Cytology Basics

Introduction

Cytology (sometimes also called *cytopathology*) is the study of the body/diseases at the cellular level. This is in contrast to *histology*, which analyzes *tissue*.

I like to think of "Cyto" as a microcosm of surgical pathology. We get to see specimens from many organ systems and scrutinize them closely. Being familiar with cyto basics, even if you don't practice cyto specifically, will only enhance your general anatomic pathology skills, so let's jump into it!

Types of *specimens*

Exfoliative cytology: cells ***shed*** by the body. They can be spontaneously shed into fluid (e.g., pleural fluid) or mechanically shed via a brushing (e.g., cervical cytology)

Examples: Pleural fluid, Abdominal fluid, Cerebrospinal fluid (CSF), Cervical brushing, Biliary brushing, Bronchoalveolar Lavage (BAL), Urine,

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Aspiration cytology: cells are ***aspirated*** (sucked out), usually via syringe into a needle. This is usually done via "Fine-needle aspiration" (FNA) either by palpation or using imaging guidance.

Examples: Ultrasound-guided FNA (e.g., thyroid, lymph node), Endobronchial ultrasound (EBUS)-guided FNA (of lung mass or mediastinal lymph node), Endoscopic ultrasound (EUS)-guided FNA (of pancreas, liver, etc.)

Imprint (touch preparation) or "squash-prep" cytology: a piece of tissue is touched or squashed onto a slide, leaving cells behind. This is often done as part of intraoperative assessment as an alternative/adjunctive study, particularly in neuropathology, as it doesn't have "freeze artifacts."

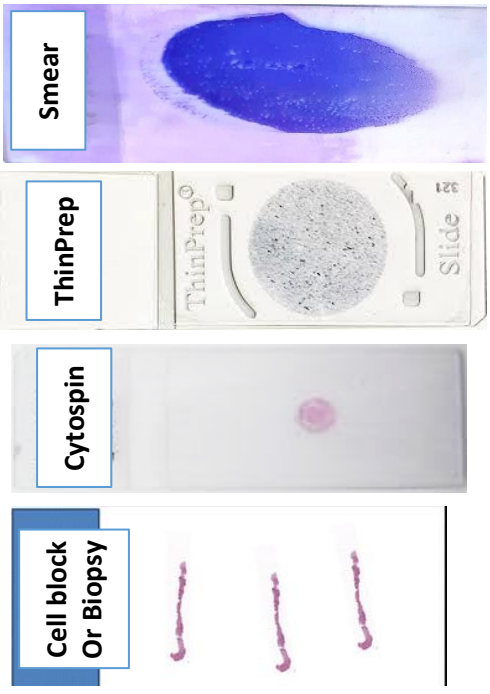
Types of *preparations*

Smear: The specimen is thinly spread directly on a slide.
Pro: Oldest, fastest, cheapest
Con: Not optimal for dilute specimens

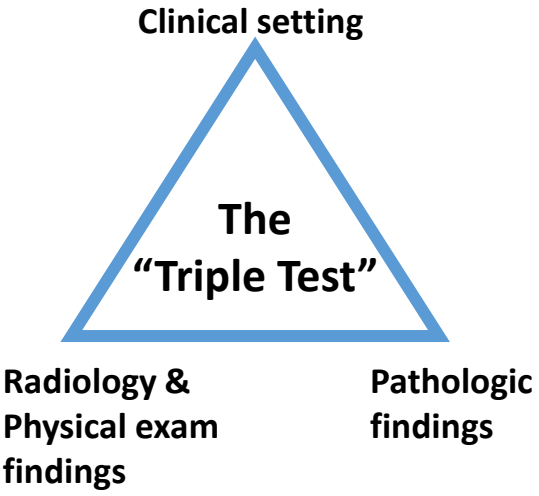
Liquid-based cytology: The specimens is preserved in a liquid suspension and then mechanically filtered, concentrated, and deposited in a thin monolayer on a slide.
Examples: ThinPrep, SurePath
Commonly used for exfoliative cytology.

Cytocentrifugation: Uses centrifugal force to concentrate and flatten cells directly onto a glass slide.
Example: Cytospin.
Most common method for CSF.

