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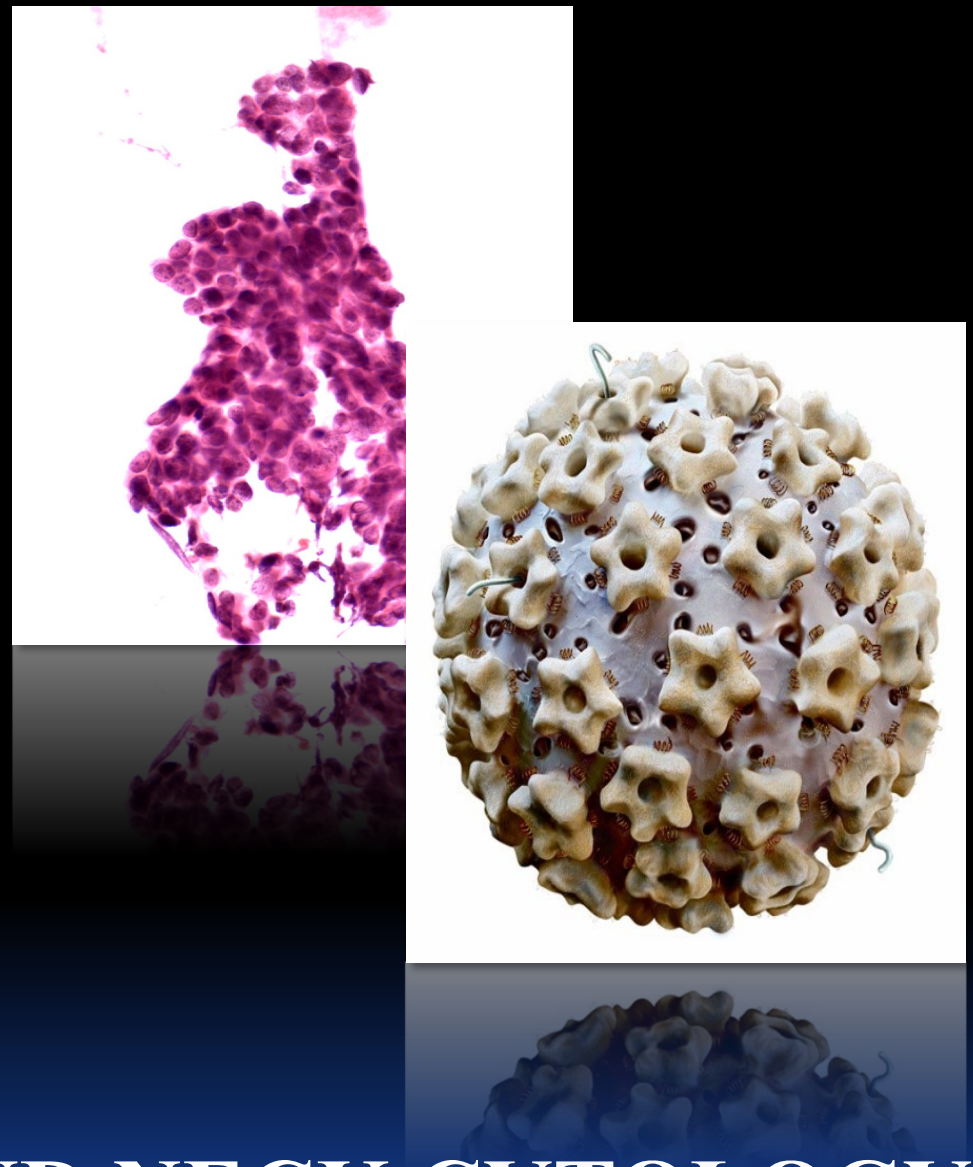


ESSENTIALS OF HEAD AND NECK CYTOLOGY

Greetings From Boston MA - USA

Bill, Boston, and Bonsai!

FNA OF THE HEAD AND NECK

- Anatomically complex region
- Wide variety of organs and tissues
- Diverse group of reactive and neoplastic lesions
- Many challenging areas including:
  - » Thyroid neoplasia
  - » Salivary gland neoplasia
  - » Squamous cysts (benign and malignant)
  - » Lymph nodes (reactive, lymphoma, metastases)
  - » Spindle cell lesions (reactive and neoplastic)
  - » Soft tissue

Differential Diagnosis of Squamous Cysts of the Head and Neck

Squamous/Squamoid Cysts of Head and Neck:

Lateral Neck:

- Epidermal inclusion cyst
- Branchial cleft cyst
- Pilomatrixoma
- Cystic squamous cell carcinoma

Upper Neck:

- Warthin tumor

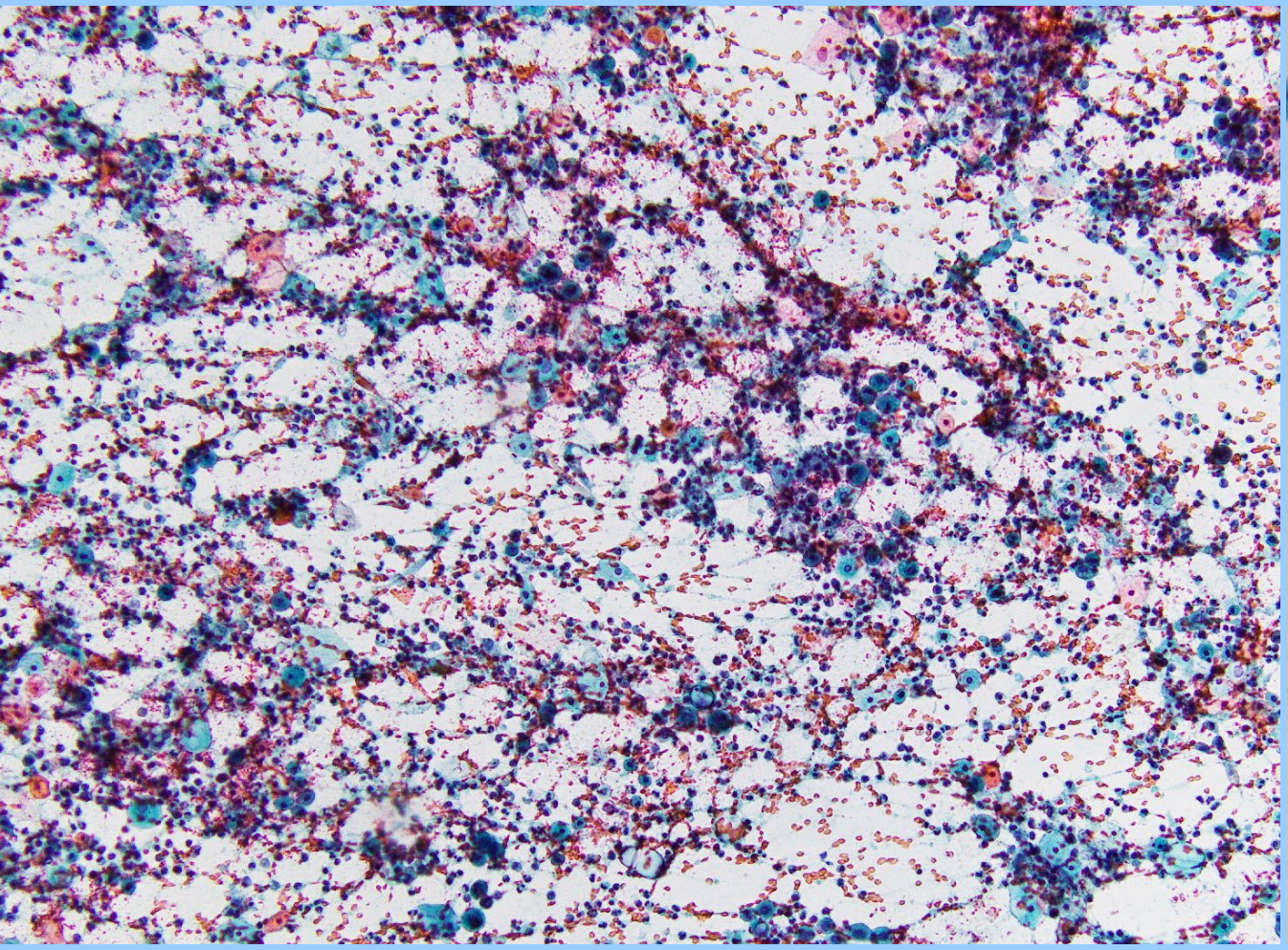
Midline Neck:

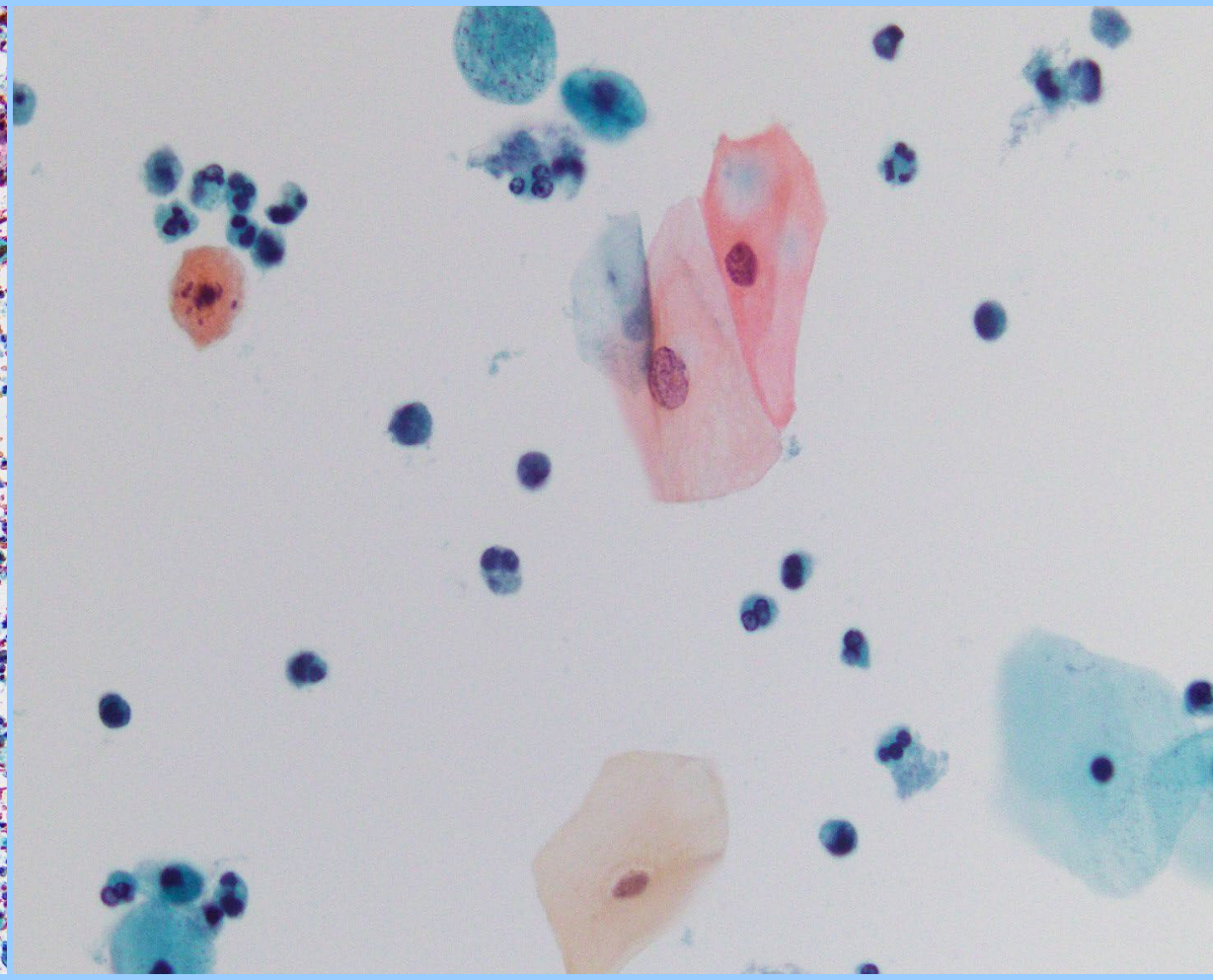
- Dermoid cyst
- Thyroglossal duct cyst
- Metastatic papillary thyroid carcinoma

CASE:

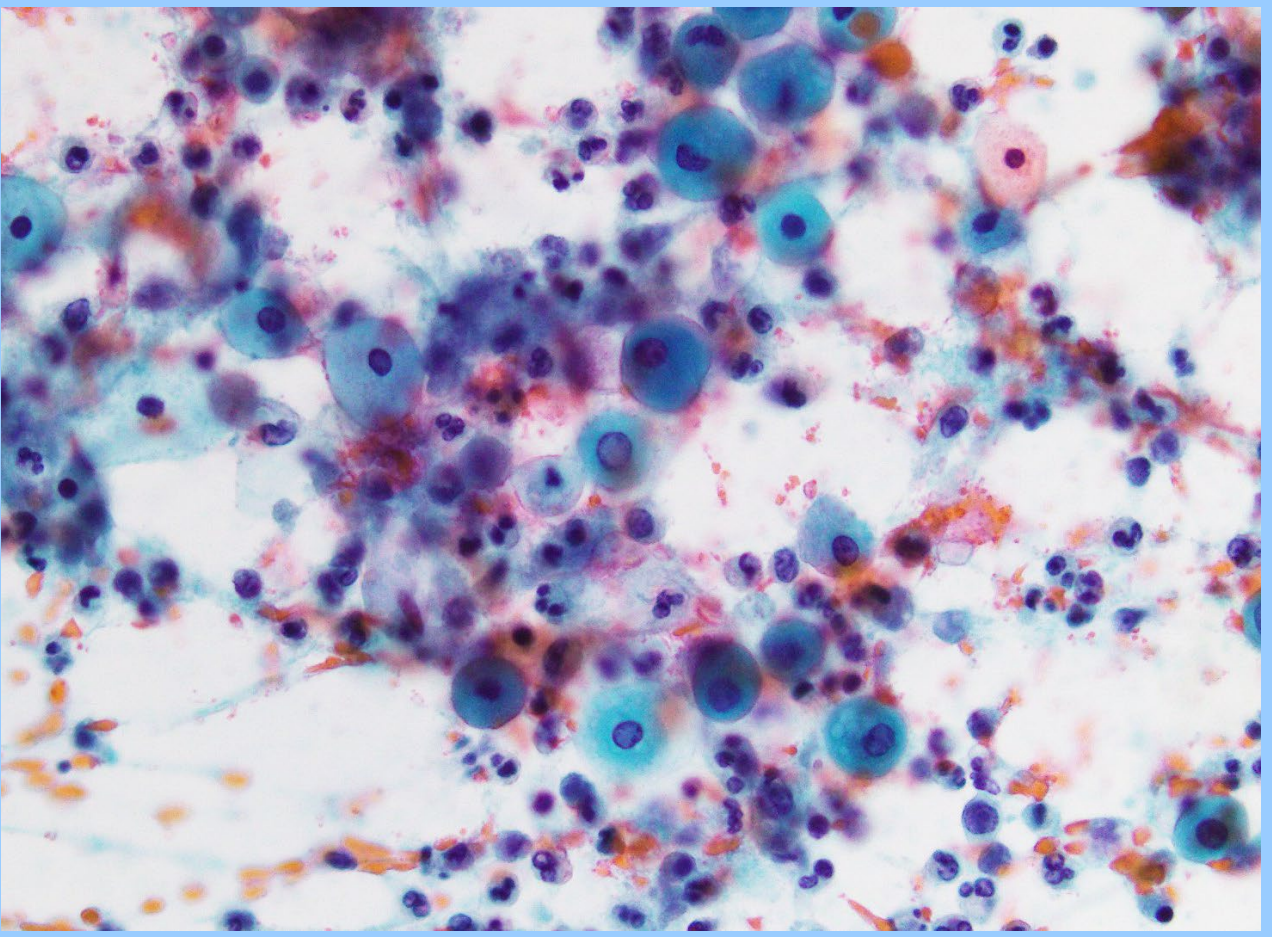
11 year-old female presenting with a cystic left neck mass. A CT showed a level 2A multilobulated, complex 3.4 cm left neck mass. An FNA was performed.

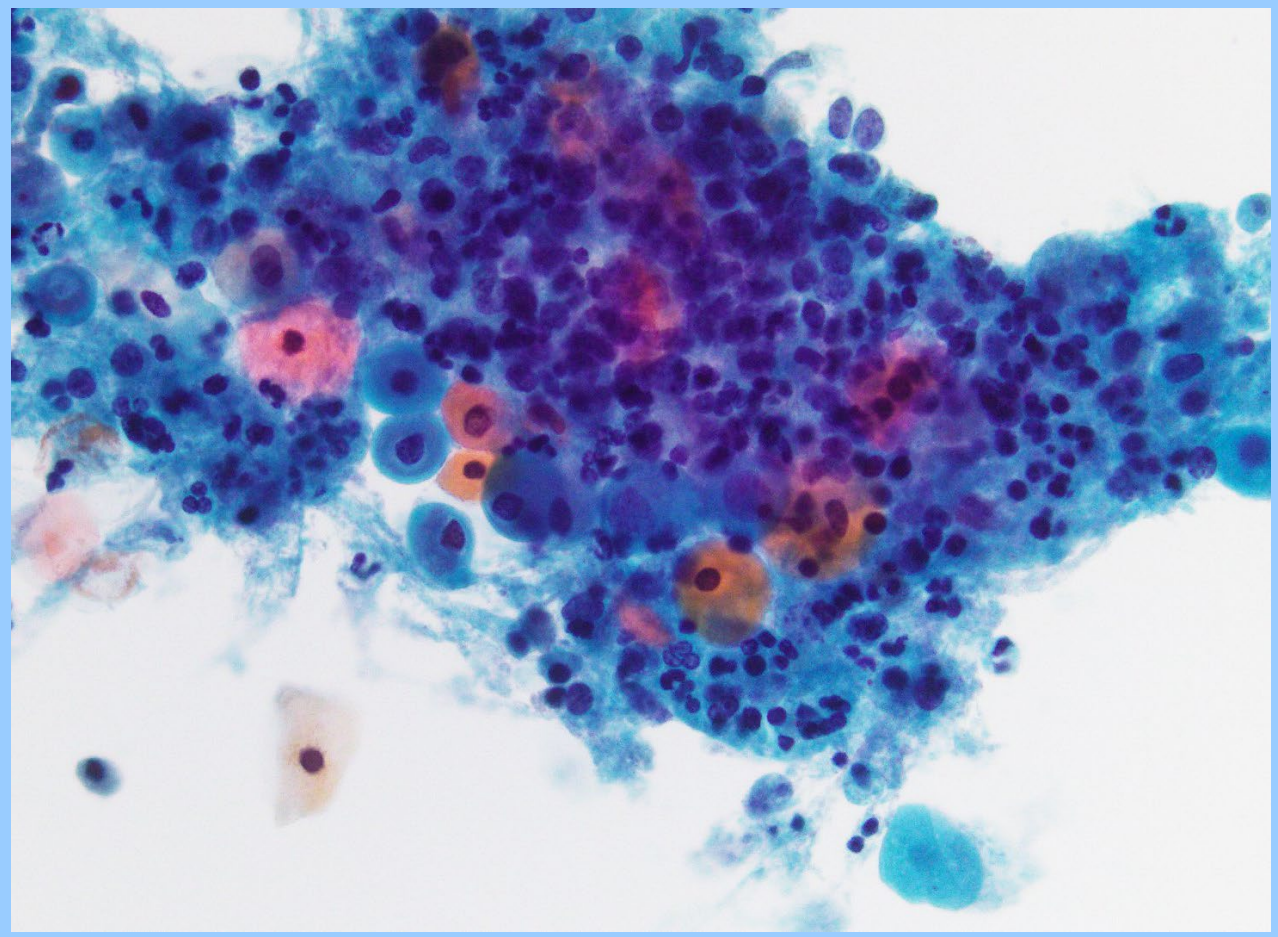
Mature squamous cells and anucleate squames admixed with blood, lymphocytes, and acute inflammation.





Abundant acute inflammation.





CYTOLOGY DIAGNOSIS:

Satisfactory for Evaluation.

BENIGN

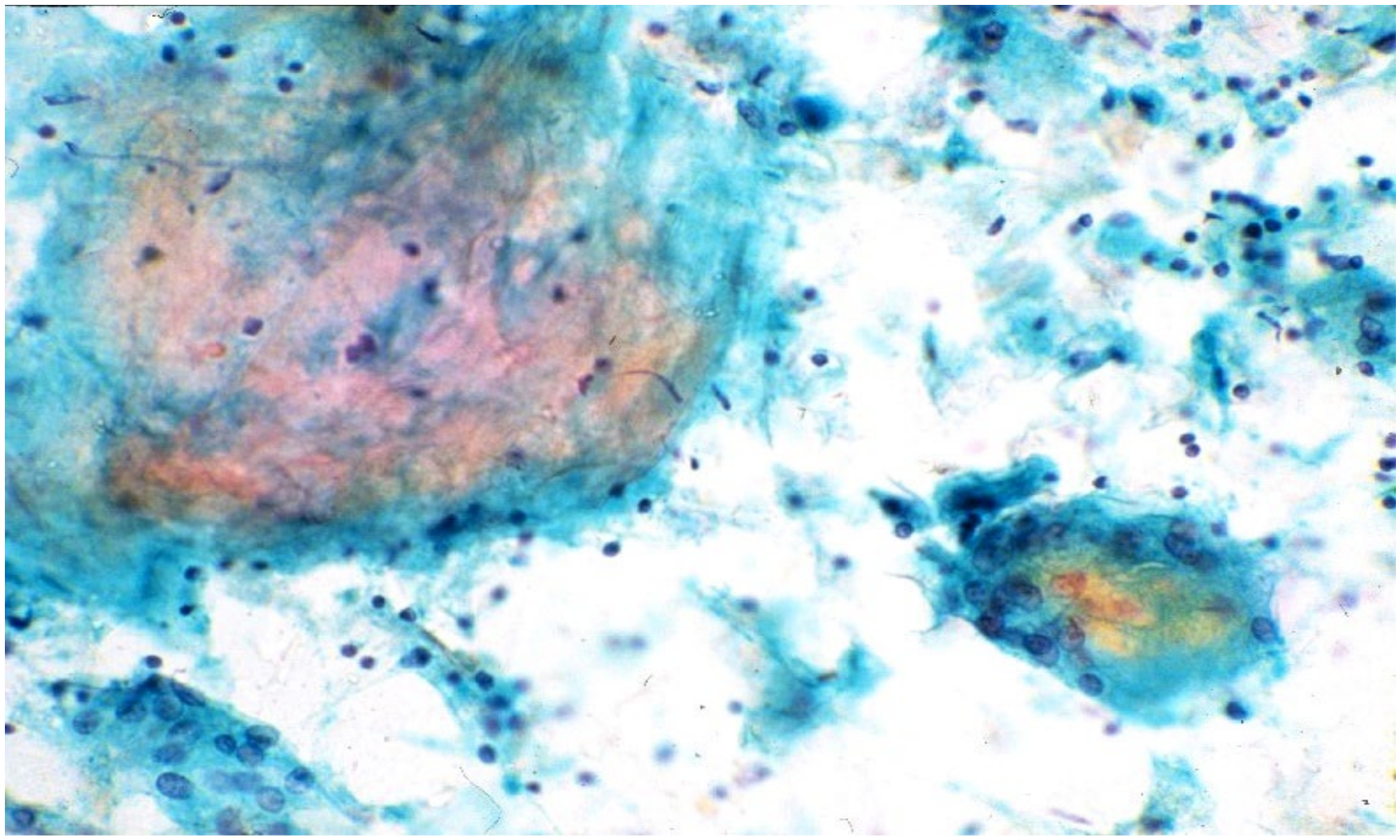
Squamous cells, reactive changes, and acute inflammation.

Note: The cytologic findings are compatible with an infected second branchial cleft cyst.

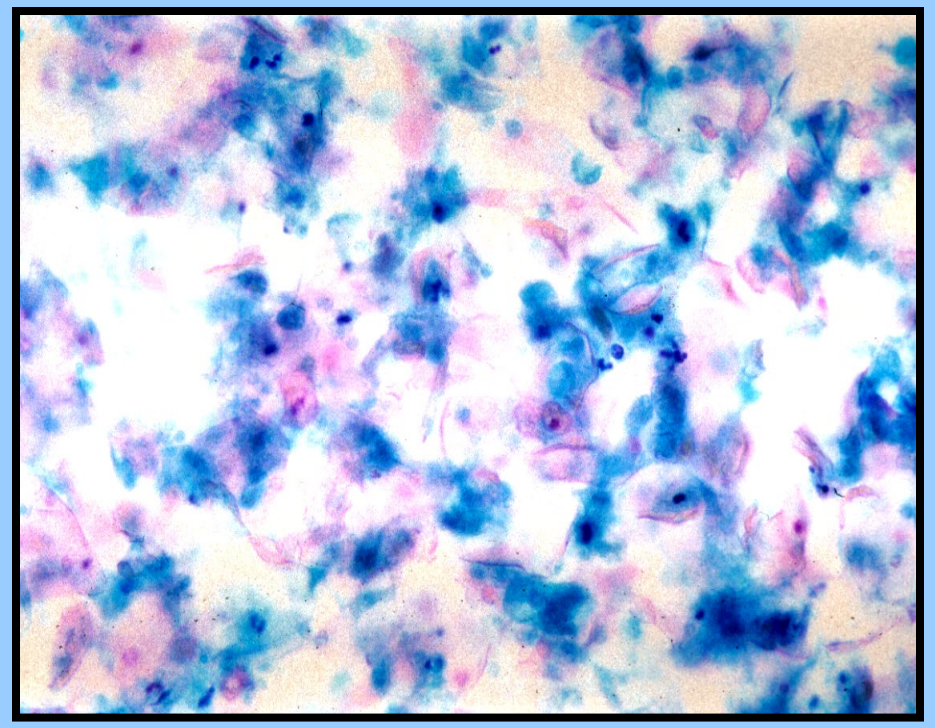
Branchial Cleft Cyst

- Very common
- Usually present in adolescents and young adults
- Anterolateral neck along SCM
- Can have squamous or respiratory epithelium
- Often inflamed and may show “atypia.”

