	Useful IHC
Mucoepidermoid carcinoma	*p63/p40 positive, SOX10 negative*
Secretory carcinoma	*S100, mammaglobin*
Acinic cell carcinoma	*NR4A3; DOG-1*
Intraductal carcinoma	
Adenoid cystic carcinoma	*MYB*
Pleomorphic adenoma	*PLAG1, HMGA2*
Basal cell adenoma	*beta-catenin*
Clear cell carcinoma	
Polymorphous adenocarcinoma	*p63 positive, p40 negative*

Diagnostic molecular findings

- Increasing number of salivary gland tumors have recurrent, defining, chromosomal rearrangements

Tumor Type	Molecular Tests
Mucoepidermoid carcinoma	*CRTC1-MAML2, CRTC3-MAML2*
Secretory carcinoma	*ETV6-NTRK3, ETV6-X*
Acinic cell carcinoma	*NR4A3-SCPP*
Intraductal carcinoma	*NCOA4-RET, TRIM27-RET*
Adenoid cystic carcinoma	*MYB-NFIB, MYBL-NFIB*
Pleomorphic adenoma	*PLAG1 and HMGA2 rearrangements*
Microsecretory adenocarcinoma	*MEF2C-SS18*
Clear cell carcinoma	*EWSR1-ATF1, EWSR1-CREM*
Polymorphous adenocarcinoma	*PRKD1/2/3 rearrangements and point mutations*

Summary

- Basaloid neoplasms and LG malignancies account for most discrepancies in salivary gland cytology
- Important to recognize high grade features
- Be aware of limitations and potential pitfalls that may lead to false negative and positive diagnoses
- Extracellular matrix may be associated with benign and malignant tumors
- Careful evaluation of matrix quality, cell-stromal interface, cell morphology, and background cells

Summary

- Minimize diagnostic errors by utilizing strict criteria
- A specific diagnosis can be rendered in many instances
- Cellular neoplasm NOS (or SUMP)
- FNA diagnosis should be interpreted in context of clinical and radiologic findings.

THANK YOU!

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