Cell block (or biopsy): Similar to conventional histology: tissue is collected, fixed in formalin, embedded in paraffin, and cut thinly and put on a slide.
Pro: Easy to do ancillary testing (e.g., IHC)



Adequacy and Diagnosis



In order to confidently render a diagnosis, we need to see a **sufficient number well-preserved representative cells from a lesion.**

The absolute number of cells varies by site, with some sites (e.g., cervix, thyroid, etc…) having well-established minimum cellularity requirements to maintain a good negative predictive value (i.e., we're sure that we got a good enough look to confidently say "there is nothing worrisome").

More broadly though, we need to consider if our findings explain the clinical and radiographic findings? This involves the "Triple test" of making sure the clinical setting, radiology, and pathology all agree.

If our findings don't fit with the radiology or clinical findings, we should reconsider if our findings are "adequate" and "diagnostic."

General Rules of Thumb

In cytology, we generally use 4 broad categories for diagnosis, which are often further pragmatically grouped for clinical management:

General Pathology Diagnostic category	Reductive clinical grouping	Clinical management
Negative/Benign	No cancer	**Observation** Possible repeat biopsy if *clinically* suspicious
Atypical		
Suspicious	Cancer *(probably)*	Proceed to **treatment**, Possible repeat biopsy for suspicious
Positive/Malignant		

Fundamentals to never forget!

1) **Don't definitively diagnose malignancy off just a couple cells**. Hold out for definite tumor. A few cells could be reactive or a contaminant. Consider saying "Atypical." (That is, don't over diagnose it, but also don't ignore it either).
2) **If there is abundant inflammation, consider reactive atypia** (and "dialing back" your diagnosis). Beware of so-called "Worrisome Atypia in Reactive/Degenerative" cells (WARD cells), which can frequently look very atypical and mimic malignancy.
3) **Don't evaluate "naked" nuclei** stripped of their cytoplasm (or at least don't place too much confidence in them). Cells need to have cytoplasm to be evaluated.

The Two Most Common Stains

Pap stain

Performed on "**wet-fixed**" smears (*not* dried)
Developed for by Dr. George *Pap*anicolaou for cervical cytology→ not surprisingly, excels at nuclear detail and squamous differentiation

Advantages:
Better nuclear detail, including nucleoli
Better for thick smears (see deeper into groups)
Highlights keratinization (pink/orange)
Less artifacts (e.g., drying artifact)

Disadvantages:
Less cytoplasmic detail
Slower

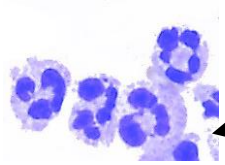
Wright-Giemsa stain

aka Romanowsky or Diff-Quik
Performed on **air-dried** smears
Also used by **hematology** labs

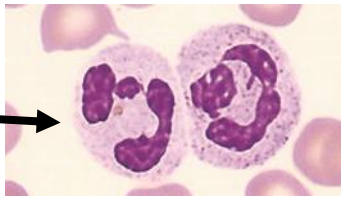
Advantages:
Better cytoplasmic detail
Better evaluation of stromal tissue.
Faster (usually)

Disadvantages:
Less nuclear detail
Prone to air-dry artifact

Neutrophils

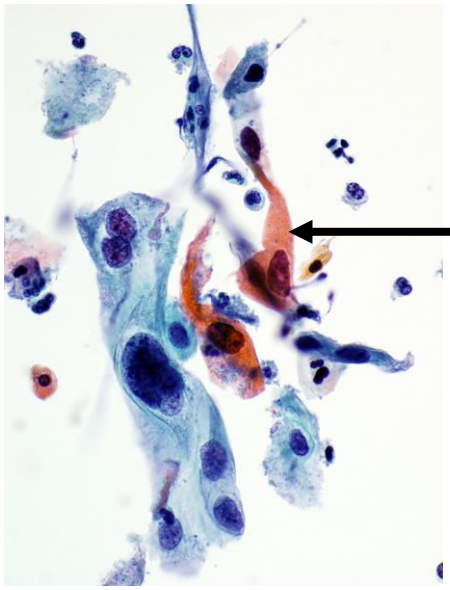


See **cytoplasmic** granules better



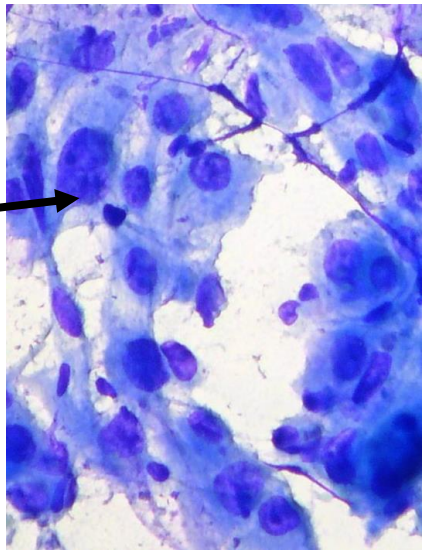
Often easier for WBCs as use similar staining in Heme lab