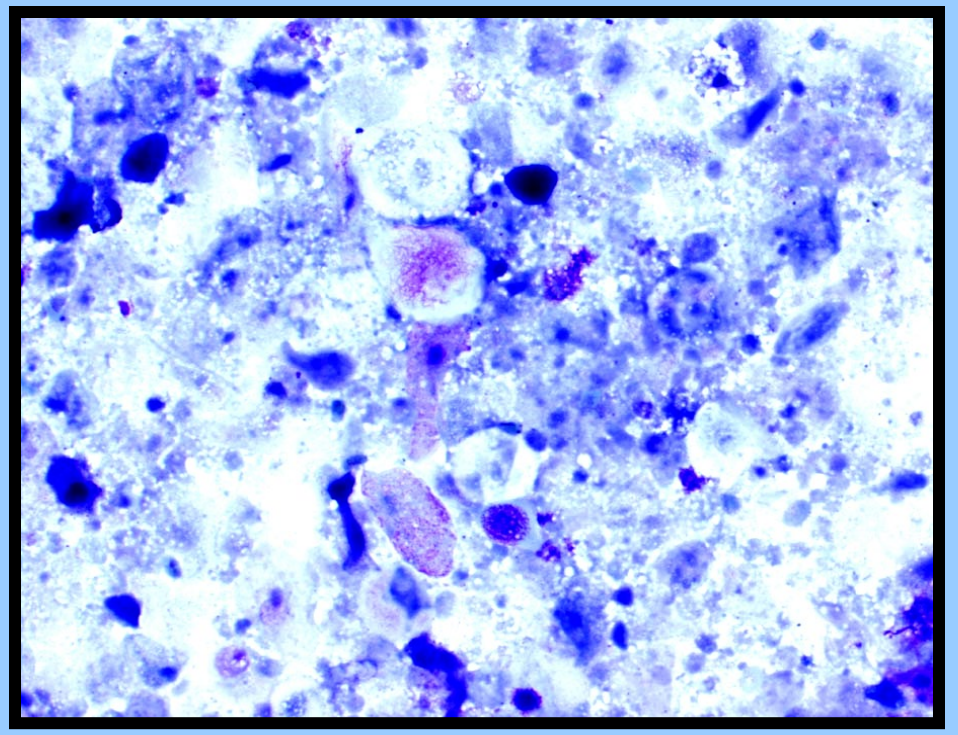
Branchial Cleft Cyst:
Mostly Anucleate Cells



Branchial Cleft Cyst:
Bland Pyknotic and Anucleate Cells

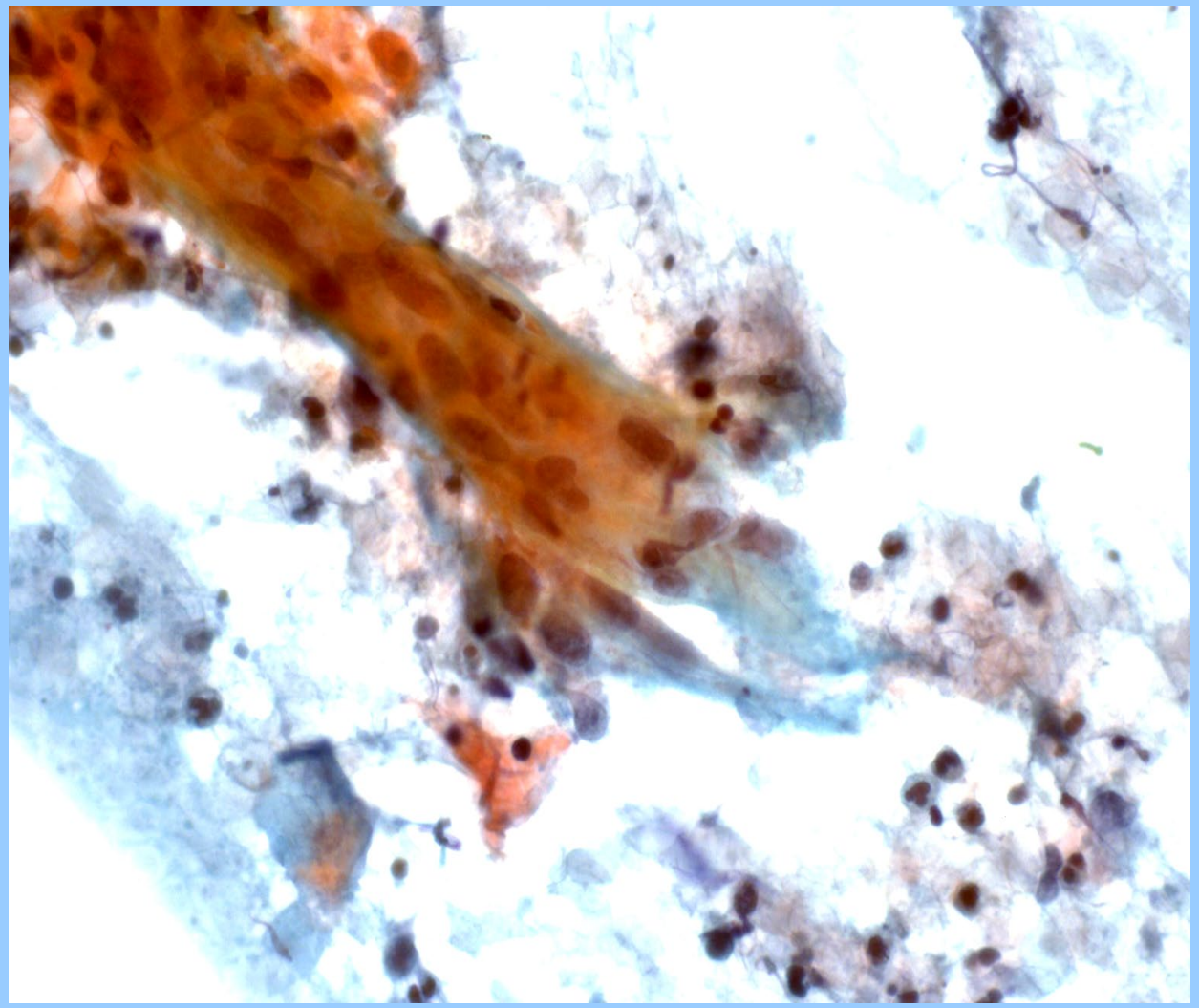


Pap Stain



Diff-Quik Stain

Inflamed Branchial Cleft Cyst: Cytologic atypia



KEY POINTS

- **Branchial cleft cyst cytology**
  - **Child or young adult = Benign**
  - **Adult = Atypical**
  - **Mixed anucleate squamous and mature squames**
  - **Acute inflammation**

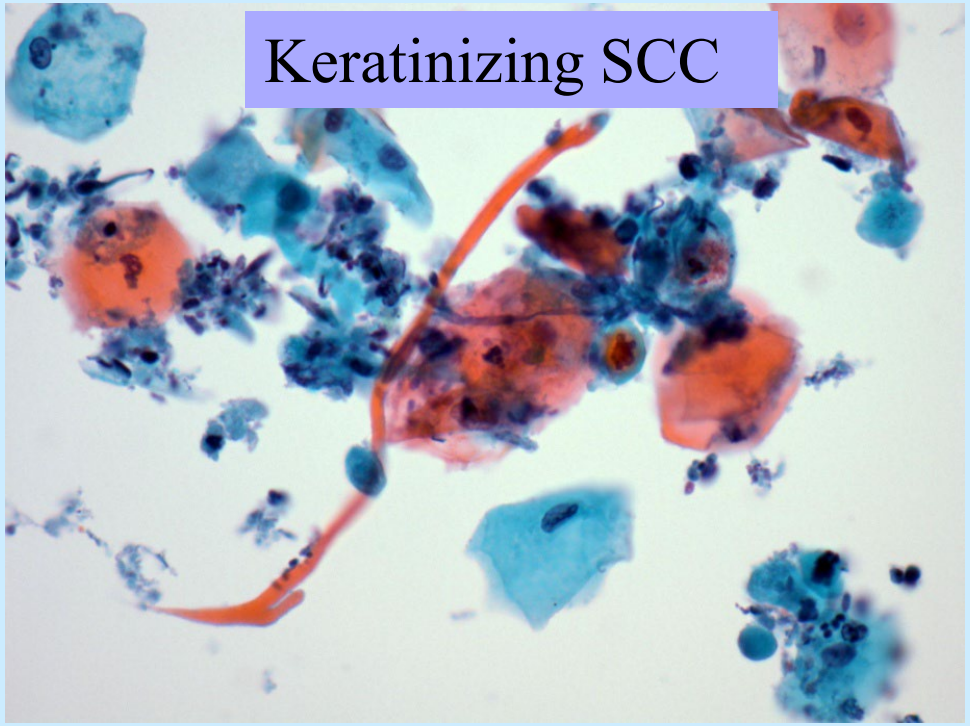
Recurring Problem in the FNA Evaluation of Head and Neck Squamous Cysts in Adults

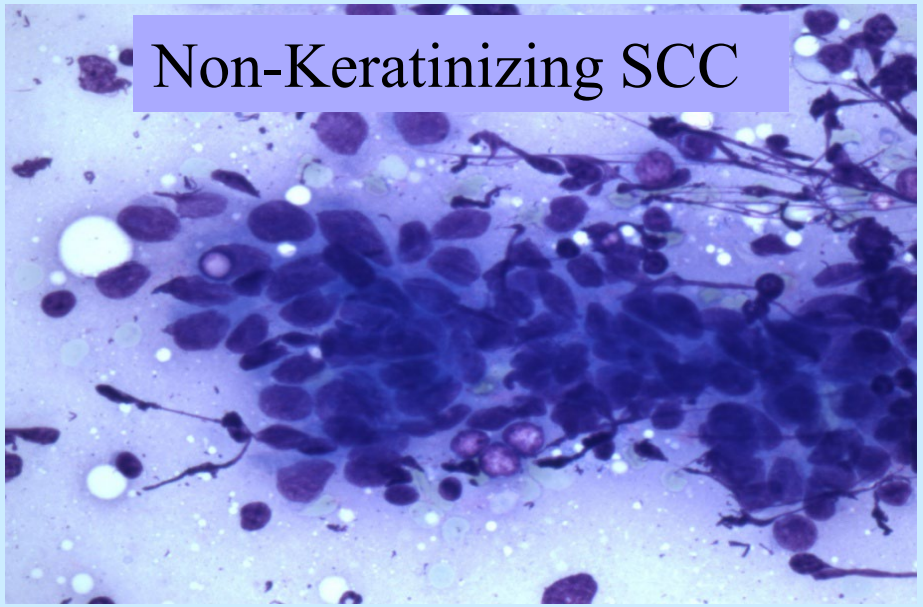
Branchial cleft cyst vs.
Well differentiated squamous cell carcinoma

How do we distinguish branchial cleft cyst from well differentiated squamous cell carcinoma?

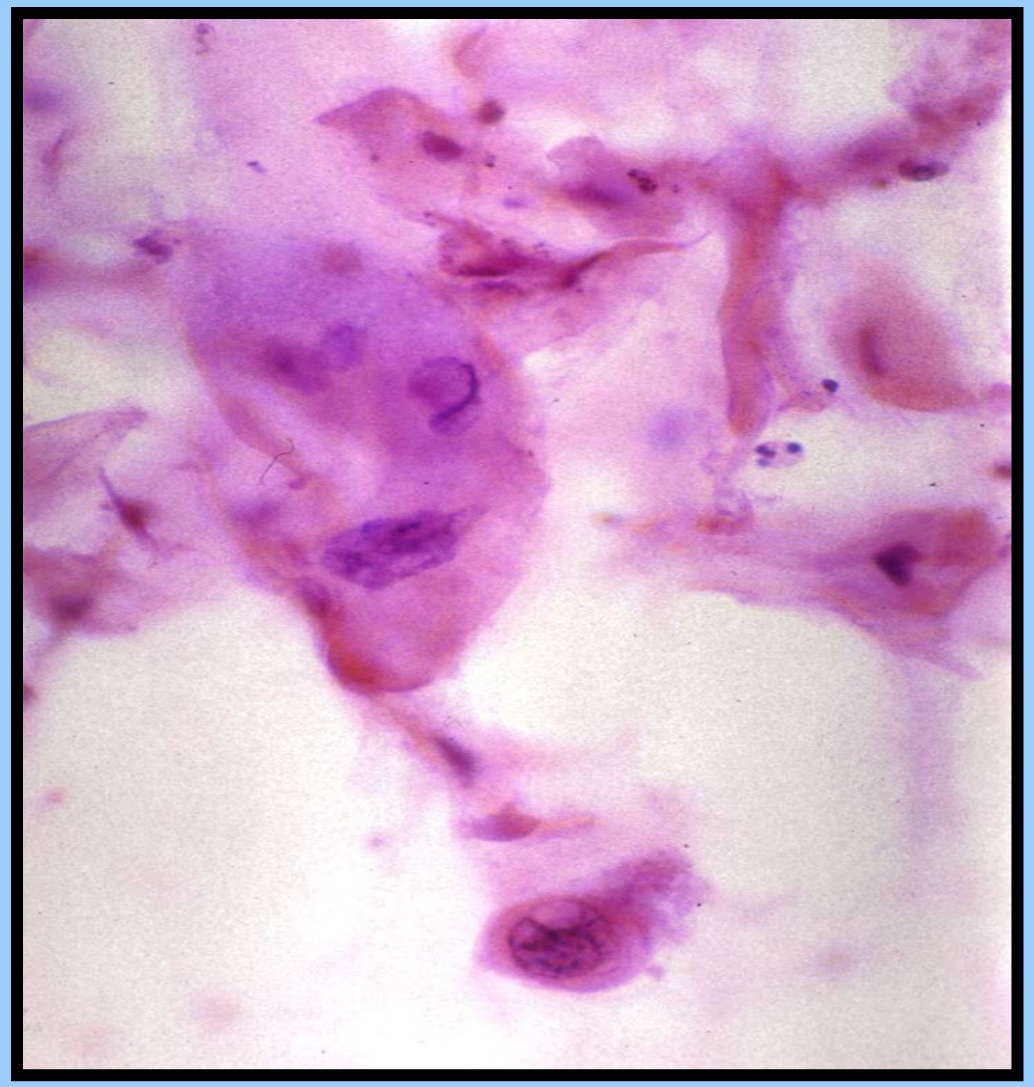
Met Squamous Cell Carcinoma

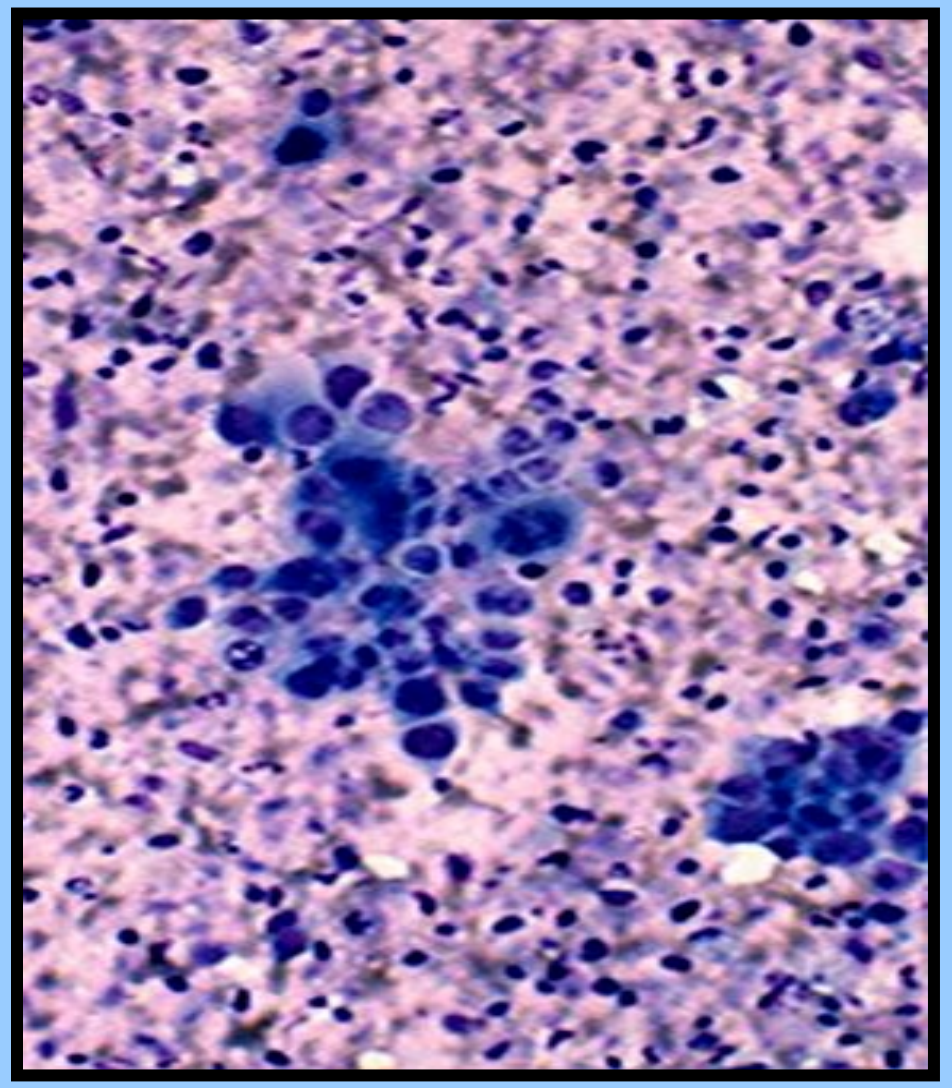
- Most common metastatic disease in HN
- Ker 5/6, p63, p40+
- Keratinizing and non-keratizing
  - UADT (oral cavity and larynx)
  - Oropharynx & nasopharynx
  - Skin
- May be cystic with numerous histiocytes