Squamous cell carcinoma

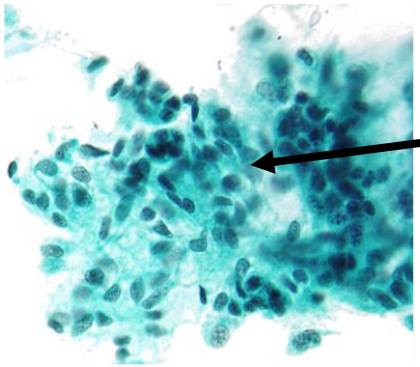


Shows **keratinization** (orange/pink)

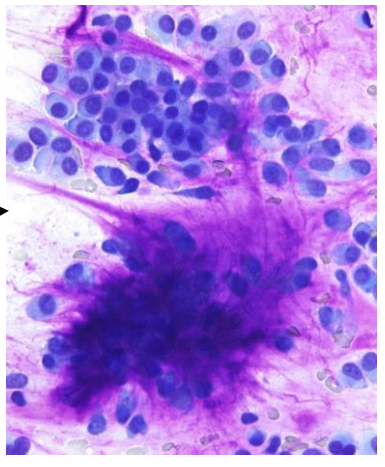
Better 3D visualization of **nuclear contours and chromatin**



Pleomorphic adenoma

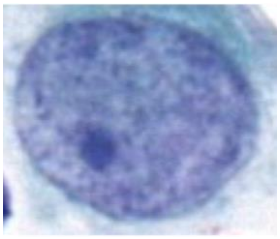


Shows **stromal tissue much better** (e.g., metachromatic stroma or colloid)



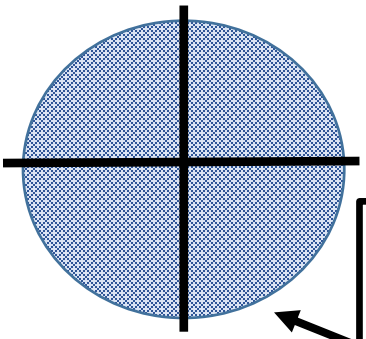
Cytology generalities

Benign

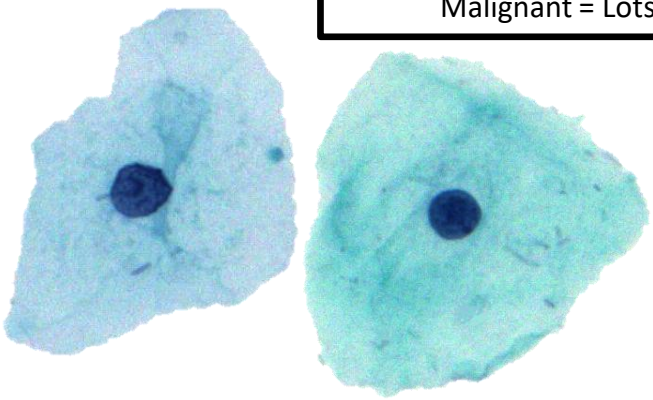


Round nuclei
"Smooth" evenly distributed chromatin

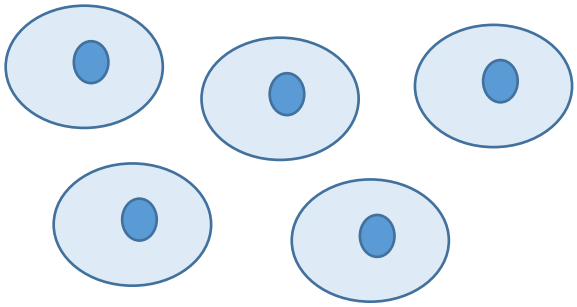
Think: Like a robin's egg or orange



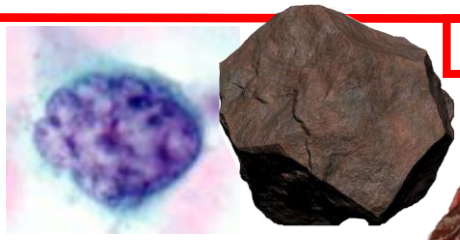
One approach: Mentally divide a nucleus into quarters and compare the chromatin and nuclear contours of each quarter.
Benign = Mostly the same
Malignant = Lots of variability

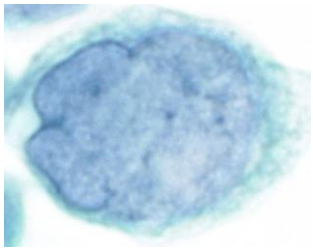


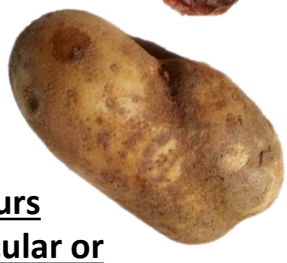
Cells/nuclei look similar to neighbors



Malignant

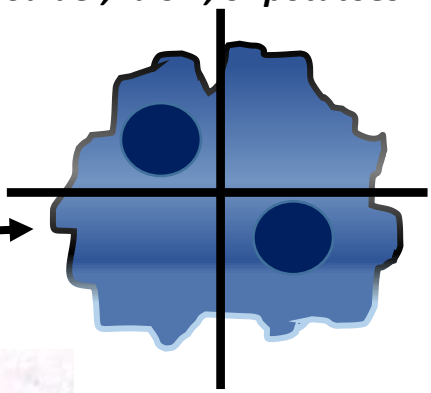


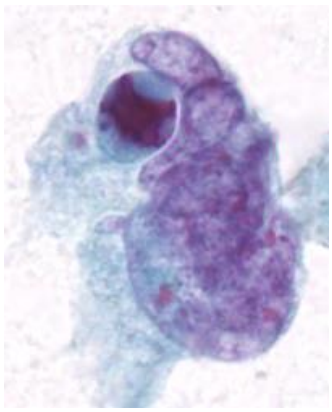


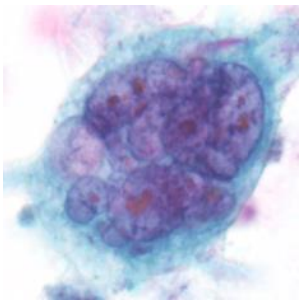


***Irregular* nuclear contours**
Clumped, uneven, vesicular or hyperchromatic chromatin

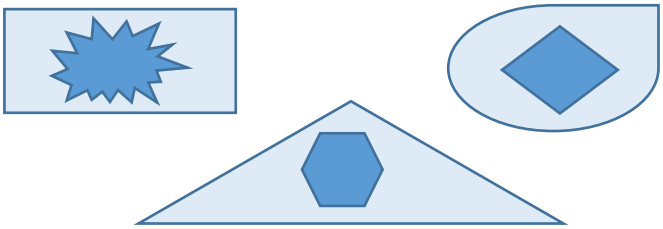
Think: Like a boulder, raisin, or potatoes