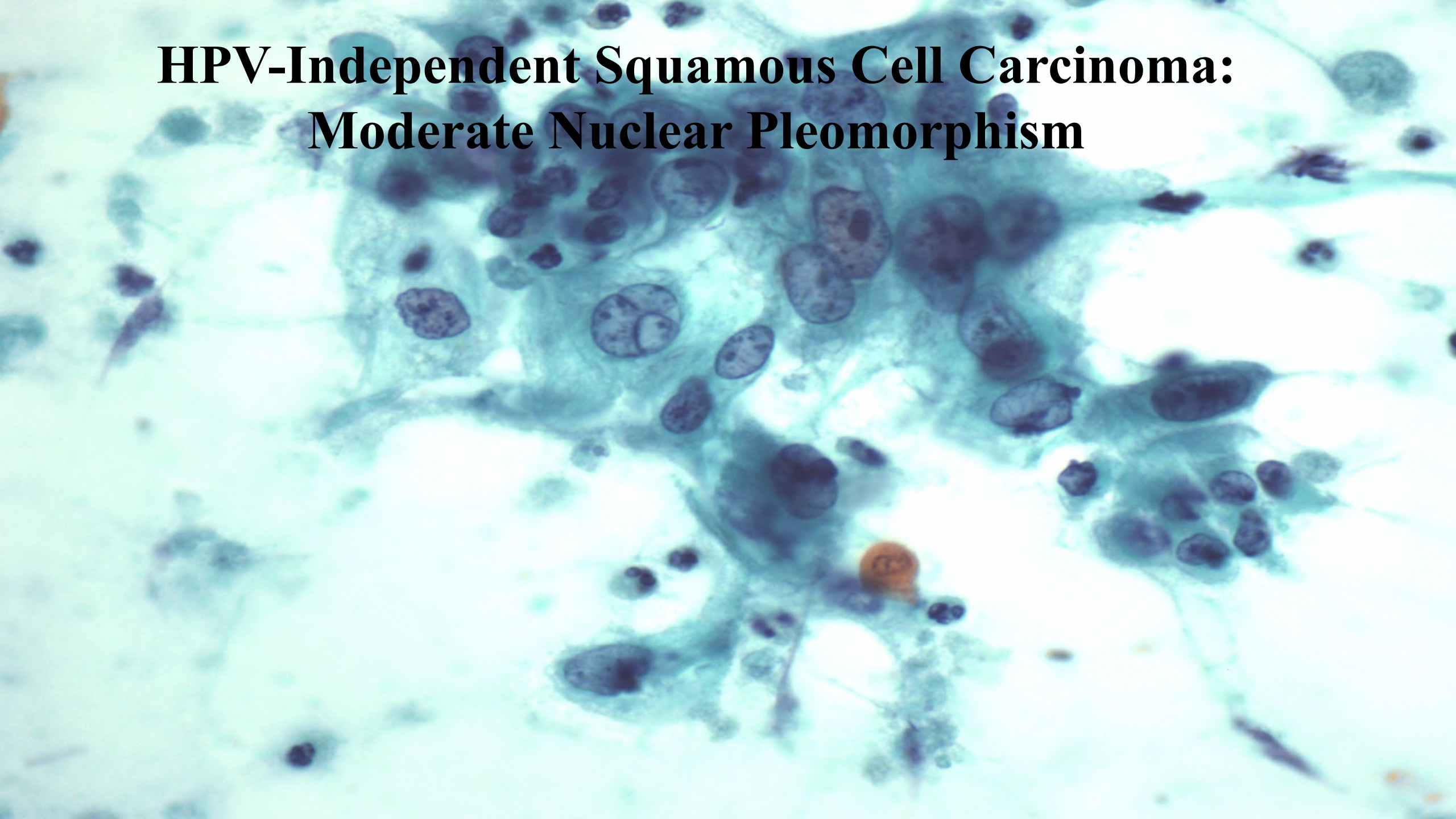


Squamous Cell Carcinoma: Mild to Severe Atypia

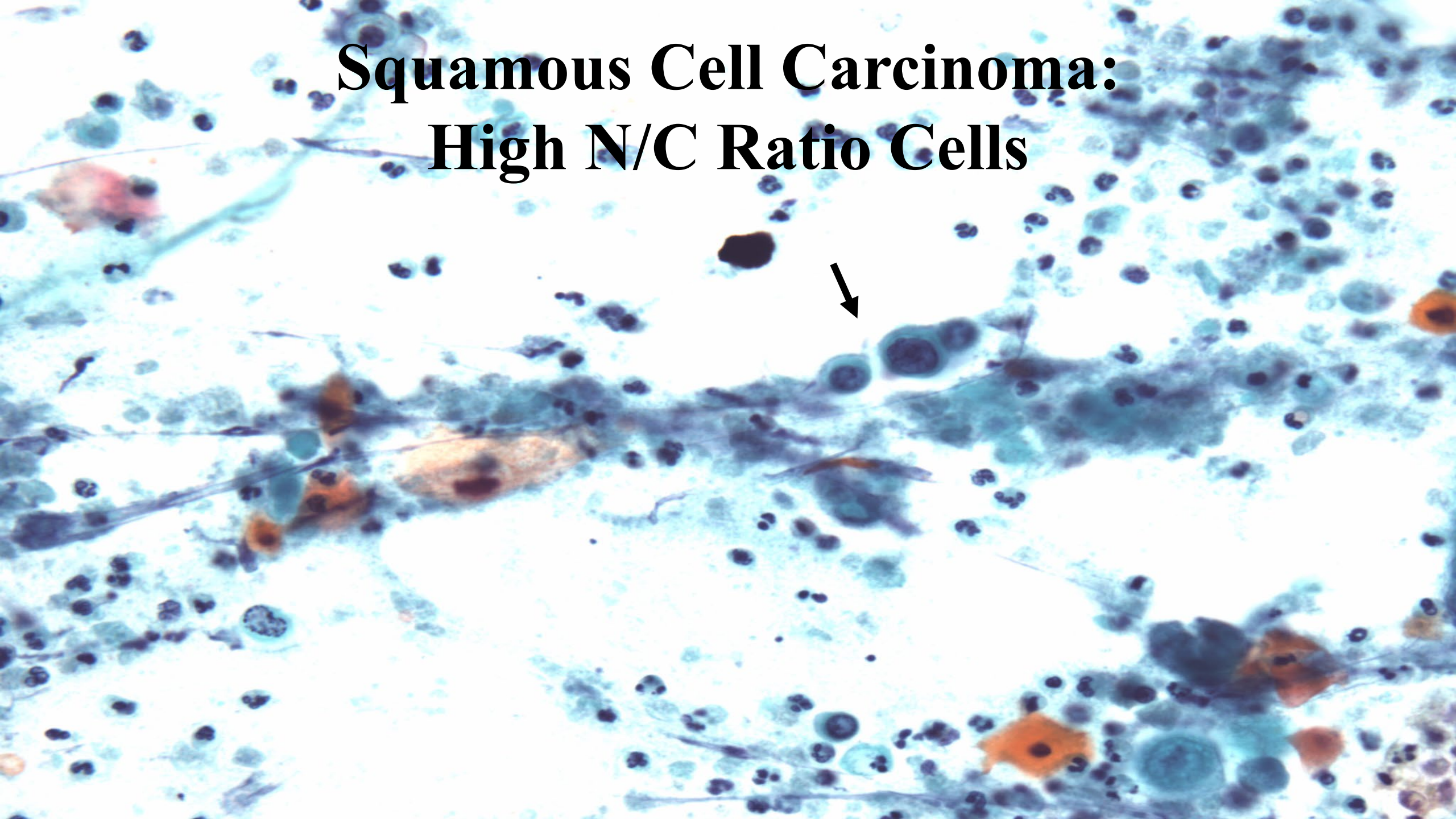




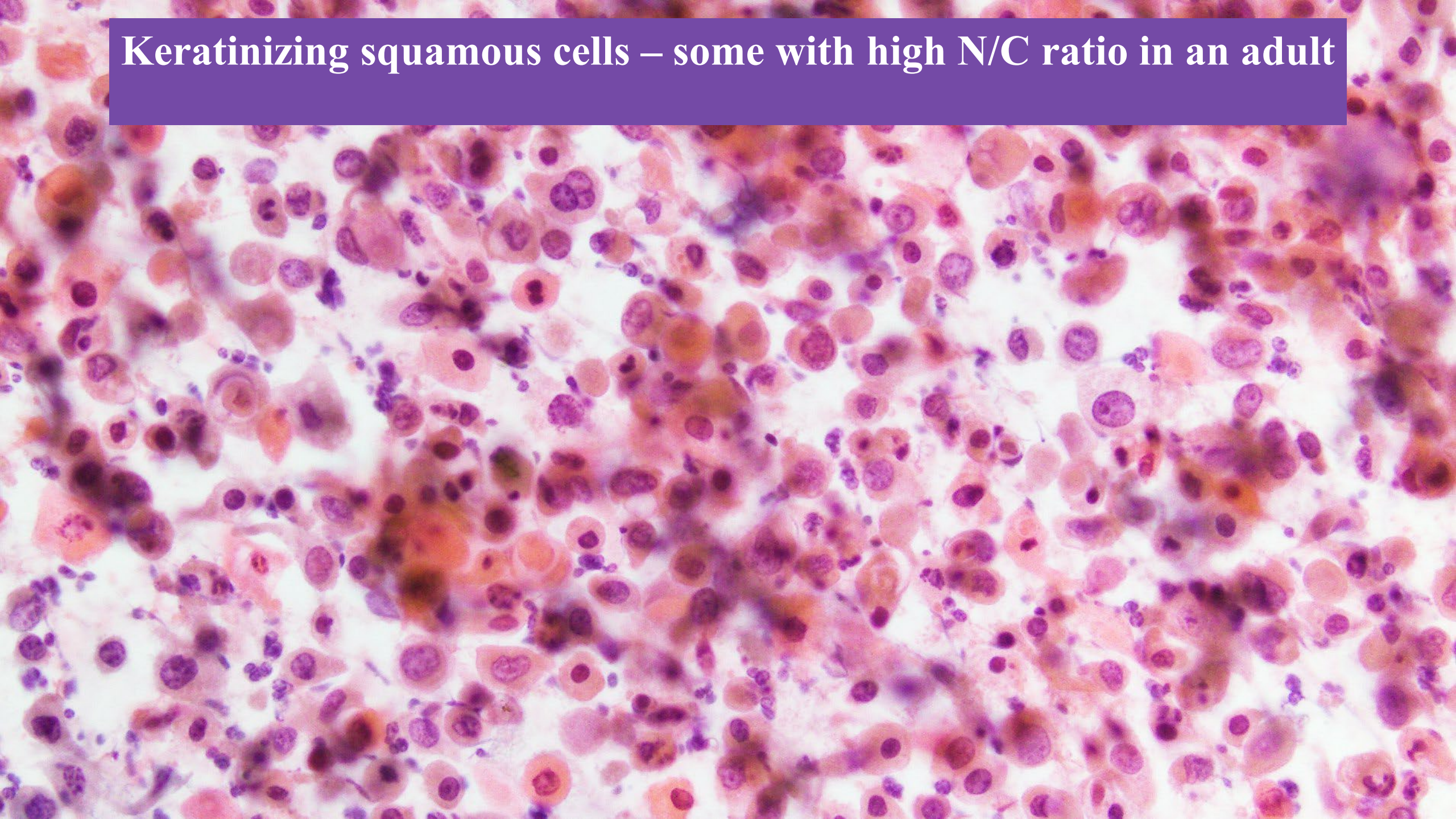
HPV-Independent Squamous Cell Carcinoma: Moderate Nuclear Pleomorphism



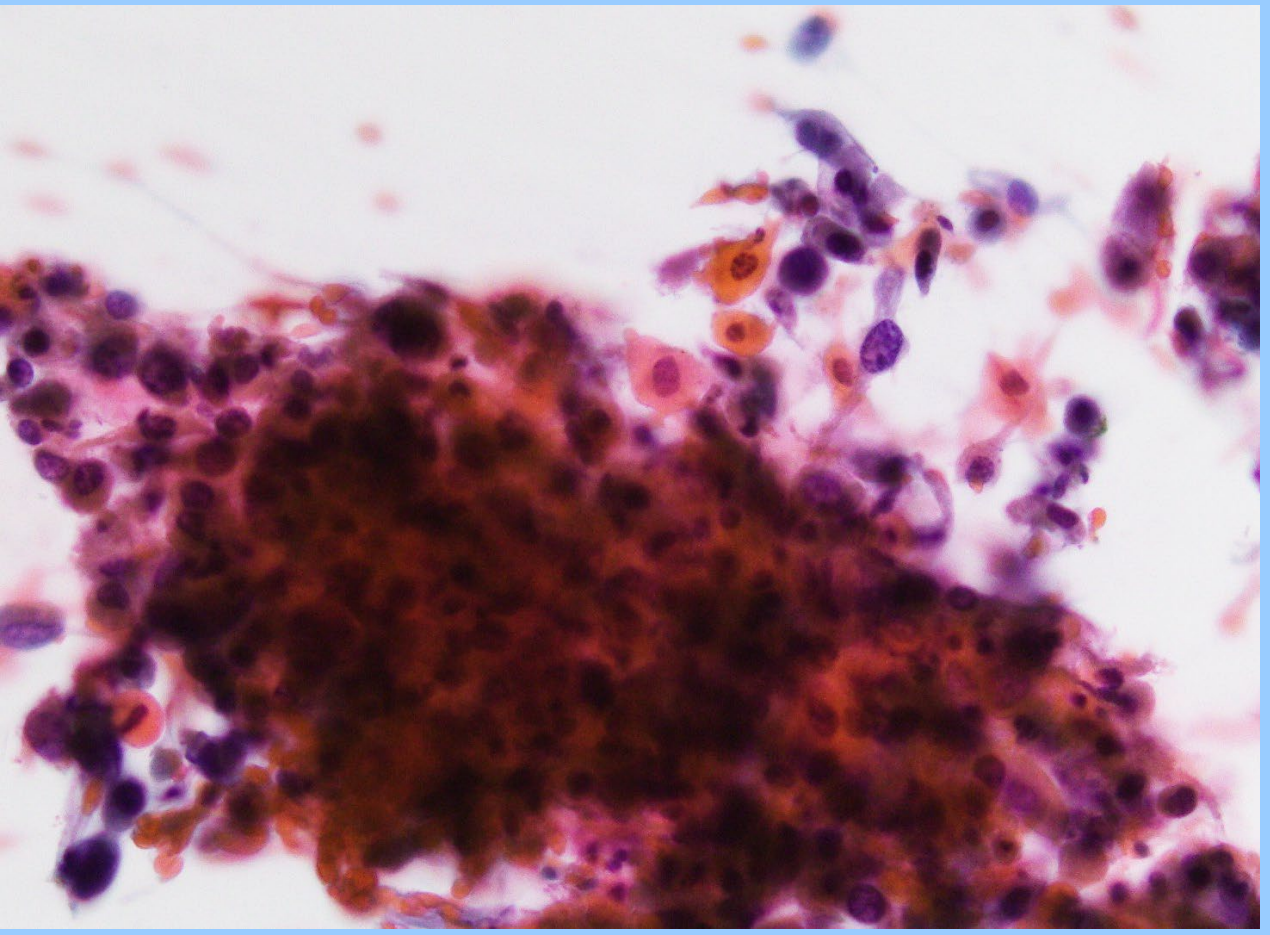
Squamous Cell Carcinoma: High N/C Ratio Cells

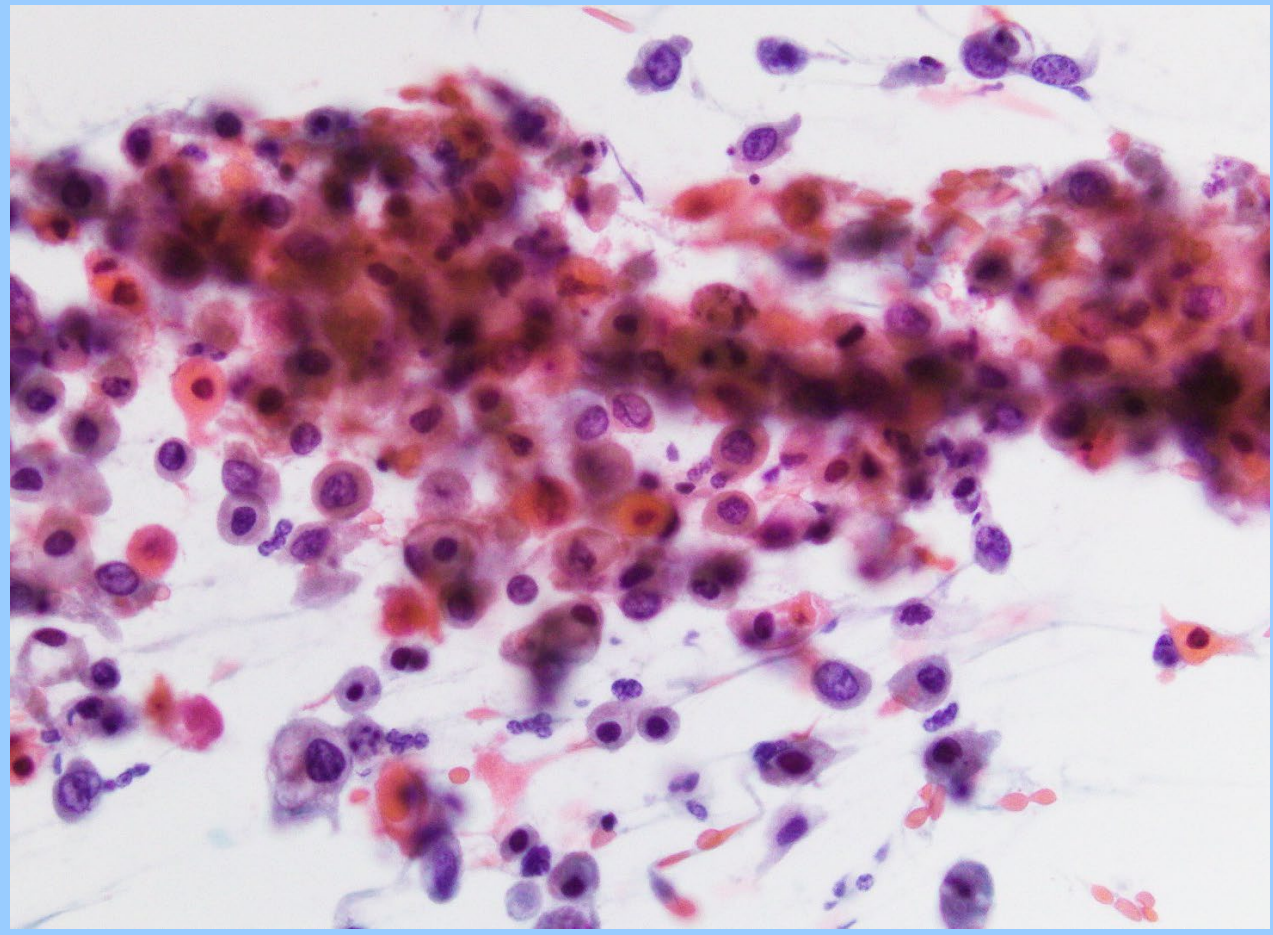


Keratinizing squamous cells – some with high N/C ratio in an adult



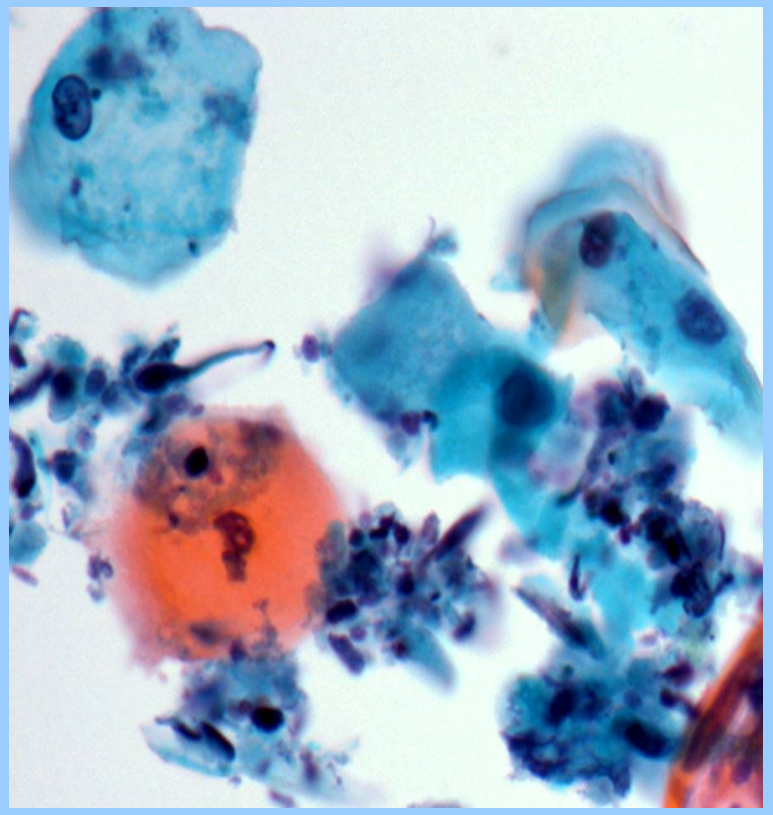
Keratinizing squamous cells with subtle atypia in an adult

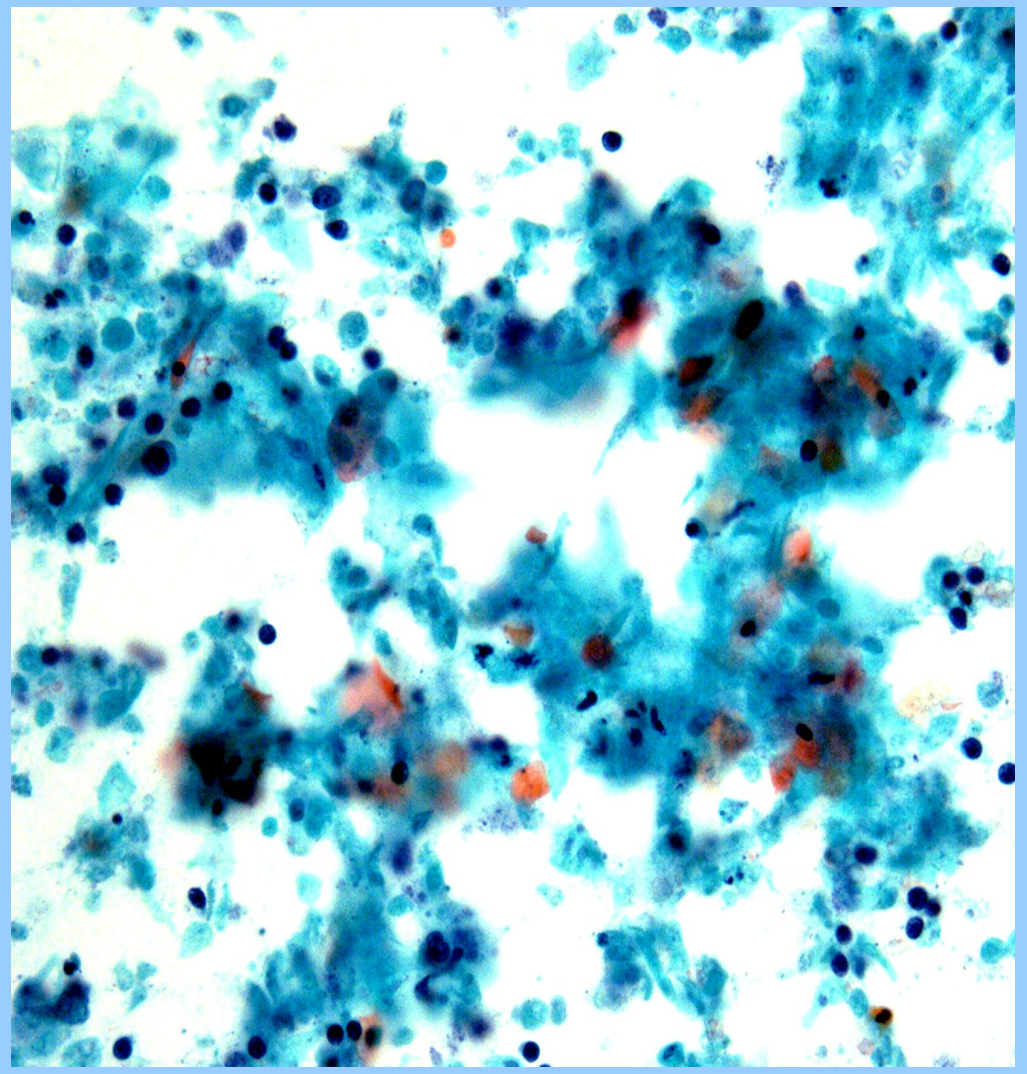




Well Differentiated Squamous Cell Carcinoma

Some cases can be very difficult – Caution!





Ancillary Markers for SCC

- **If you are not sure if it is squamous, use IHC on a cell block.**

<u>SCC is positive for:</u>

***p63, p40, keratin 5/6**

***Sometimes Ki-67, p53, GLUT-1 but of limited value!**

FNA of Cystic Squamous Lesions of the Head and Neck

KEY POINT:

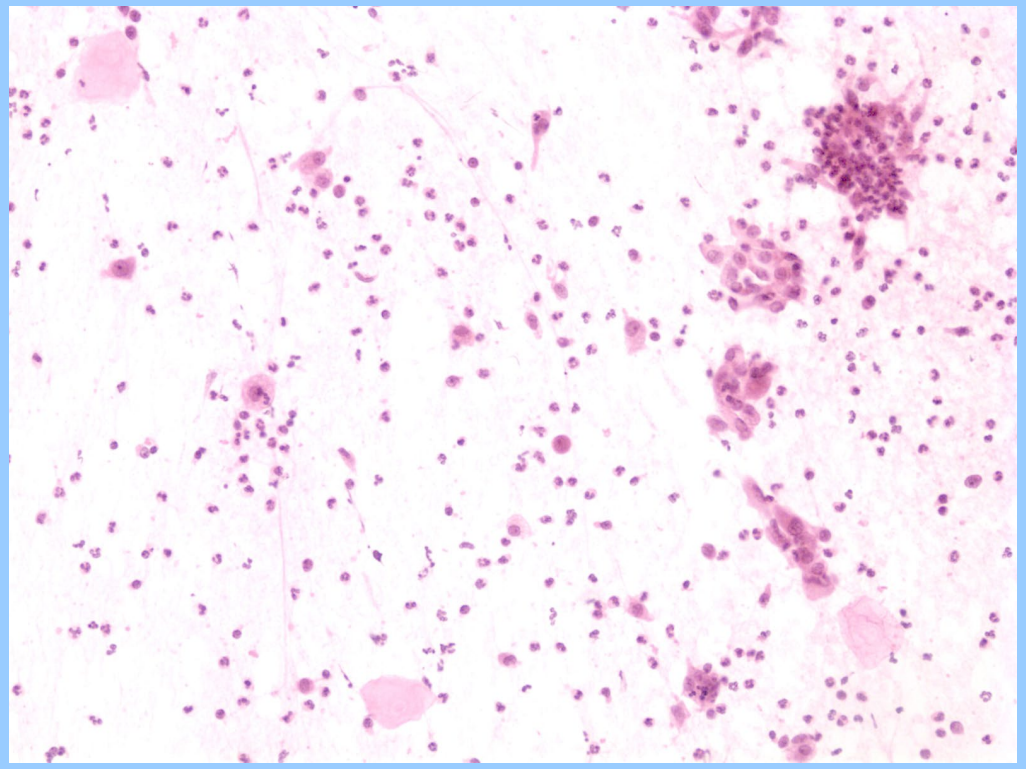
FNA of any cystic squamous lesion of the neck in a patient over 35 years old should be evaluated with caution!

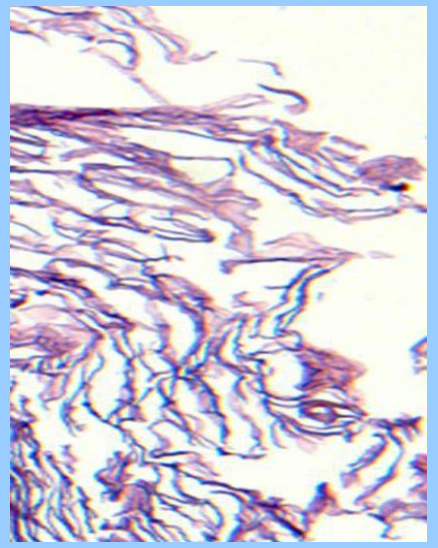
KEY POINTS

- **Metastatic SCC cytology**
  - **Adult = Atypical, Susp, or Malignant**
  - **Search carefully for marked nuclear atypia**
  - **Acute inflammation**
  - **Ancillary stains have limited value!**

FNA of 2 Head and Neck Skin Cysts - One common, one uncommon!