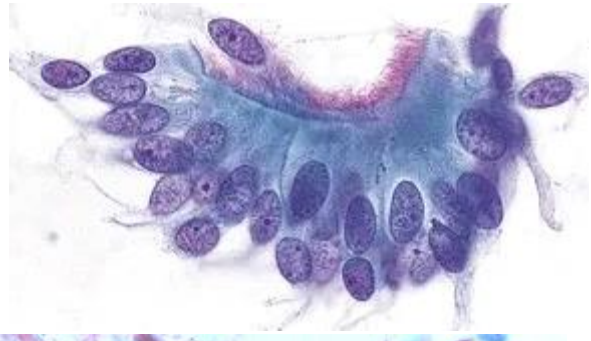


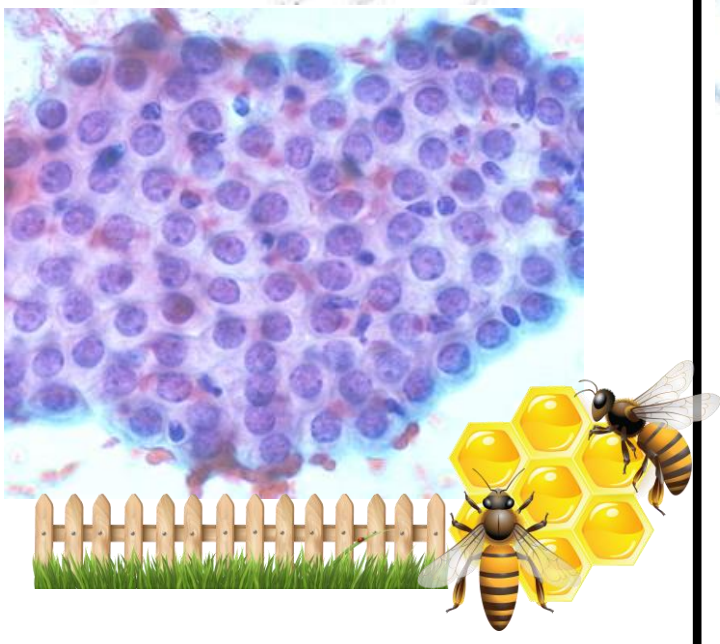
Lots of variation in size/shape of neighboring cells (Pleomorphism)



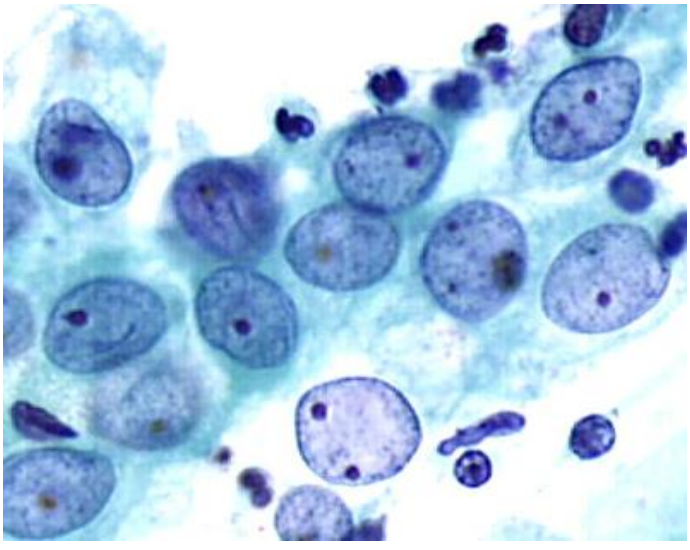
Benign

Organized cell clusters
Polarized cells (that know which way is up)
"Honeycomb" or "Picket-fence" glandular architecture