FNA of Epidermal Inclusion Cyst





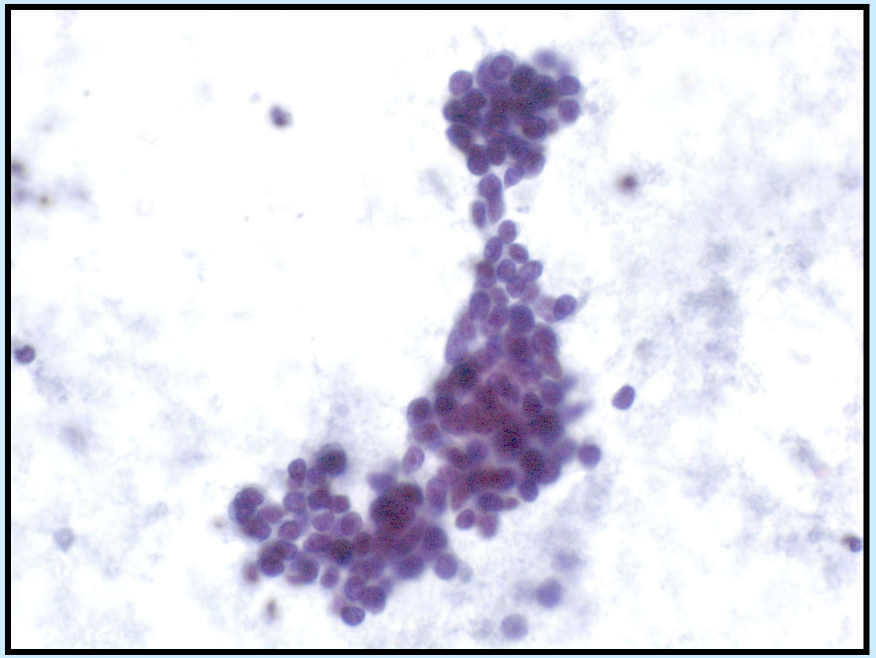
- Common cystic lesion of the head and neck
- Keratin debris and anucleate squamous cells
- Ruptured cysts can produce clusters of epithelioid histiocytes and giant cells

PITFALL: Pilomatrixoma

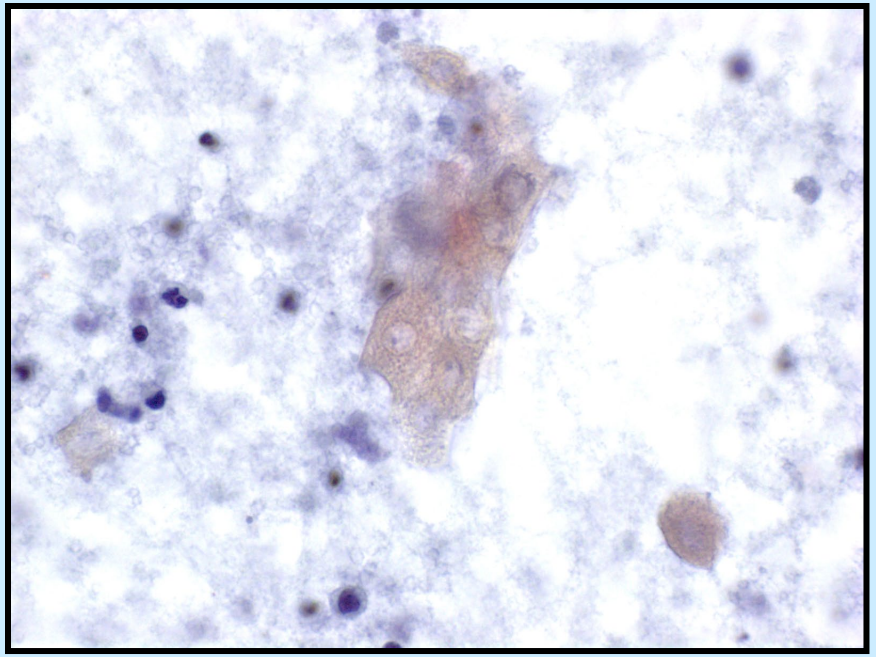
(aka: Pilomatricoma; Calcifying Epithelioma of Malherbe)

- Rare benign dermal lesion
- Most common in children and young adults
- Mimics sebaceous cyst

Two Key Cytologic Features

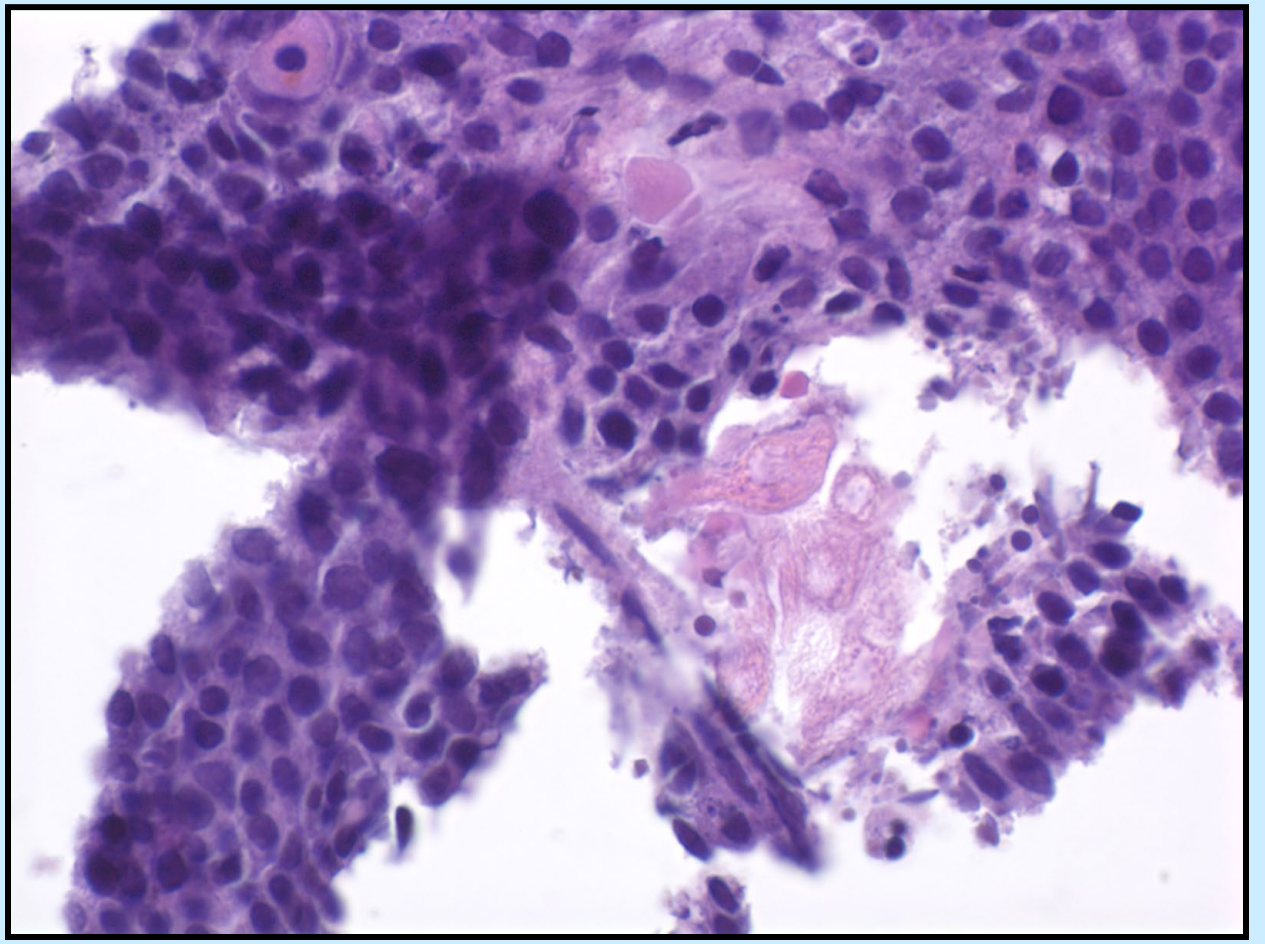


Basaloid cells



Ghost cells

Cell block:
Basaloid cells + ghost cells



****How do we evaluate lymph nodes for metastatic carcinoma in the head and neck?**

AND

****What are the most common metastases to cervical lymph nodes?**

FNA of Enlarged Cervical Lymph Nodes

- **Indications:**
- **Lymph nodes larger than 1-2 cm are generally cause for concern in adults --- FNA!**
  - **For children and young adults – benign lymphadenopathy is more common**

Metastases to Cervical Lymph Nodes

- High sensitivity and specificity for detection of LN mets
- Tumor cells are usually abundant relative to lymphoid tissue
- Majority of patients have history of cancer
  - » Clinical correlation is essential
- Approx 20-30% are unknown primaries (CUP)

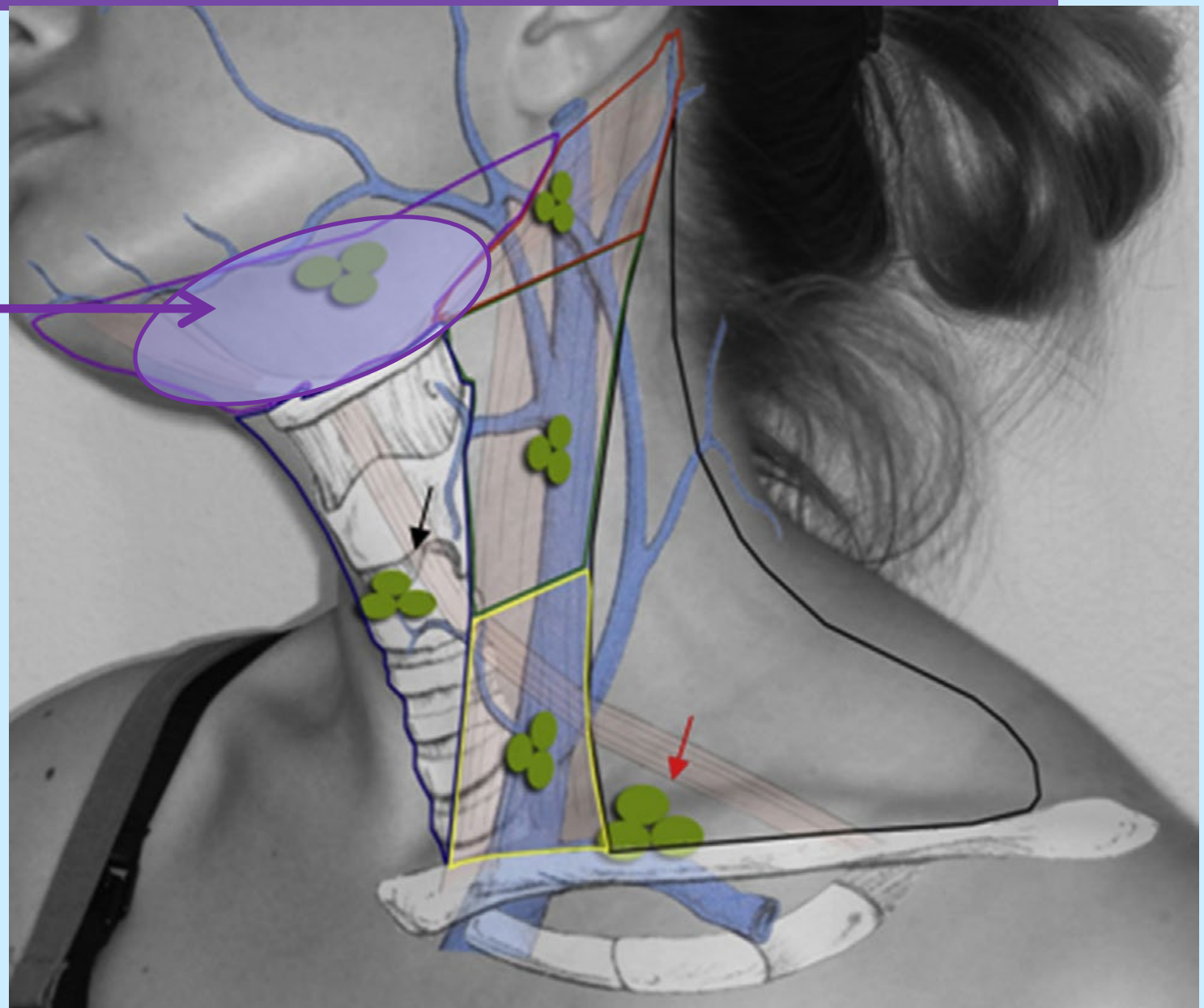
A Challenge When Assessing Cervical LN Mets is Determining the Primary Site and Tumor Classification

- **The site of metastasis can offer important clues to the site of origin!**

Cervical Lymph Nodes: Level I

Level I:
- **IA: Submental**
- **IB: Submandibular**

- **Oral cavity**
- **Submandibular gland**

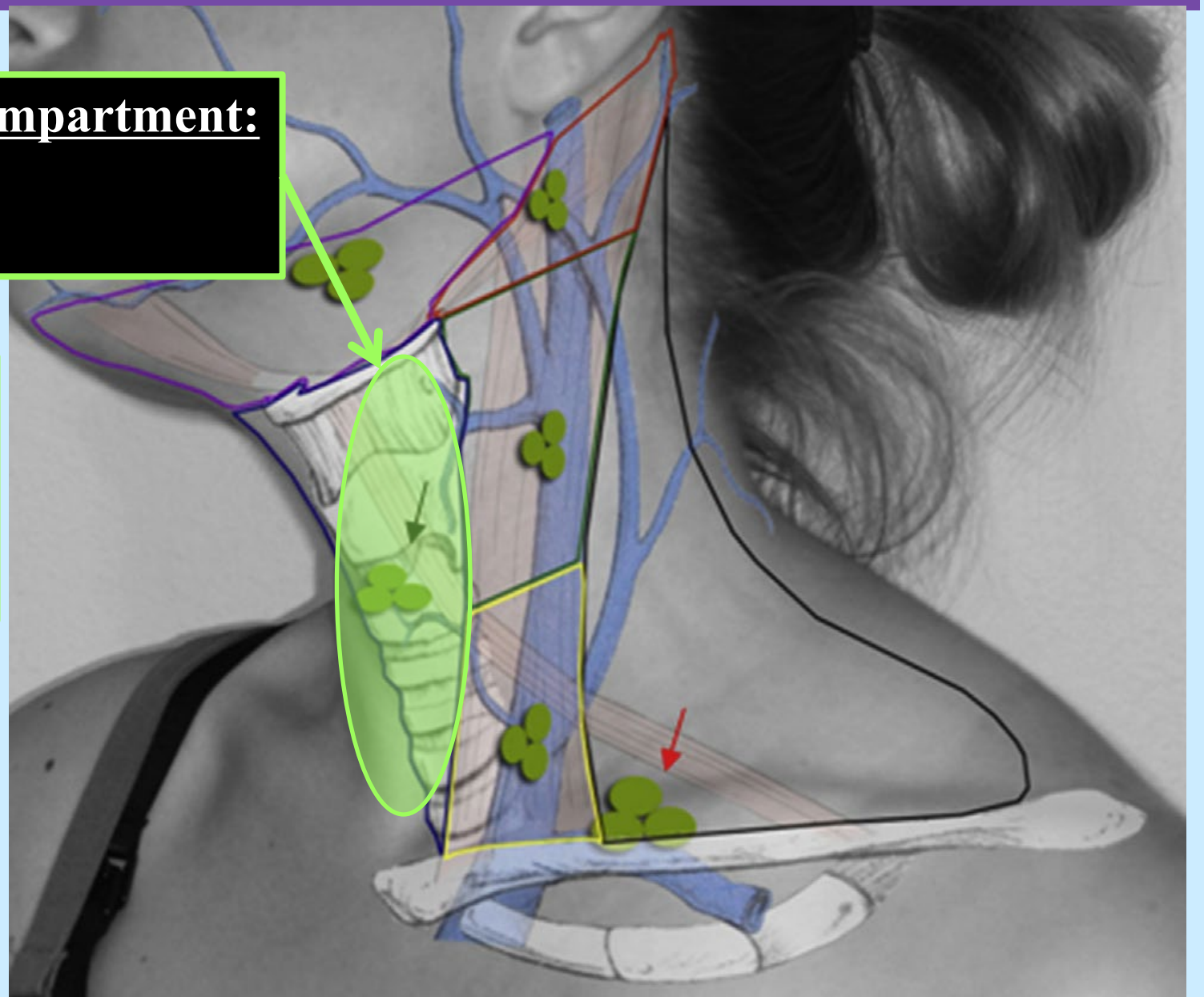


Cervical Lymph Nodes: Level VI & Delphian

Level VI Central Compartment:

- Prelaryngeal
- Paratracheal

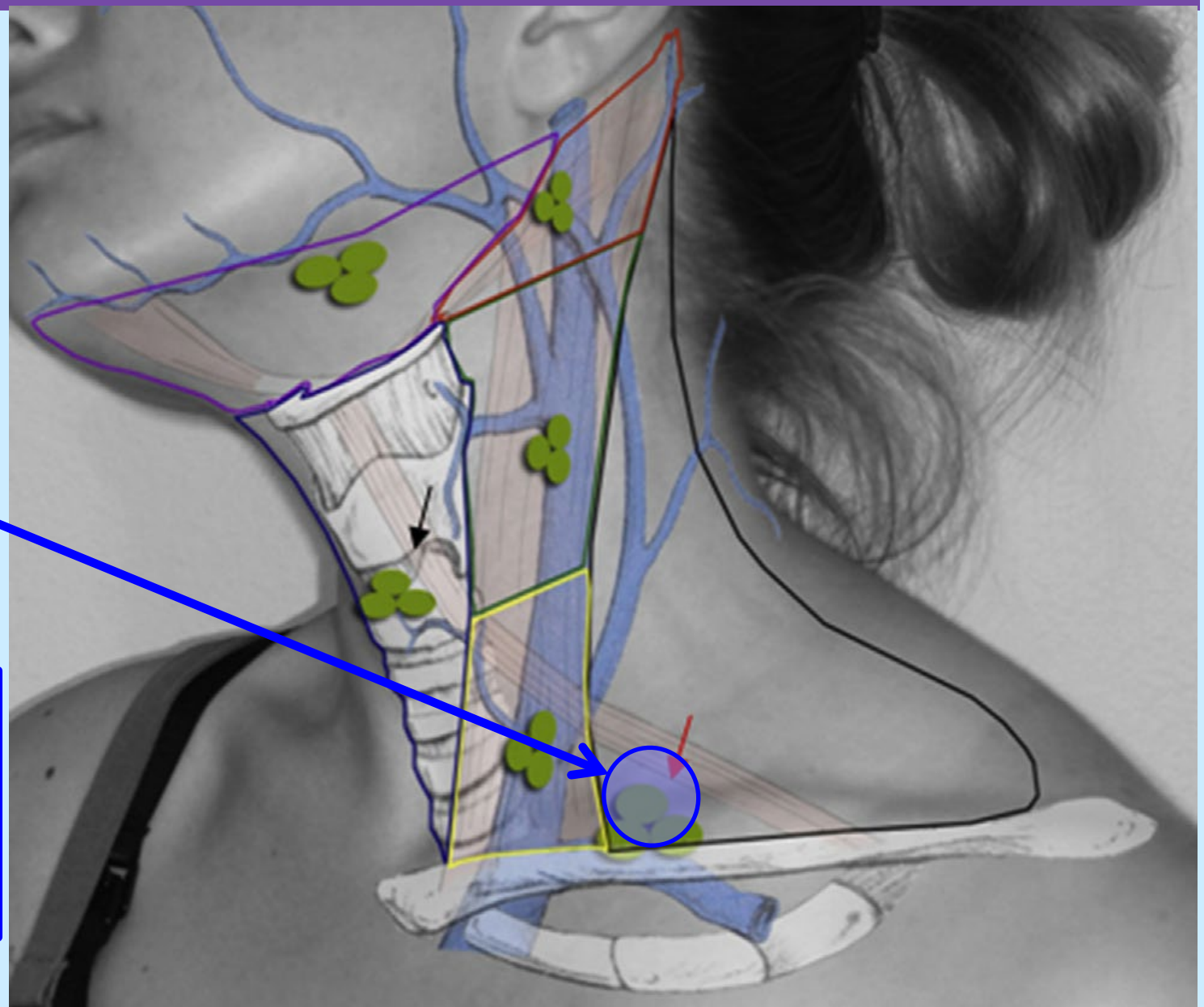
- Thyroid
- Hypopharynx
- Larynx
- Cervical esophagus



Lymph Nodes: Supraclavicular and Virchow LN

Virchow LN
- Supraclavicular
- Thoracic duct & left subclavian v

Distant mets:
- Gastric/GI cancer
- Lung cancer
- GU



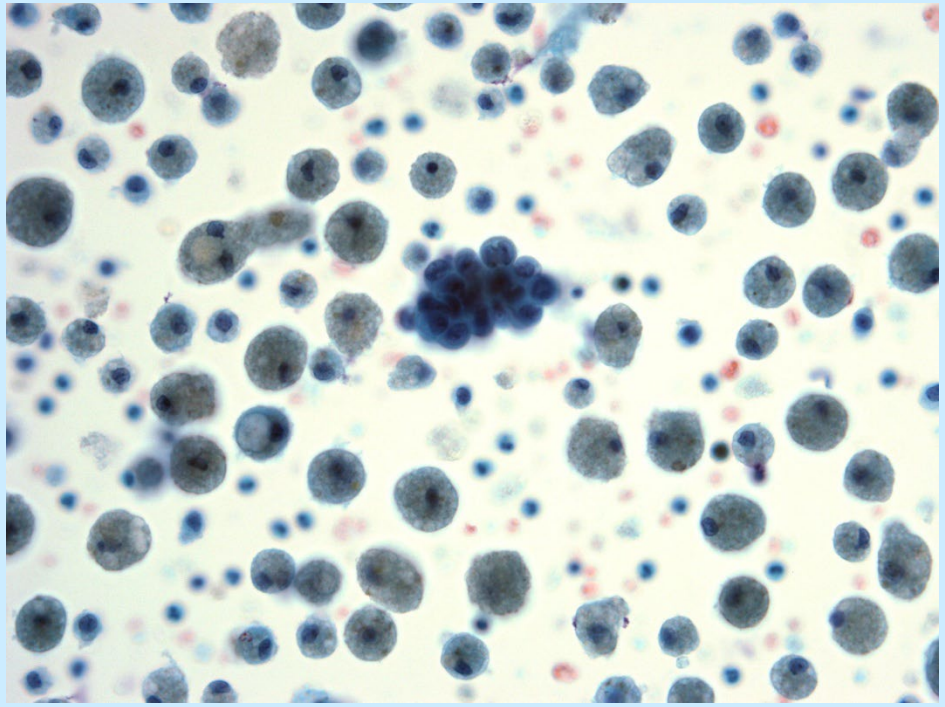
FNA of Enlarged Cervical Lymph Nodes: Metastatic Disease

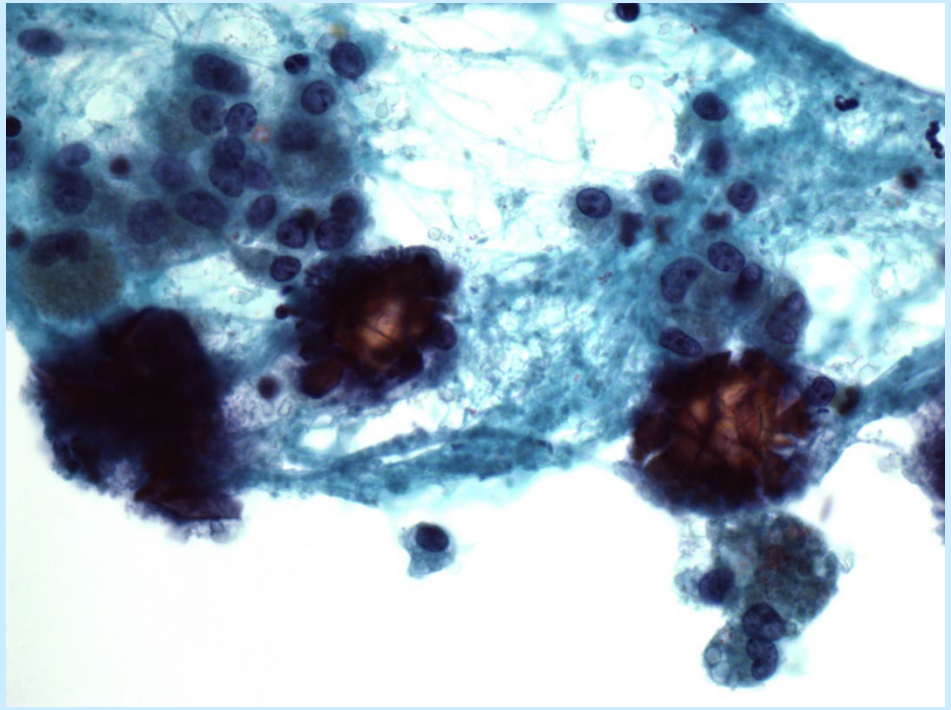
Most common metastatic tumors in HN:

- Squamous cell carcinoma (HPV+/-)
- Thyroid carcinoma (PTC, MTC, UTC)
- Malignant melanoma
- Nasopharyngeal carcinoma (EBV+)
- Neuroendocrine carcinoma (small cell, MDNC, Merkel cell)
- Distant metastases:
  - Lung
  - Breast
  - Kidney
  - Ovary
  - Pancreas
  - Germ cell tumors
  - Sarcomas

Metastatic Papillary Thyroid Carcinoma, Level VI

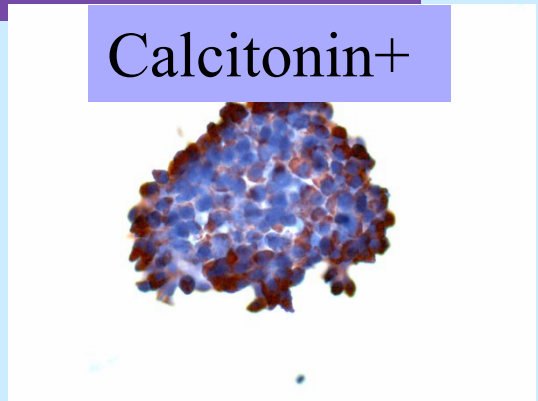
- Often Cystic – pitfall
  - Cyst fluid Thyroglobulin can be helpful
- LN involvement: Level VI, as well as levels II-V
- Cytologic features can be very subtle: histiocytic appearance
- Ker +, TTF-1+, PAX-8+, thyroglobulin+, BRAF+

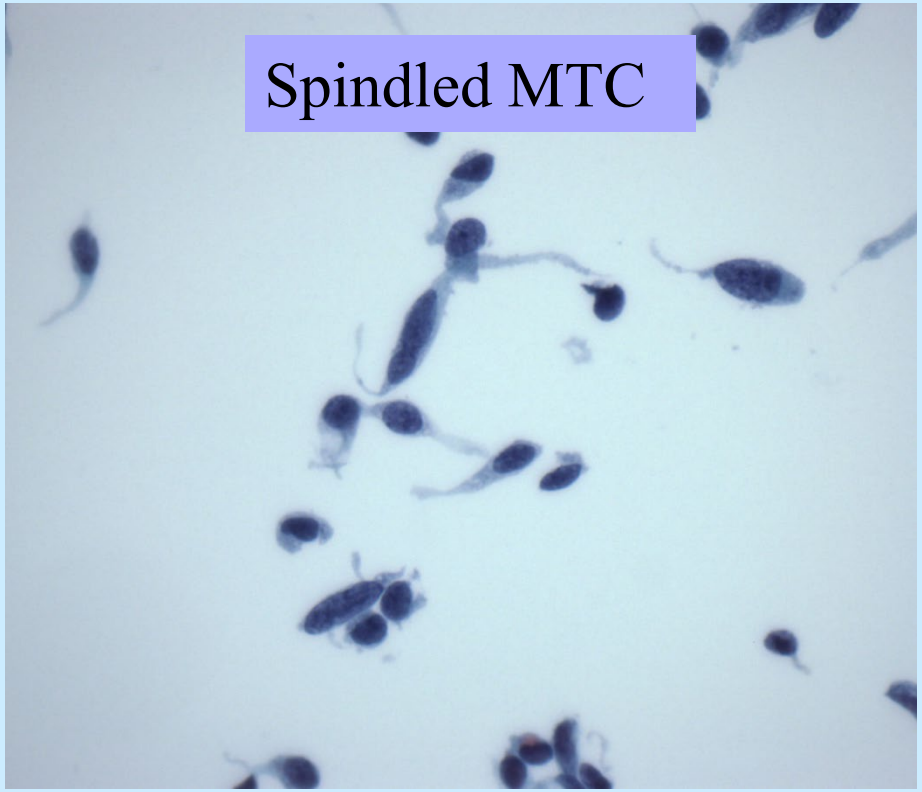


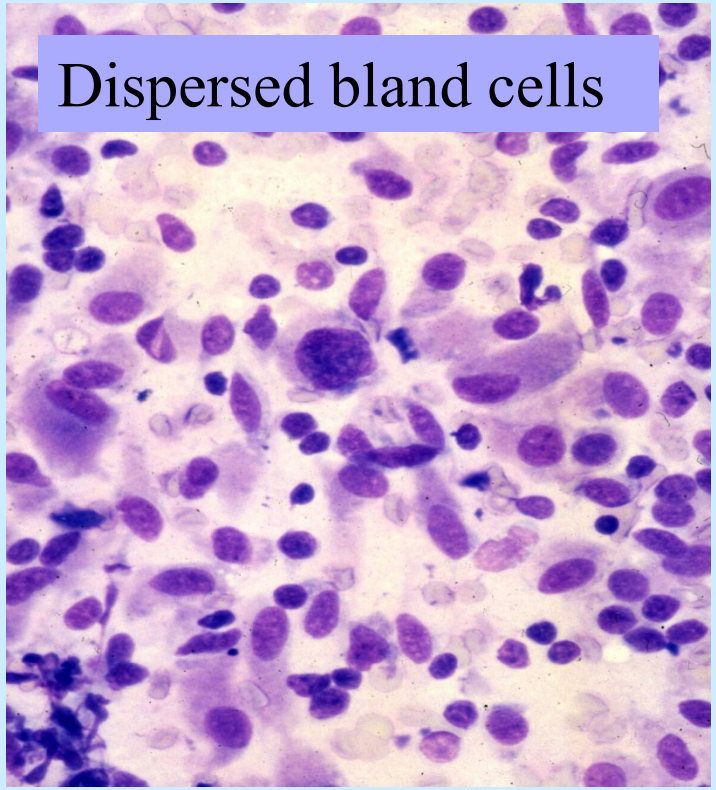


Metastatic Medullary Carcinoma

- Frequently presents as LN met
- Often bland cytology- many appearances
- Ker+, Calcitonin+, Chromo+, TTF-1+, CEA; elevated serum calcitonin
- LN metastases to Levels VI, and II-V

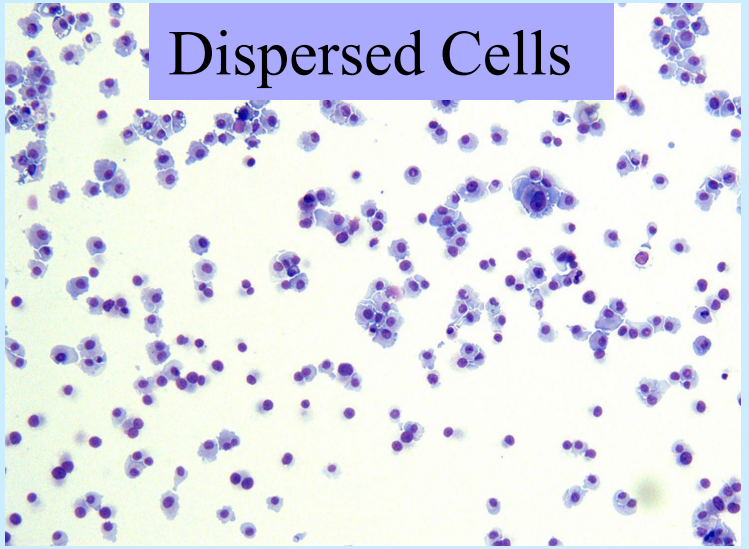


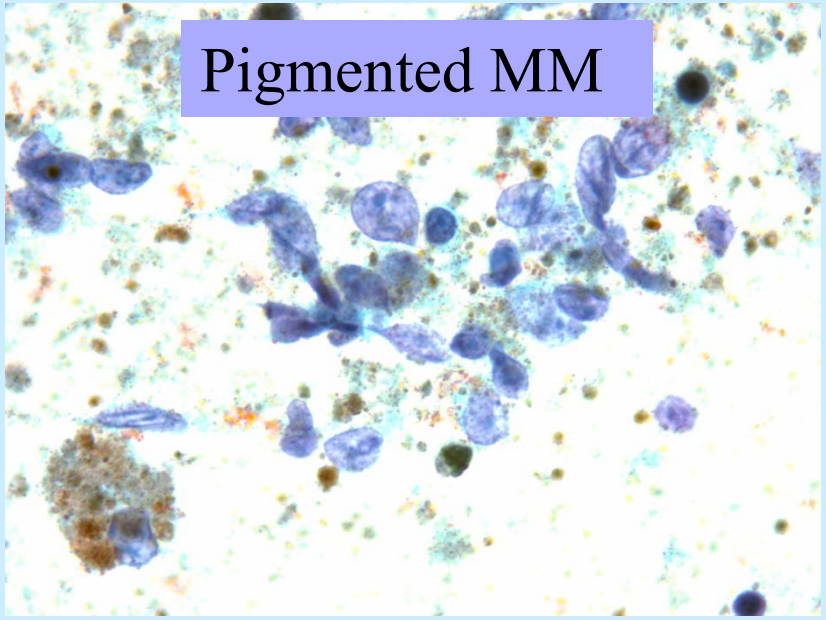


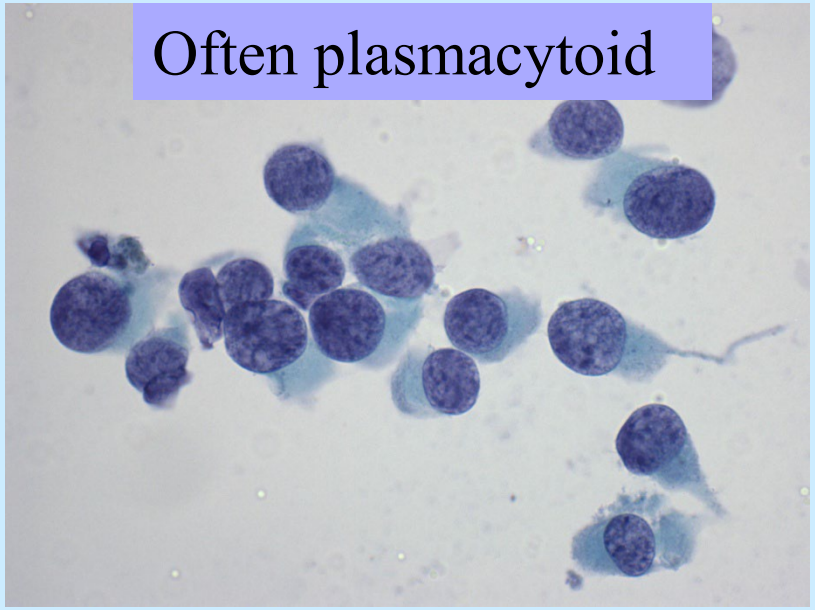


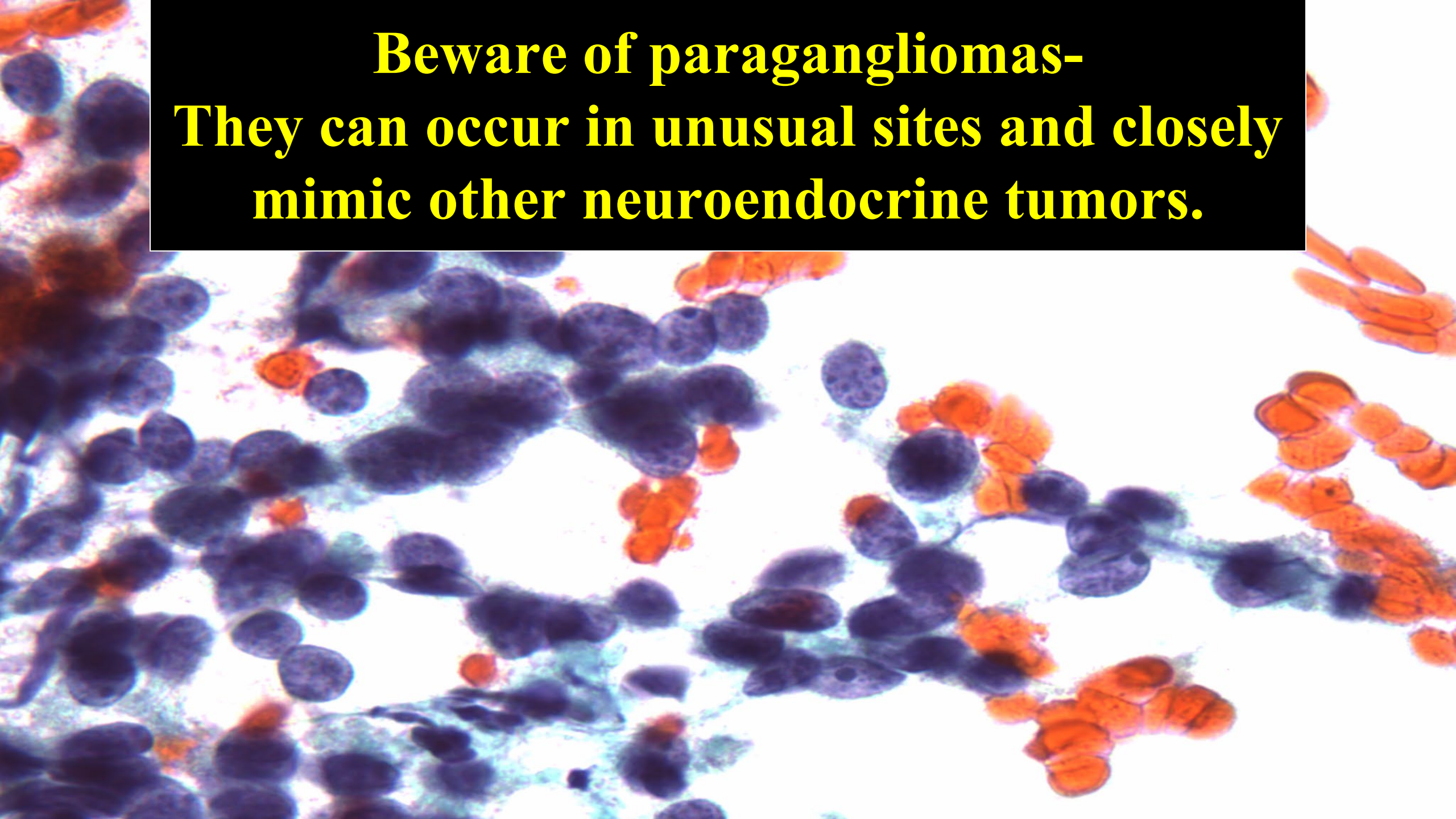
Metastatic Melanoma (levels I, II, V)

- Great Masquerader
- Less than 50% have melanin
- Binucleation and nuclear inclusions
- Can mimic DLBCL
- S-100+, MART-1+, HMB-45+, Mitf+, Melan-A+, Ker-, CD45-



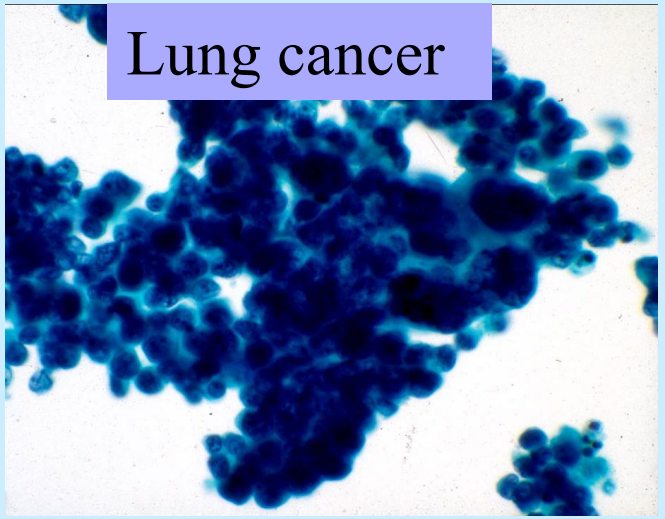


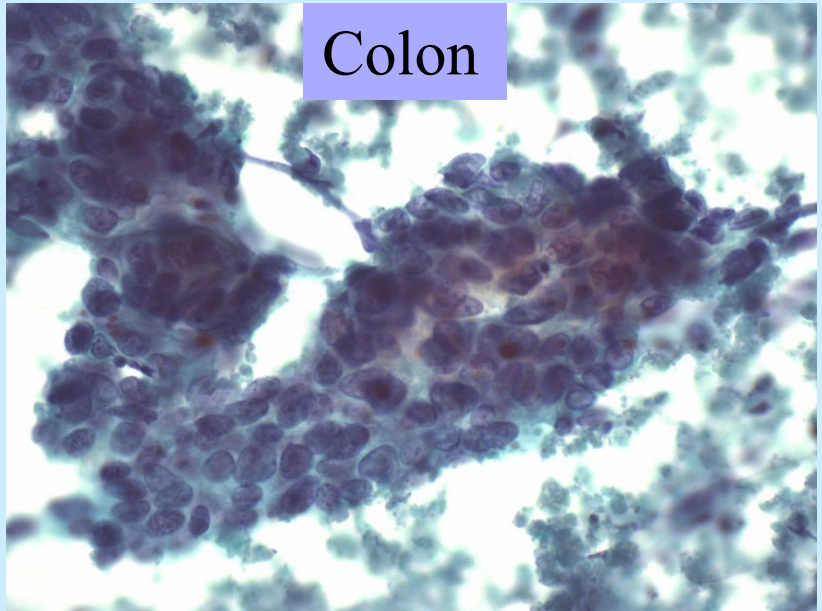


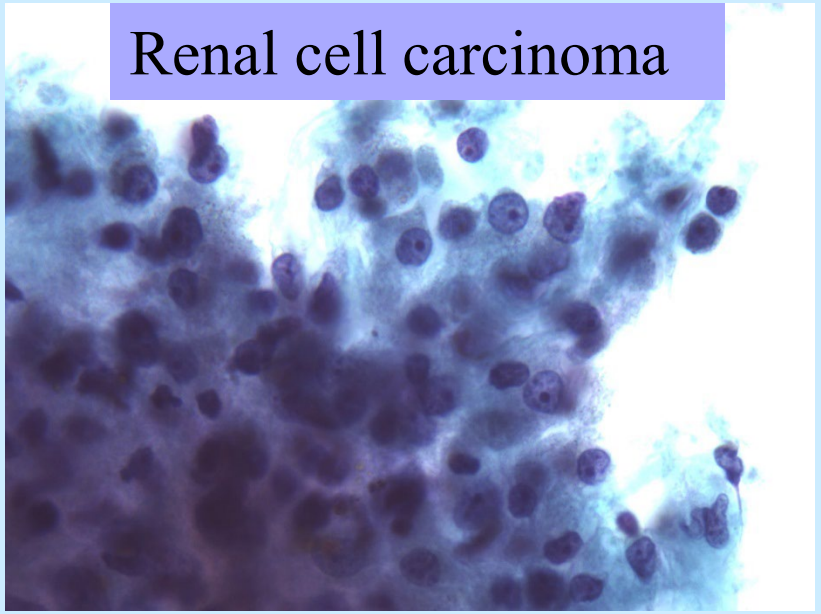

Beware of paragangliomas-
They can occur in unusual sites and closely mimic other neuroendocrine tumors.

Distant Metastases

- **Supraclavicular LNs**
- **Lung** — **TTF-1, Napsin-A**
- **Breast** — **GATA-3, Mammaglobin, ER/PR, GCDPF**
- **GI** — **Ker 20,**
- **Renal** — **PAX-8, RCC, CD10,**
- **GYN** — **PAX-8, ER/PR**
- **Prostate** — **PSA, PLAP, Racemase, NKX3.1**





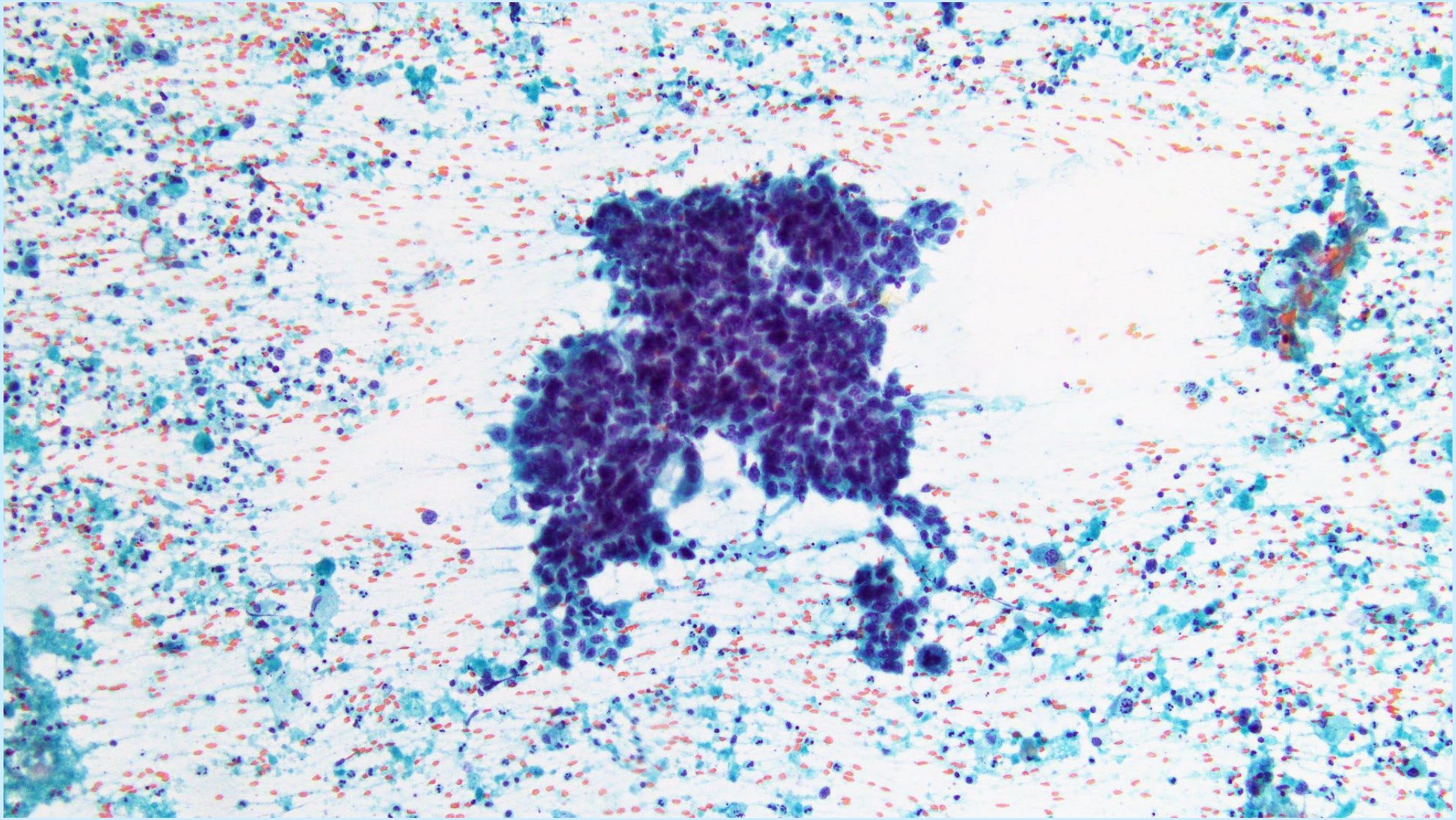


What about HPV and Head and Neck Cancer???

CASE:

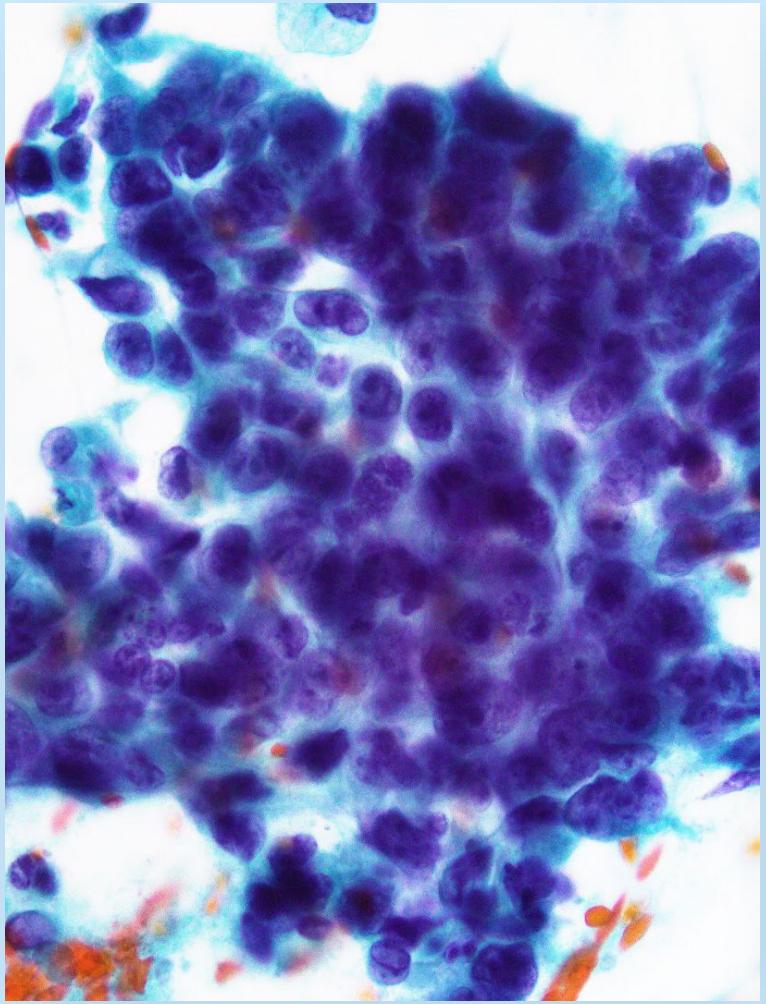
61 year-old male presenting with a left neck mass. A CT showed a level 2, partially cystic 2.5 cm left neck mass. An FNA was performed.