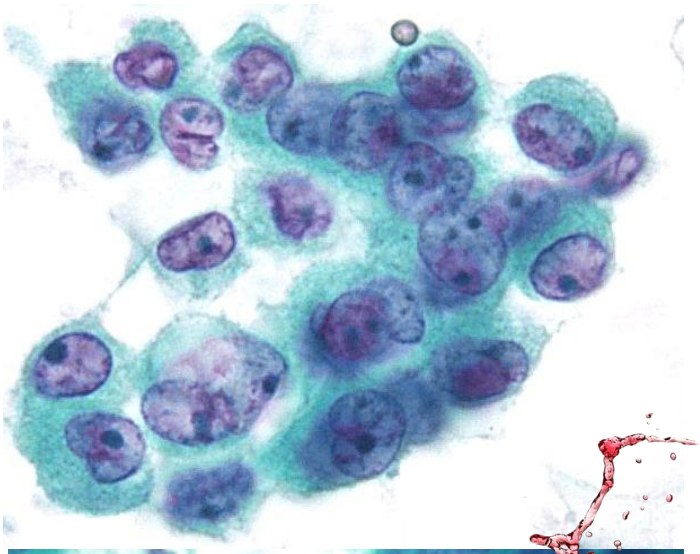


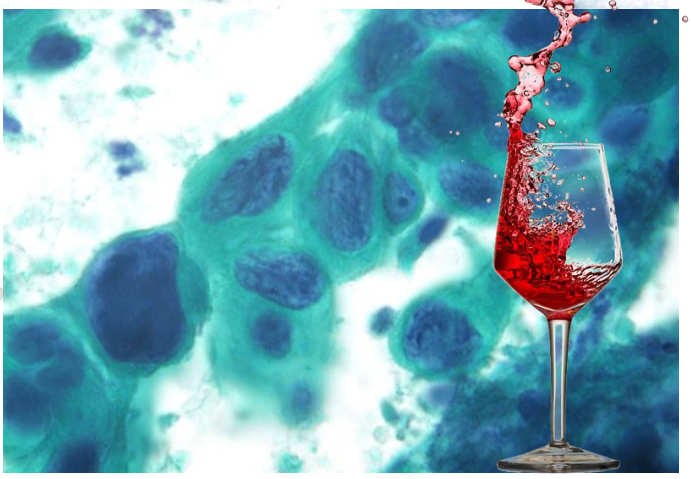
Small, usually inconspicuous nucleoli
Rare mitoses



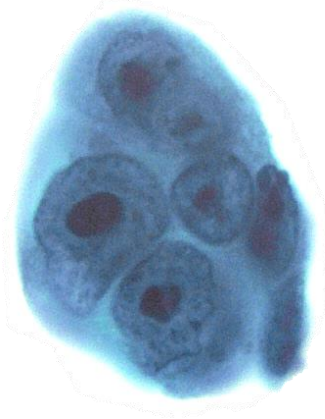
Malignant

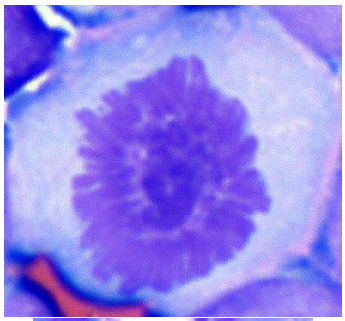
Irregular "Drunken" architecture
Tightly packed together