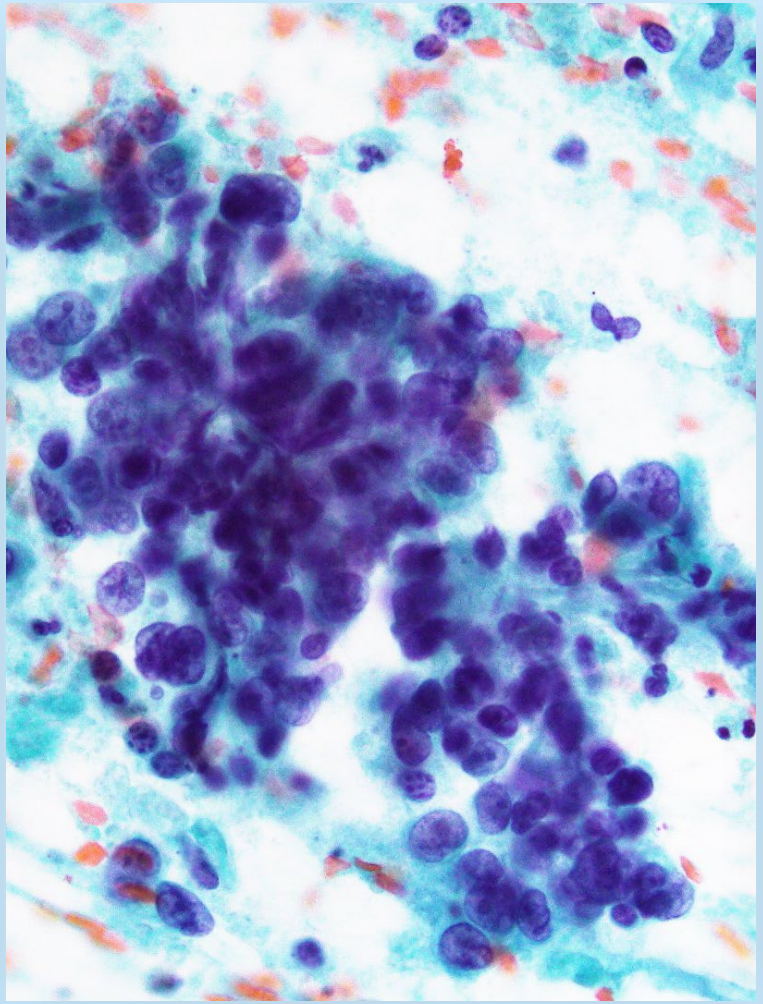
Basaloid cells in necrotic background

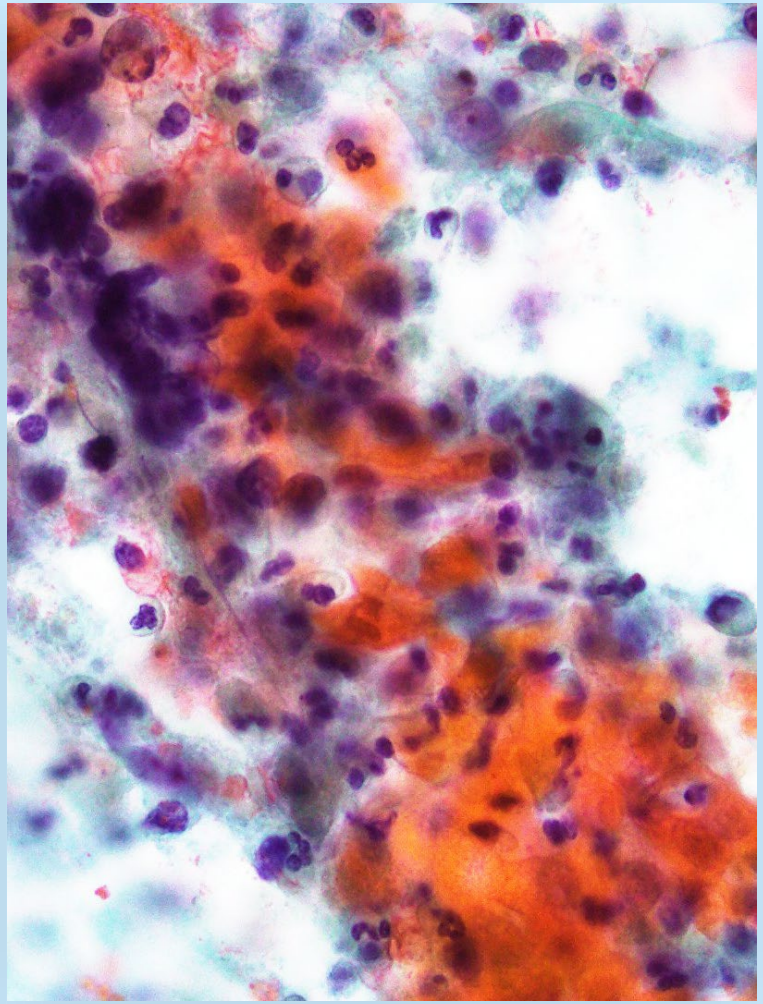


FNA: Squamous cell carcinoma, focally keratinizing

Carcinoma of unknown primary (CUP)







HR-HPV analysis performed on FNA liquid-based SP specimen using BD-Onclarity PCR-based assay is POSITIVE for HR-HPV 16

CYTOLOGY DIAGNOSIS:

Satisfactory for Evaluation.

Malignant

Metastatic HPV-associated squamous cell carcinoma.

Note: The findings suggest an oropharyngeal primary carcinoma.

Summary of HPV-Associated Head and Neck Cancer

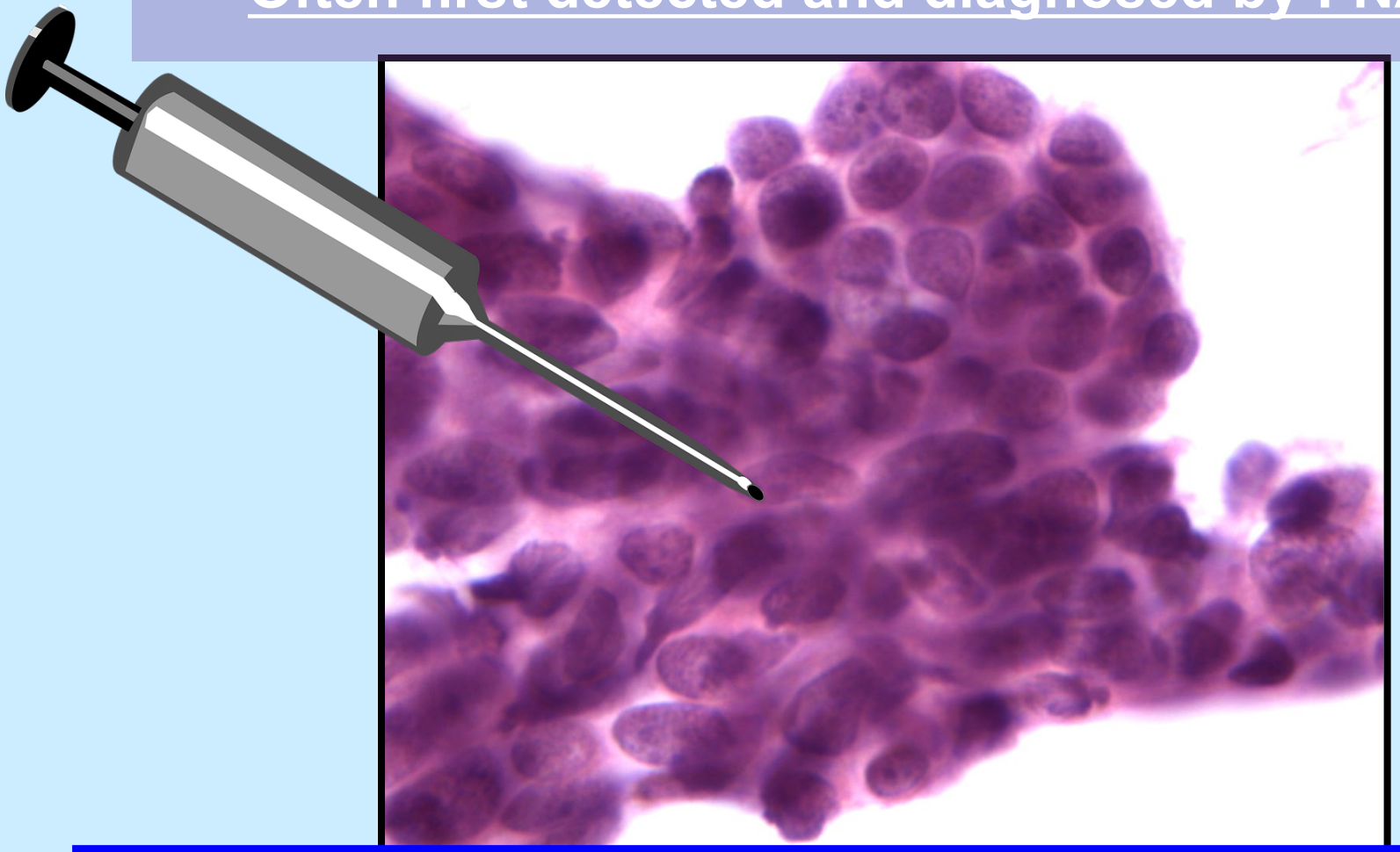
Nodal Metastases in HPV-positive OP SCC

Ang et al. *NEJM* 2010; 363: 24.
Jordan et al. *Am J Surg Pathol* 2012; 36: 945.
Lewis Jr. et al. Am J Surg Pathol 2010: 1044:38.

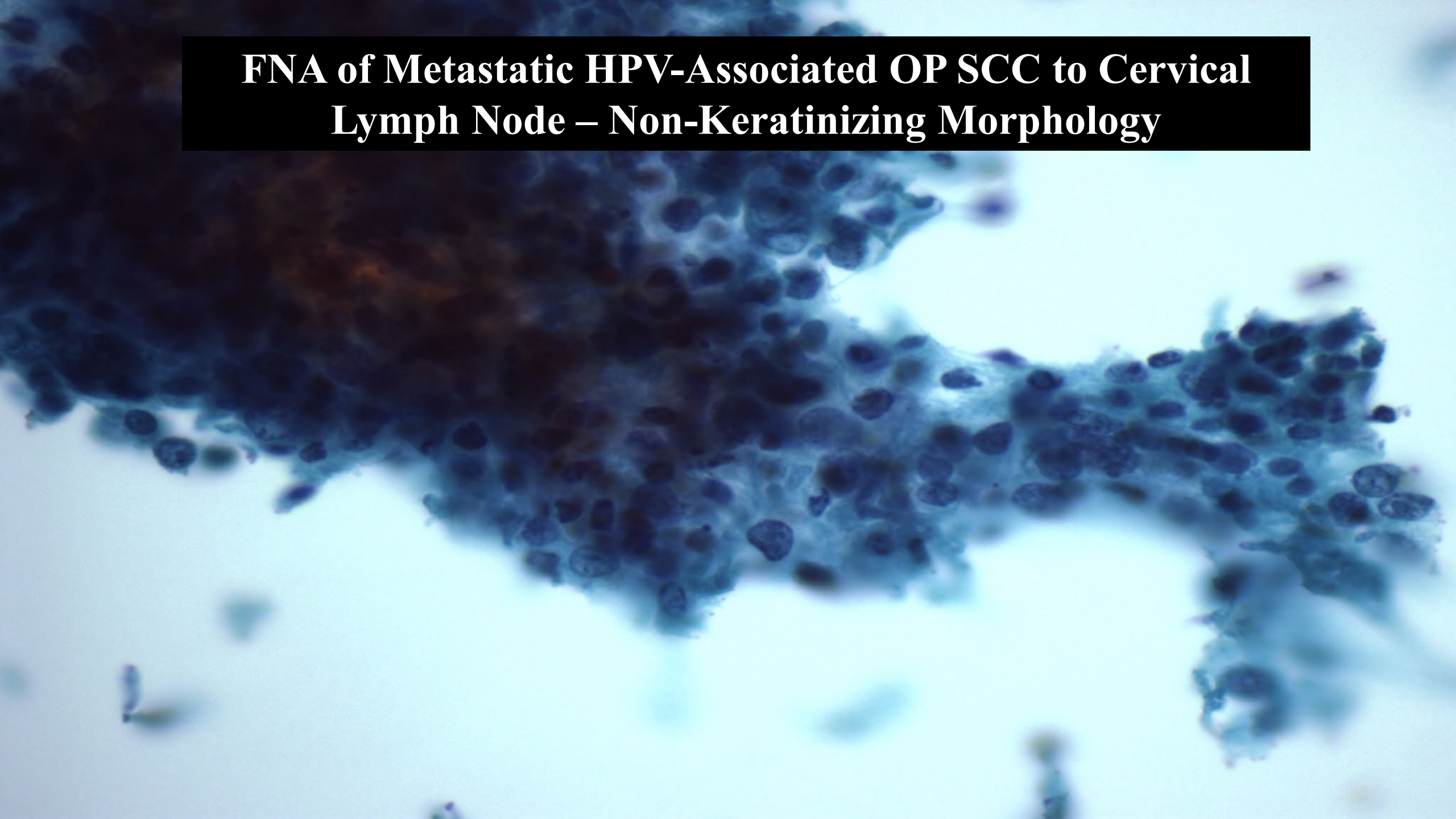
Nodal metastases to Level II or III are present at presentation in approx 80-85% of all HPV-associated OPSCC.

HPV-Positive Oropharyngeal SCC:

Often first detected and diagnosed by FNA !



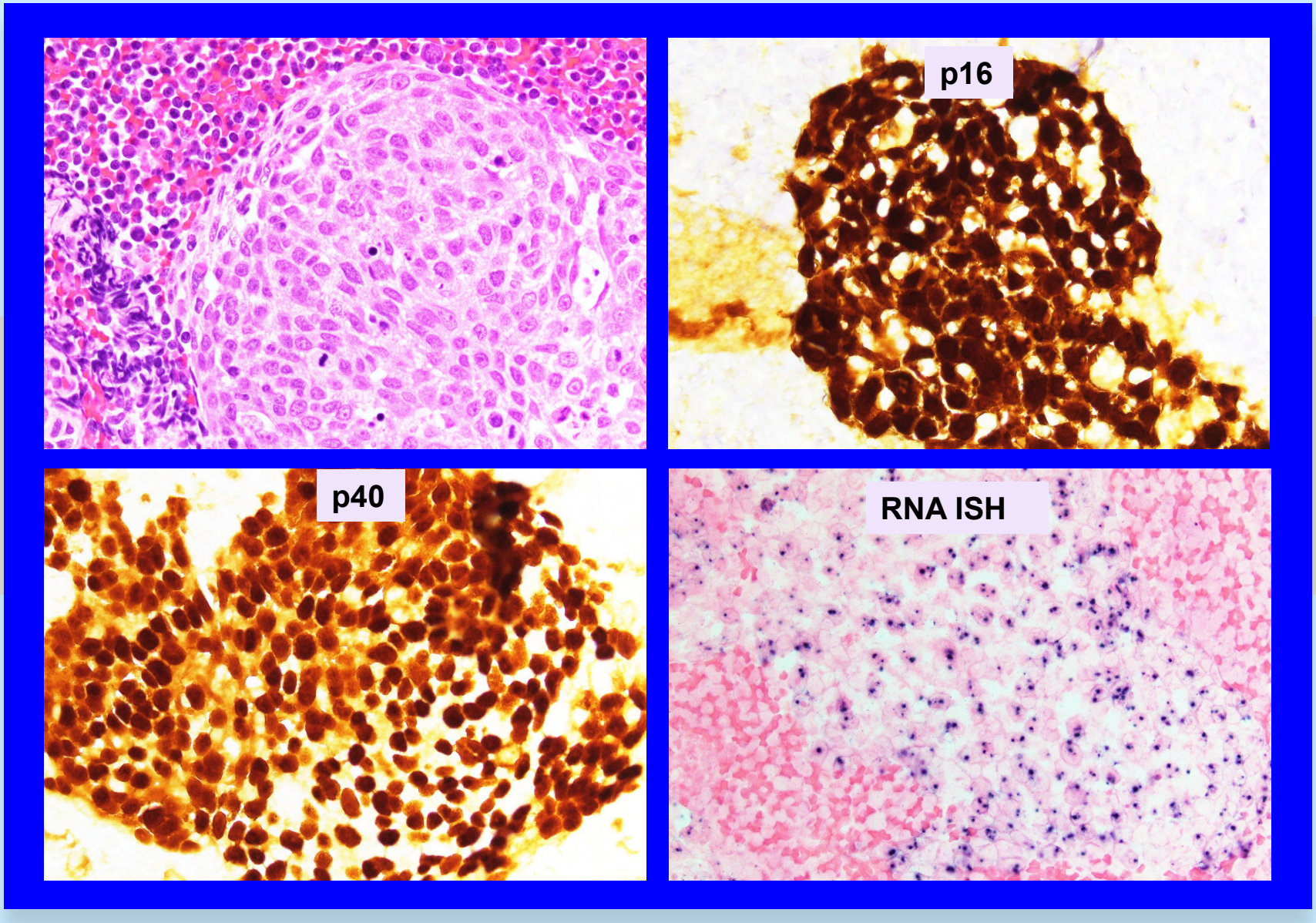
FNA is a key method used in the initial detection of these metastatic cancers.


FNA of Metastatic HPV-Associated OP SCC to Cervical Lymph Node – Non-Keratinizing Morphology

How should HR-HPV testing be done in FNA specimens?

Testing for HR-HPV in FNAs of HNSCC

CELL BLOCK



HR-HPV testing in FNAs of HNSCC CUP: Which test to use?

- P16 is no longer recommended as a stand-alone test or a screening test for FNA specimens
- HPV-specific testing is preferred!!!
  - RNA ISH for HR-HPV works well
  - Liquid-based HR-HPV testing works well

HR-HPV in FNAs of HNSCC

- **HPV-Specific Liquid-phase testing:**
  - **Advantages over cell block (FFPE)**
  - **Objective result with clear-cut scoring**
  - **Can be automated**

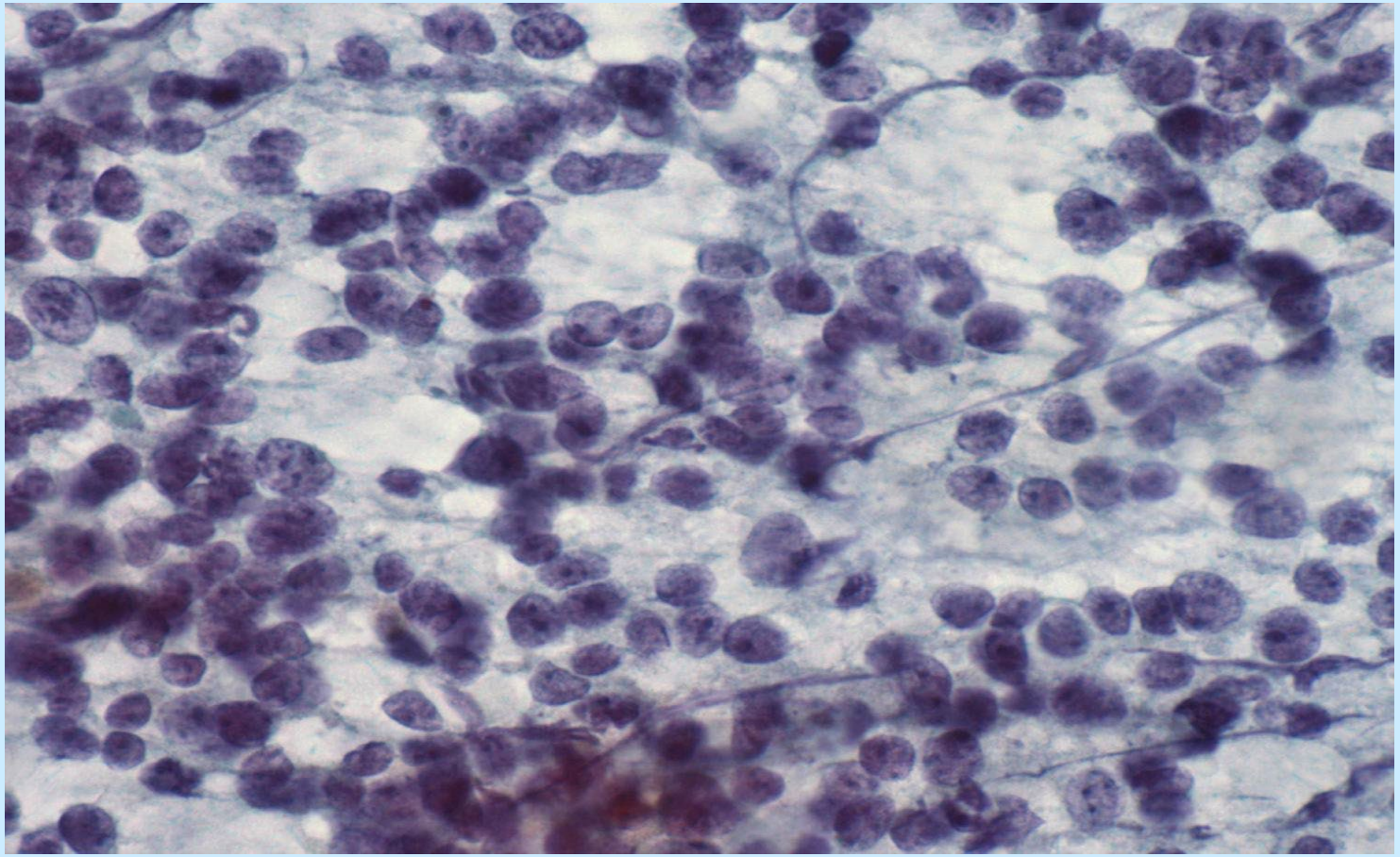
  <u>Several have already been validated:</u>
    - » **Hybrid Capture II**
    - » **CervistaTM HPV HR**
    - » **CervistaTM HPV 16/18**
    - » **Roche cobas® HPV test**
    - » **APTIMA® HPV Assay**
    - » **BD Onclarity**

KEY POINTS

- **Metastatic SCC of unknown primary (CUP)**
  - **Test for HR-HPV**
    - **HPV-specific testing is preferred over p16**
  - **If HPV negative, Test for EBV**

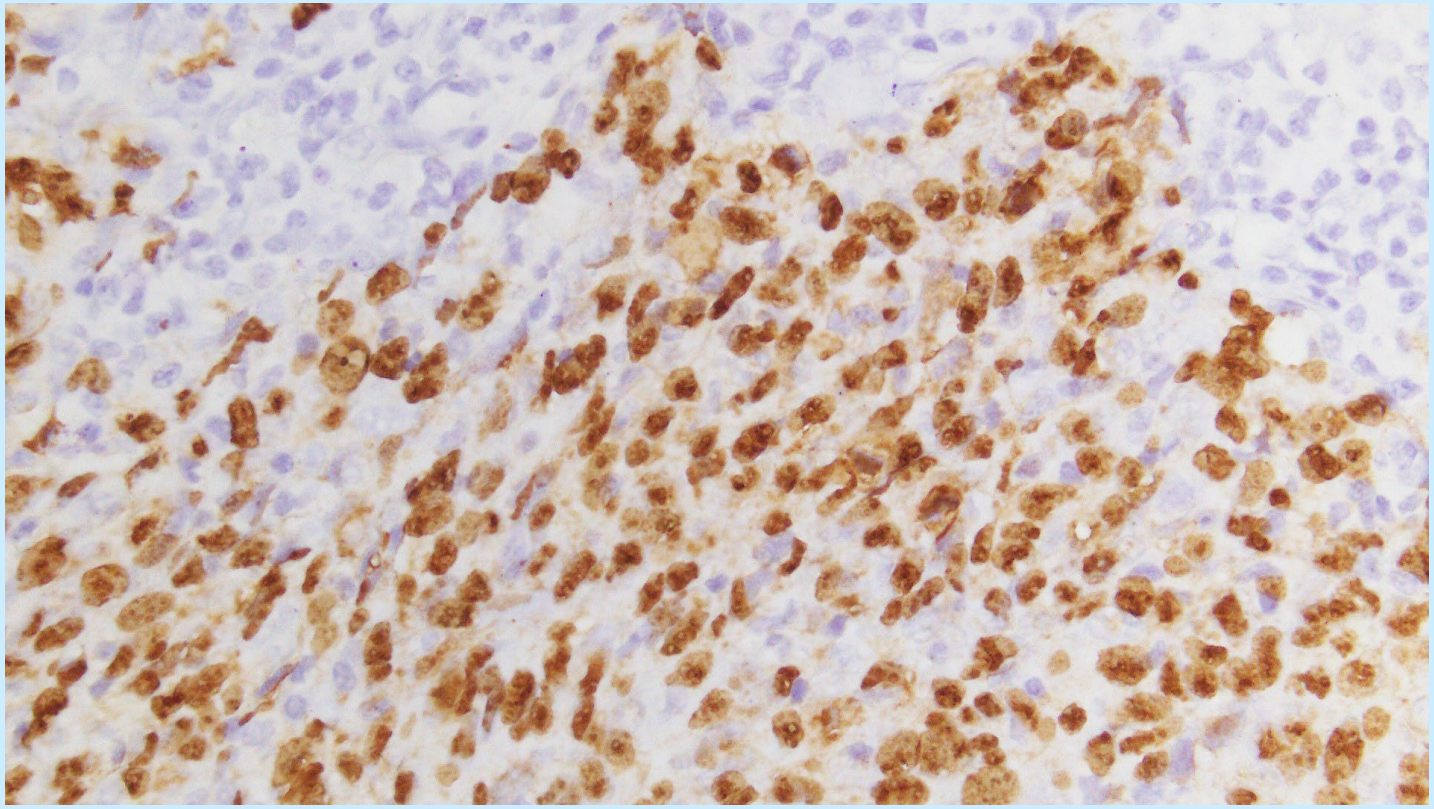
Metastatic Nasopharyngeal Carcinoma

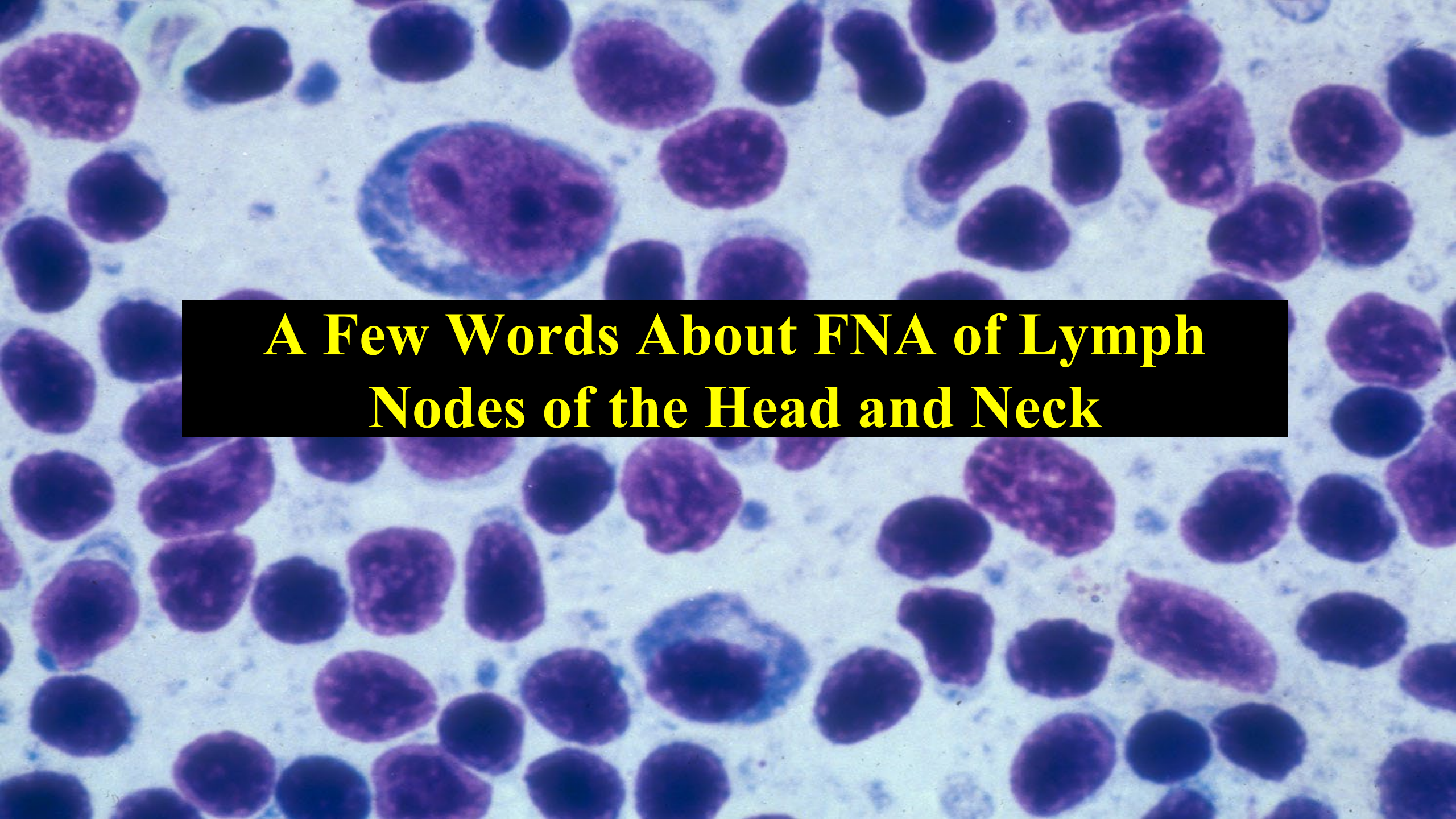
- Usually non-keratinizing
- Undifferentiated-appearing
- Ker 5/6, p63, p40+, EBER+



NOTE:
When HPV testing is NEGATIVE, consider testing for EBV to diagnose nasopharyngeal carcinoma.

ISH for EBER in Metastatic NPC

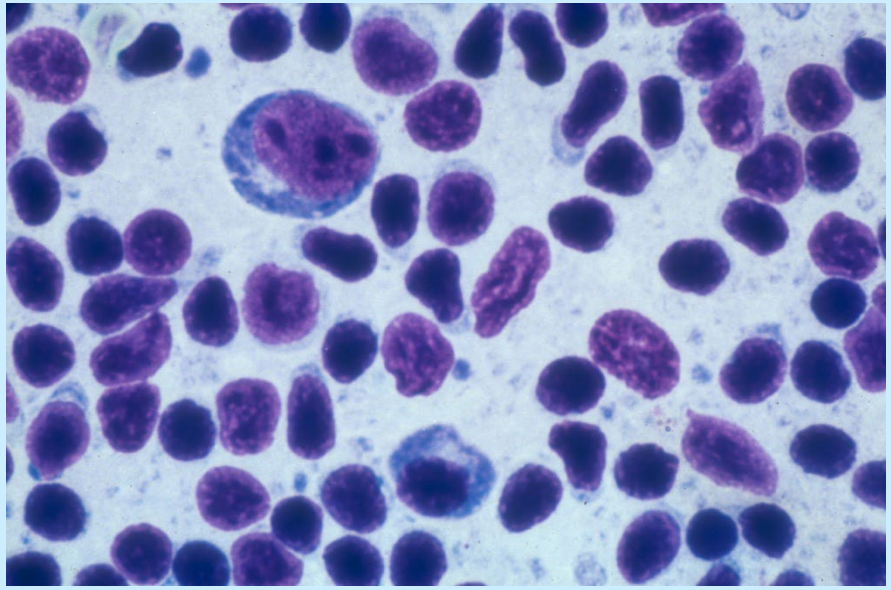


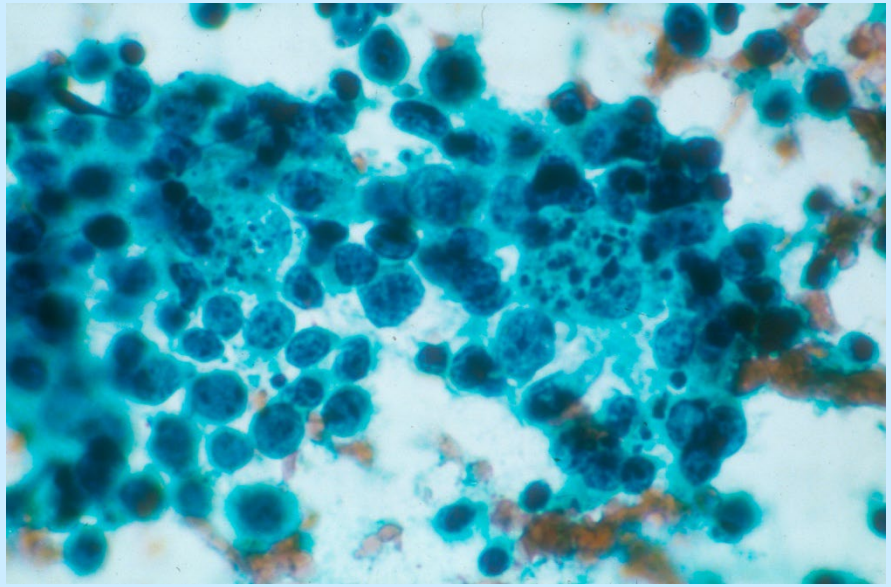


A Few Words About FNA of Lymph Nodes of the Head and Neck

Reactive Lymphoid Hyperplasia:

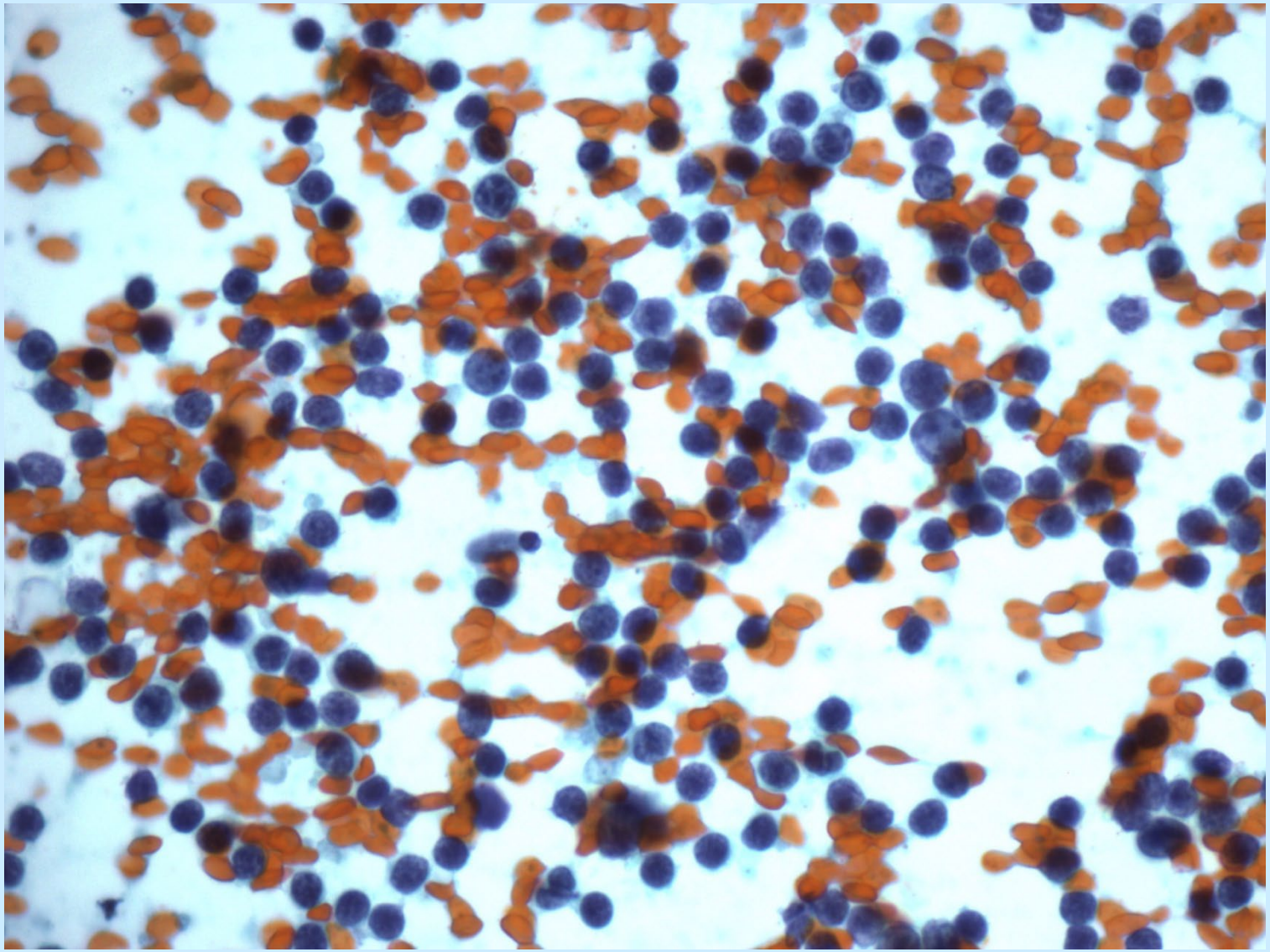
- Children & young adults
- Single enlarged LN
- Cytology:
  - » Wide variety of cell types
  - » Cellular
  - » Predominance of small lymphocytes
- Immunophenotype:
  - » Polyclonal





Reactive LN: Mixed Lymphoid Population

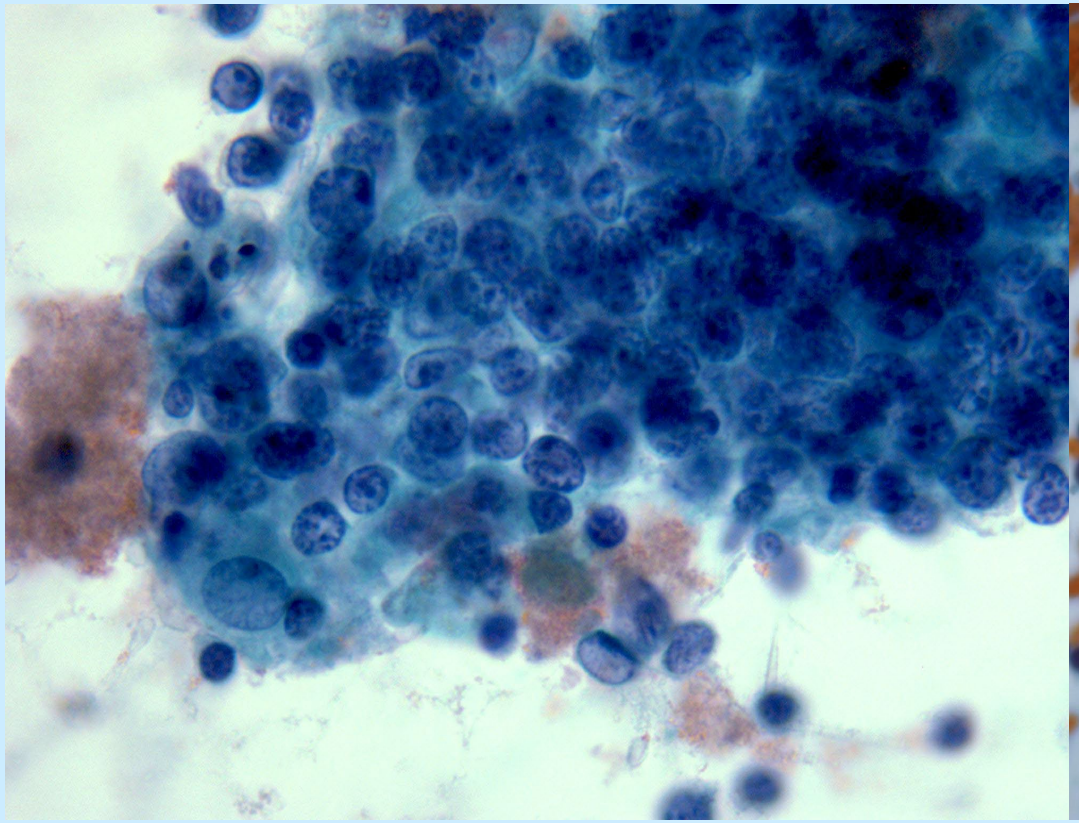
Predominance of small lymphocytes (Mixed B & T cells)

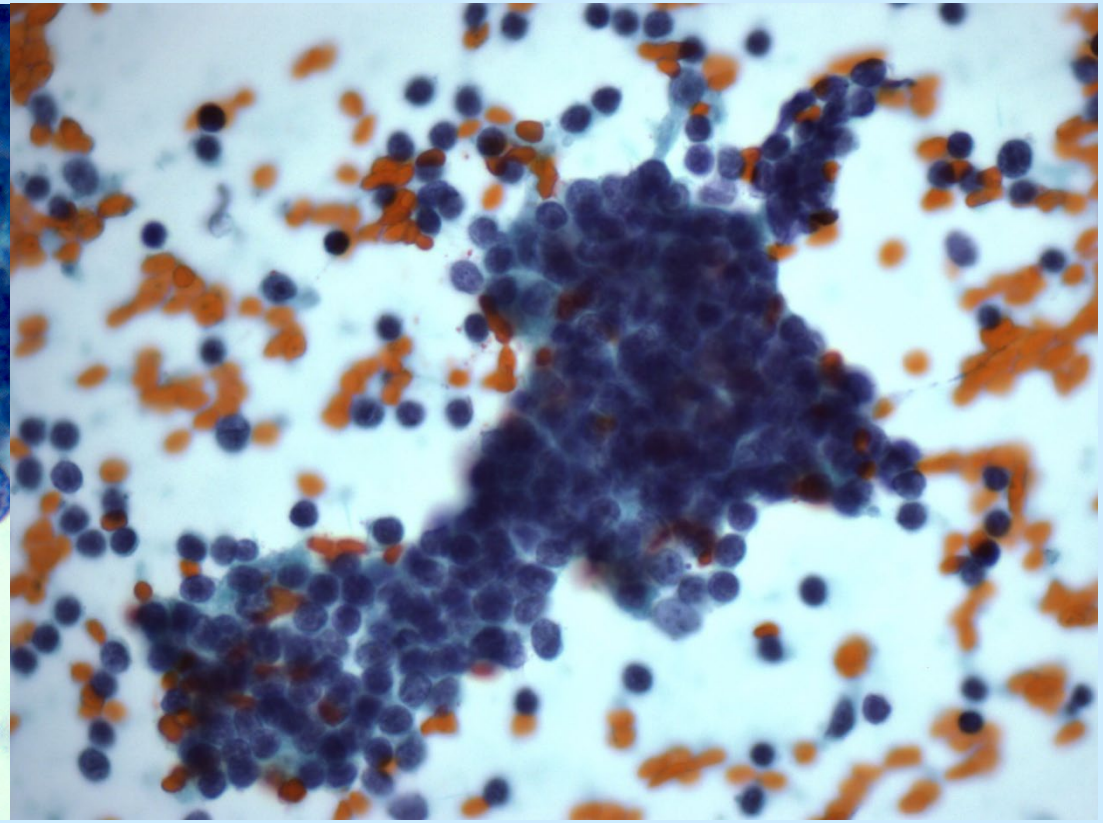


Reactive LN: Germinal Center Fragments

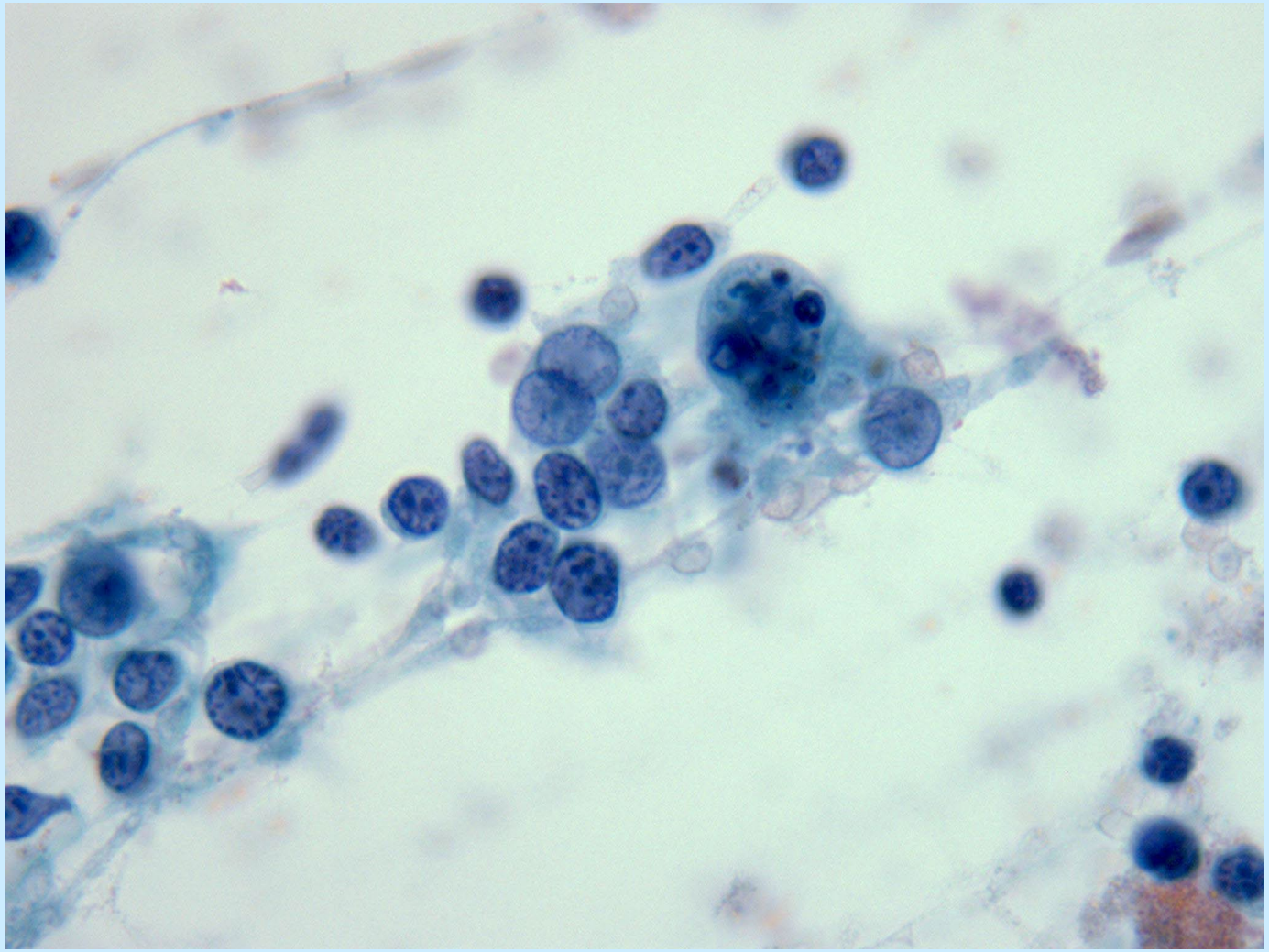
(aka: Lymphohistiocytic aggregates)

Do not confuse these cohesive groups with metastatic disease!





Reactive LN: Tingible Body Macrophage



Reactive Process VS Lymphoma

- **IMMUNOPHENOTYPING combined with cytomorphology is the key to distinguishing lymphoma vs reactive LN hyperplasia.**

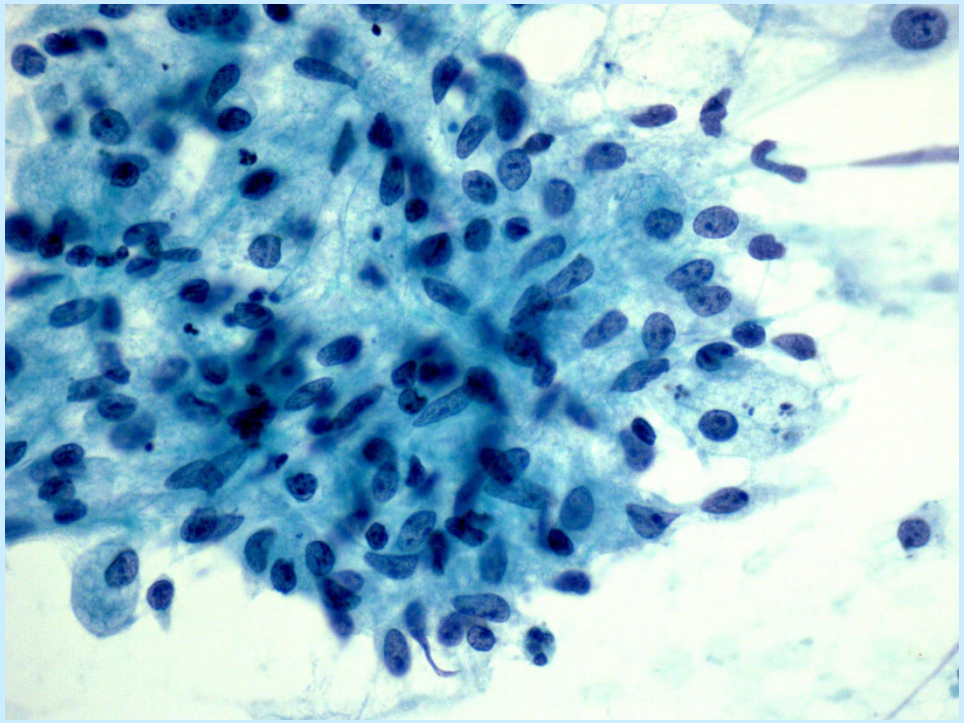
KEY POINTS

- **Reactive/Benign Lymph Node Cytology:**
  - **Mixed population of lymphocytes**
  - **Tingible body macrophages**
  - **Germinal center fragments**
  - **Polyclonal**

What are examples of specific causes of enlarged lymph nodes?

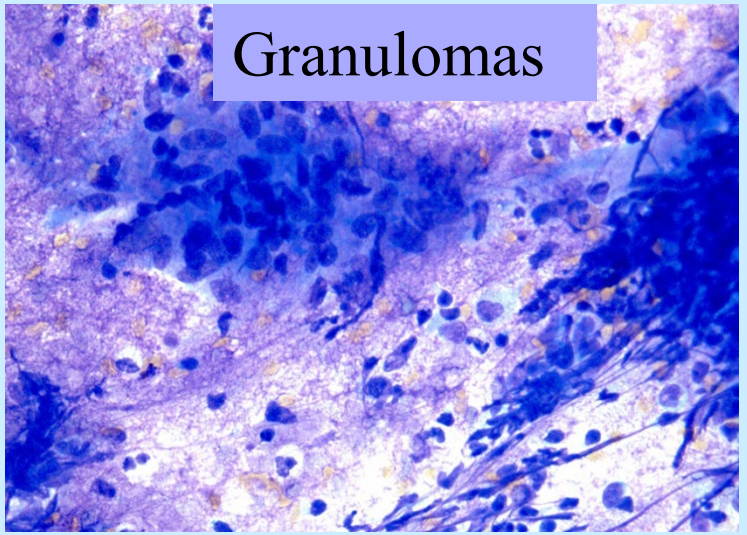
Sarcoidosis

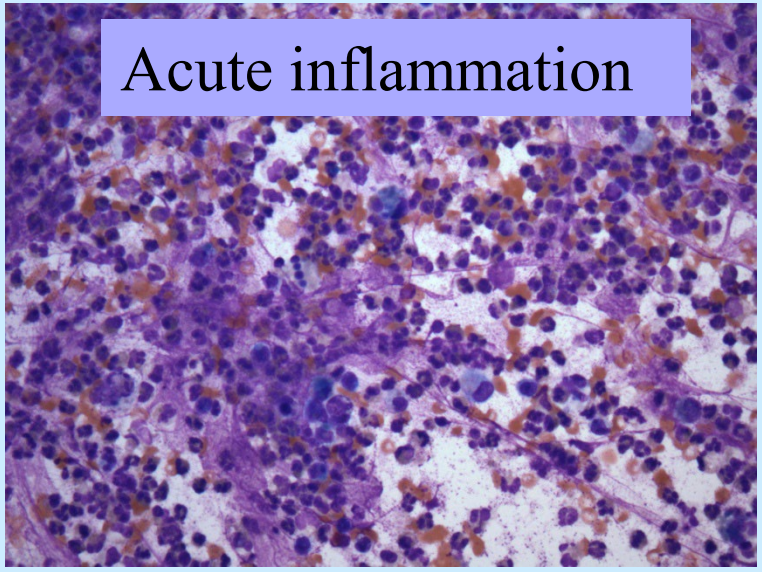
- Young to middle age adults
- More common in African Americans
- Diagnosis of exclusion/clinical correlation
- Cytologic features:
  - Non-caseating granulomas
  - Epithelioid histiocytes
  - Multinucleated giant cells
  - Lymphocytes
  - Clean background
- DDX:
  - Fungal or mycobacterial infection
  - Malignancy-associated granulomas
  - Foreign body reaction



Cat Scratch Disease

- Self-limited; resolves in 1-4 months
- LNs of groin, axilla, neck
- *Bartonella henselae* (serologies/Steiner stain)
  - Gram neg coccobacillus
  - PCR, serology, IF
- Cat bite or scratch reported in 50-70%
- Cytology (suppurative granulomatous lymphadenitis):
  - Acute inflammation
  - Granulomas
  - Necrosis





How do we diagnose lymphoma by FNA???

Non-Hodgkin Lymphomas

- Divided into B- and T-cell types
  - B-cell NH lymphomas represent 90%
    - DLBCL and follicular lymphoma represent >75%
  - T-cell NH lymphomas represent approx 10%
- Useful to divide into small and large cell lymphomas for FNA dx

KEY POINTS

- **Small cell lymphomas**
  - **Often MONOTONOUS pattern**
  - **Often ATYPICAL**
  - **Needs immunophenotyping**
- **Large cell lymphomas**
  - **Usually OVERTLY malignant**
  - **Must distinguish from other malignancies**

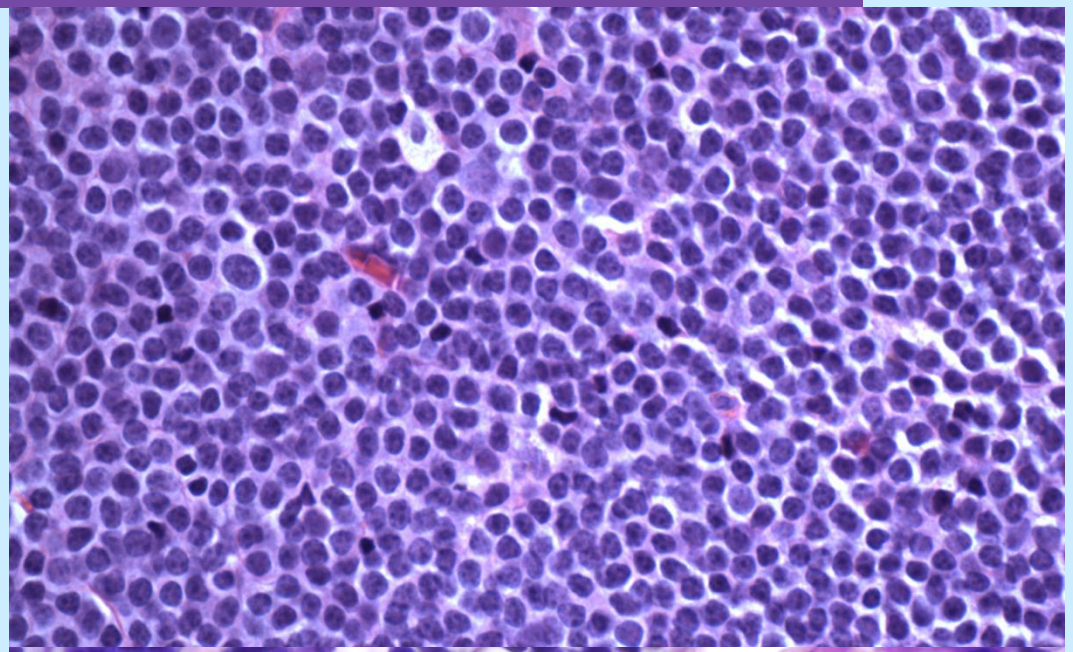
Subclassification of the 4 Major Small Cell NH Lymphomas:

An Algorithmic Approach by Immunoprofile

- **CD5 coexpression: B-CLL/SLL vs. Mantle cell**
  - **Light chain dim: favors B-CLL/SLL**
  - **CD5+CD23+ = B-CLL/SLL**
  - **CD5+CD23- = Mantle cell**
- **CD5 negative: MALT vs. Follicular**
  - **CD10+ = Follicular lymphoma**
  - **CD10- = unresolved (may require open biopsy)**
    - **Can use IHC on cell block for CD10 and Bcl-6**
    - **Cytoplasmic Ig = MALT**
    - **CD43+ favors MALT**
    - **Bcl-6+ favors follicular lymphoma**
    - **Clinical: extranodal favors MALT; nodal favors follicular lymphoma**

Small Lymphocytic Lymphoma

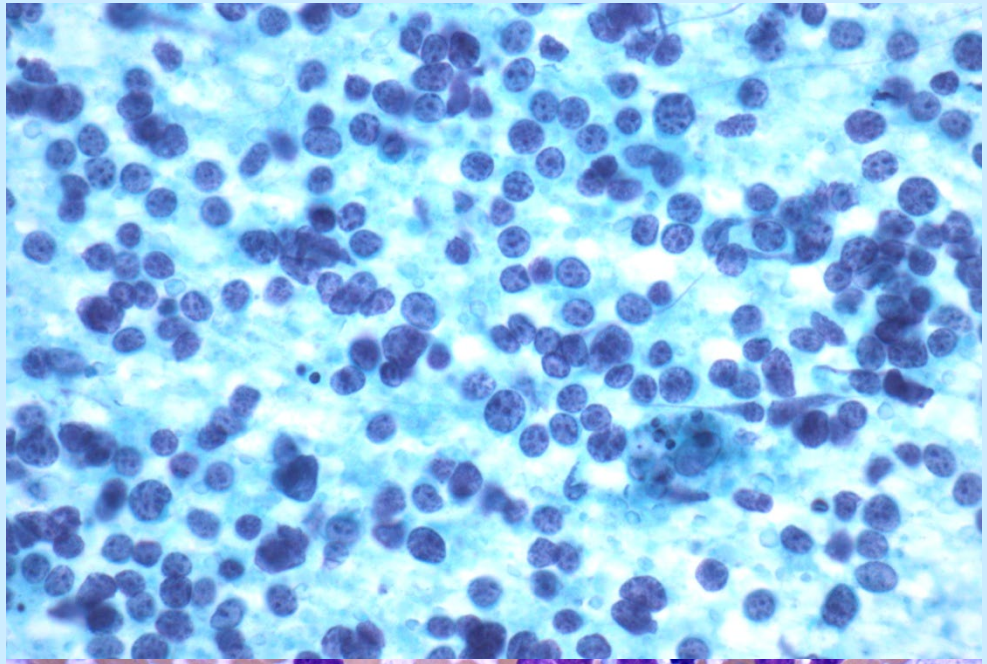
- **Cytologic features:**
  - **Monomorphous small lymphocytes**
  - **Round nuclei with clumped chromatin**
  - **Smudged cells on smears**
- **IHC and Molecular:**
  - **CD5+,CD23+, CD10- is characteristic**
    - **CD5, CD20, LC are usually dim or weak**
  - **Trisomy 12 (30%), others**

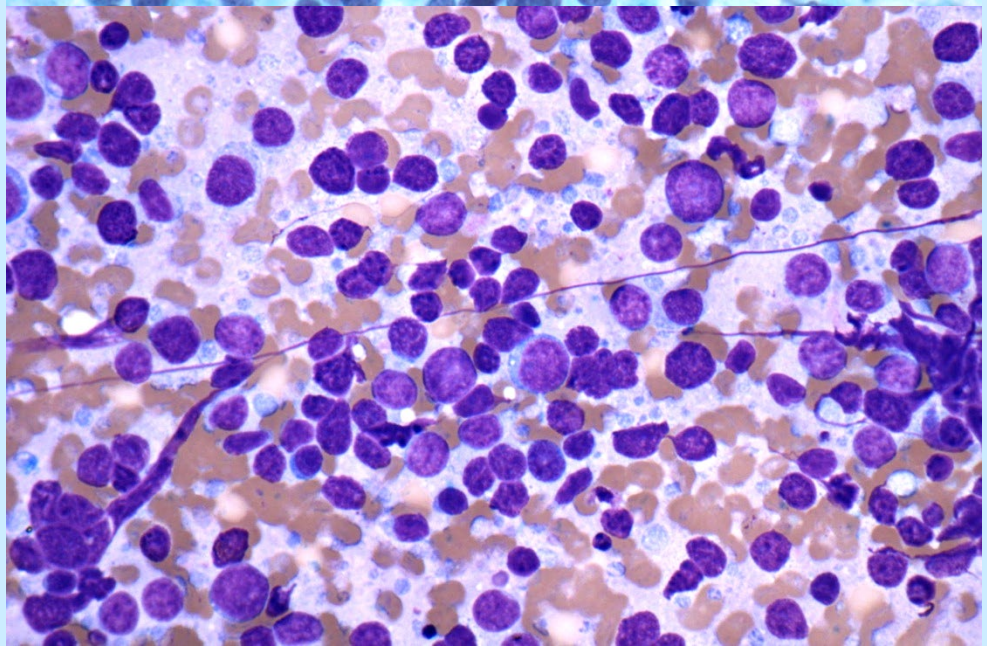


Smudged cells on smears

Marginal Zone Lymphoma

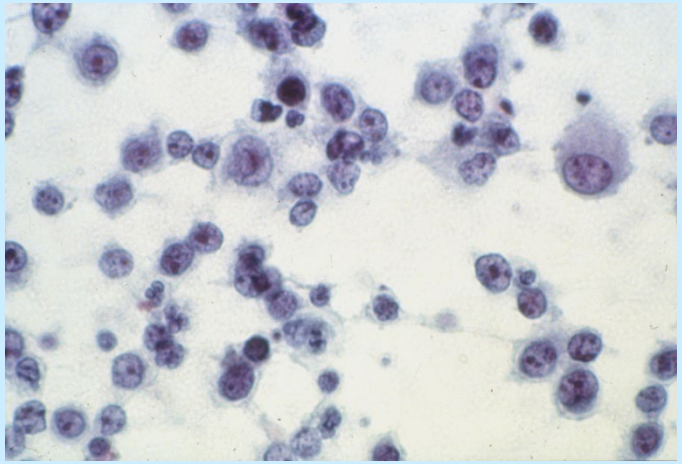
- Cytologic features:
  - Mixed/polymorphous pattern resembling reactive hyperplasia
  - Monocytoid cells
  - Plasma cells
  - Follicular dendritic cells, tingible-body macrophages, reactive immunoblasts, follicular aggregates
- IHC and Molecular:
  - CD5-, CD10-, Bcl-6-, cyclin D1-
  - Trisomy 3 or 18, t(11;18), others
- DDX:
  - Reactive lymphoid hyperplasia
  - CD10- Low grade follicular lymphoma

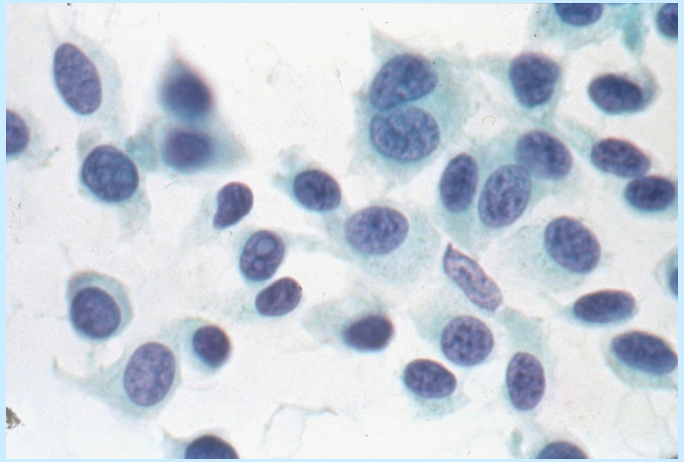




Large Cell Lymphoma
Must distinguish from other single cell cancers

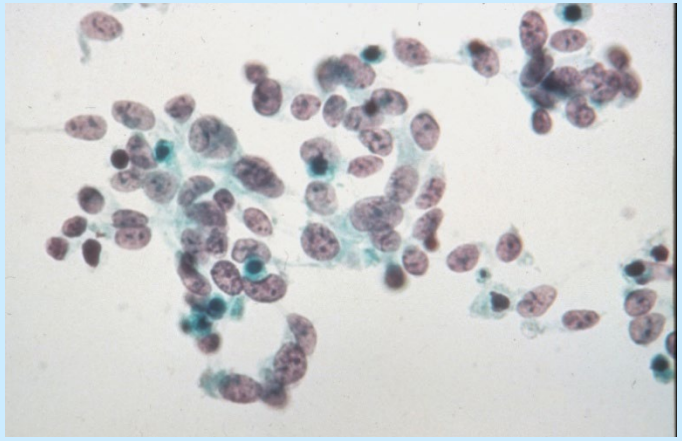
Large B-cell Lymphoma
CD45+, CD19+
Lympho-Glandular bodies

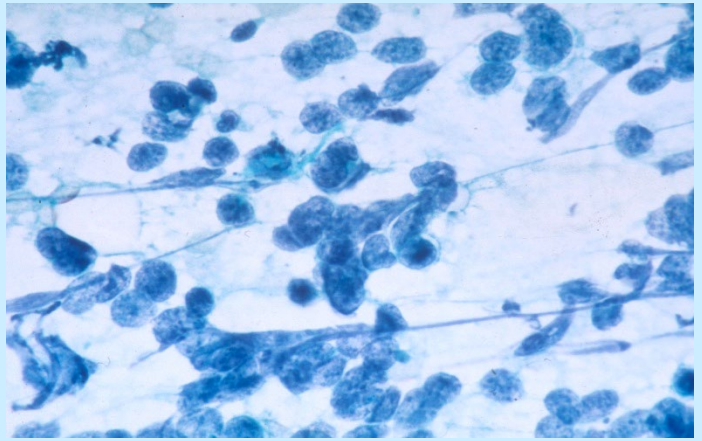




Malignant Melanoma
S-100+, HMB45+

Medullary Carcinoma
Ker+, Calcitonin +



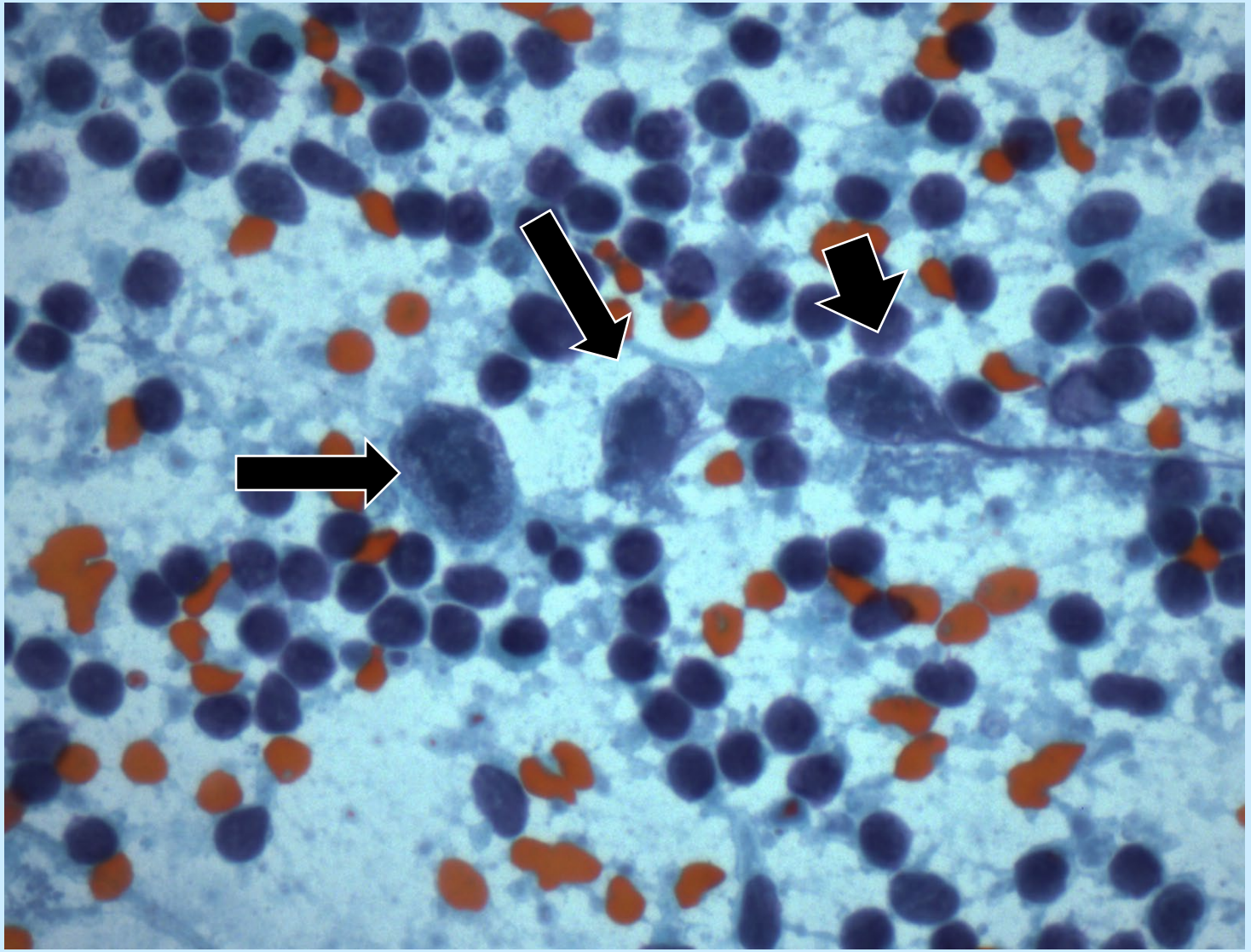


Small Cell Carcinoma
Ker+, Syn+, molding

Don’t forget about Hodgkin lymphoma!

FNA of Classical Hodgkin Lymphoma:

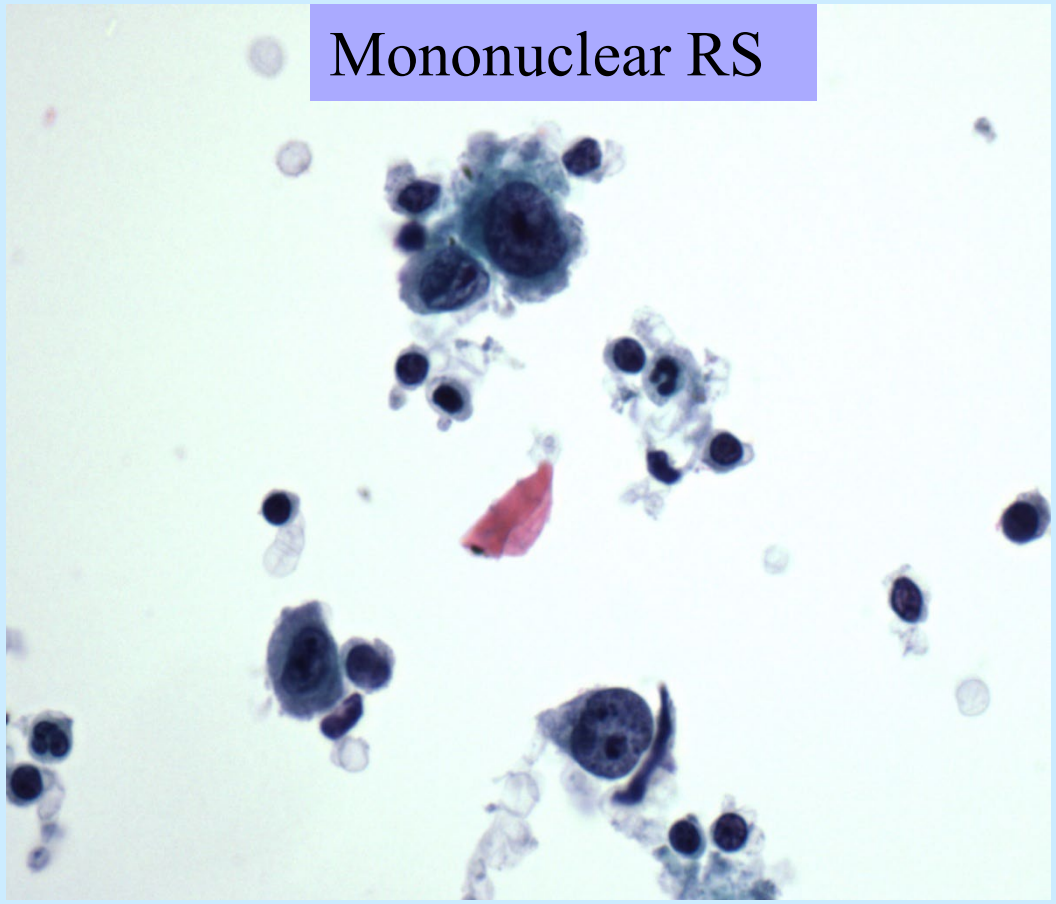
Easy to "miss" the RS cells



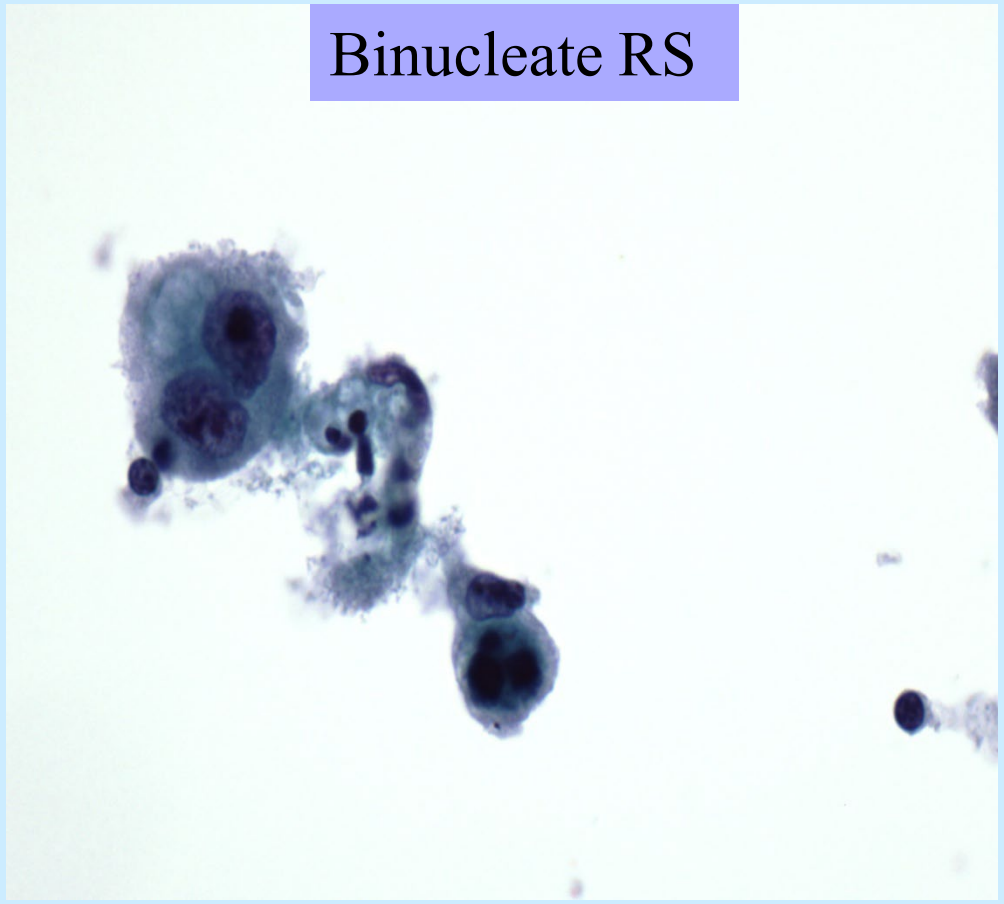
FNA of Classical Hodgkin Lymphoma: RS Cells

Pitfall: A cause of a false negative FNA is the Nodular Sclerosis Type of Classical HL due scant cellularity --- Search carefully for RS cells!

Mononuclear RS



Binucleate RS



KEY POINTS

- **Hodgkin lymphoma: Beware!**
  - **Negative flow cytometry**
  - **Reed Sternberg cells may be scant**
  - **Background lymphocytes resemble a reactive lymph node**

Thank You!