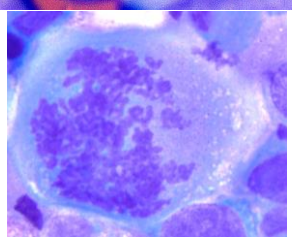




Large, prominent nucleoli (sometimes)
Frequent mitoses, especially atypical

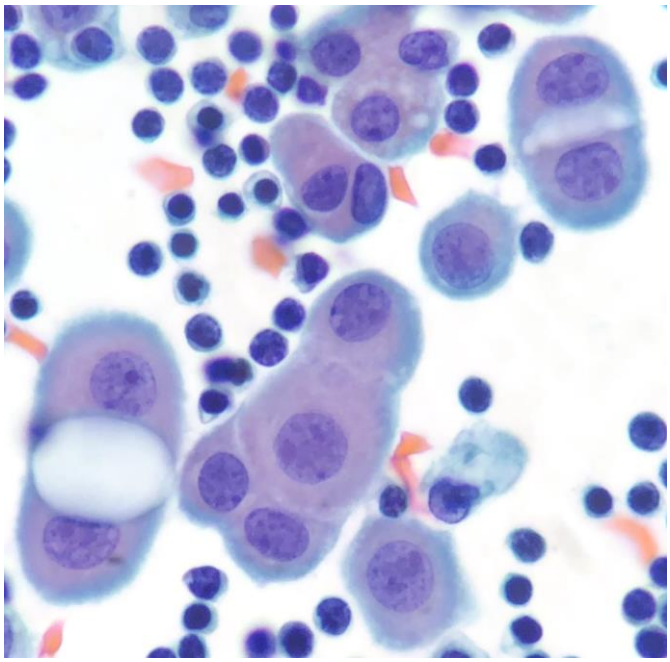




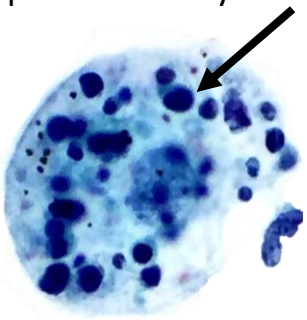


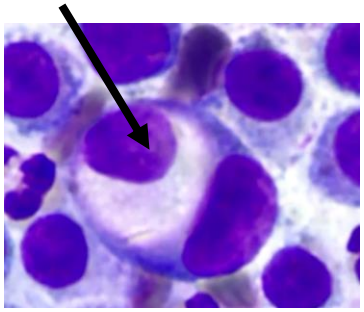
Benign

No nuclear molding



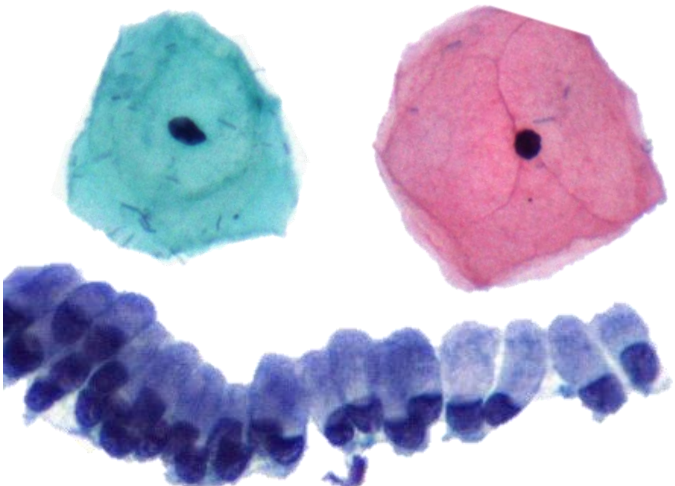
The main phagocytosis of cells in benign processes is by macrophages





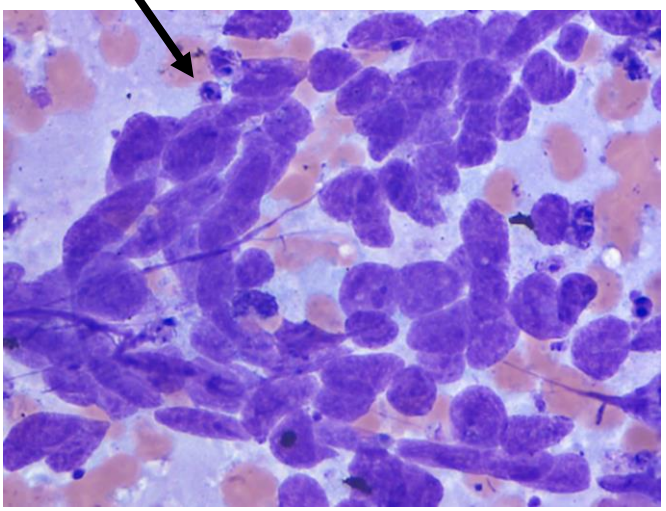
Often Low N:C Ratios

(However, there are obvious exceptions to this, like benign lymphocytes or reserve cells having scant cytoplasm)



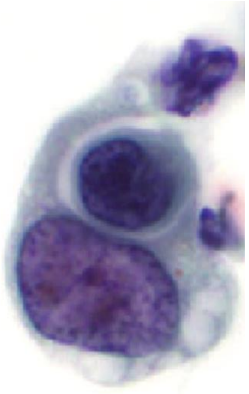
Malignant

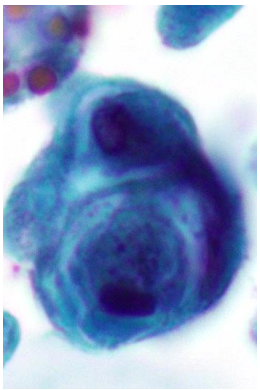
Nuclear molding



"Cannibalism"

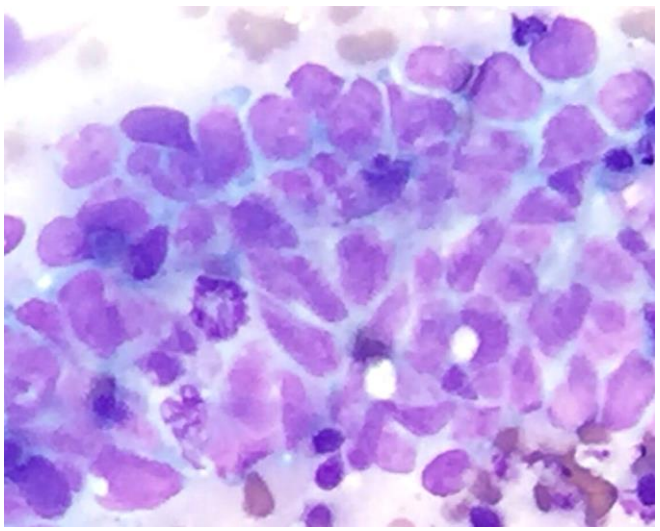
(tumor cells eating other tumor cells)





Often High N:C ratios

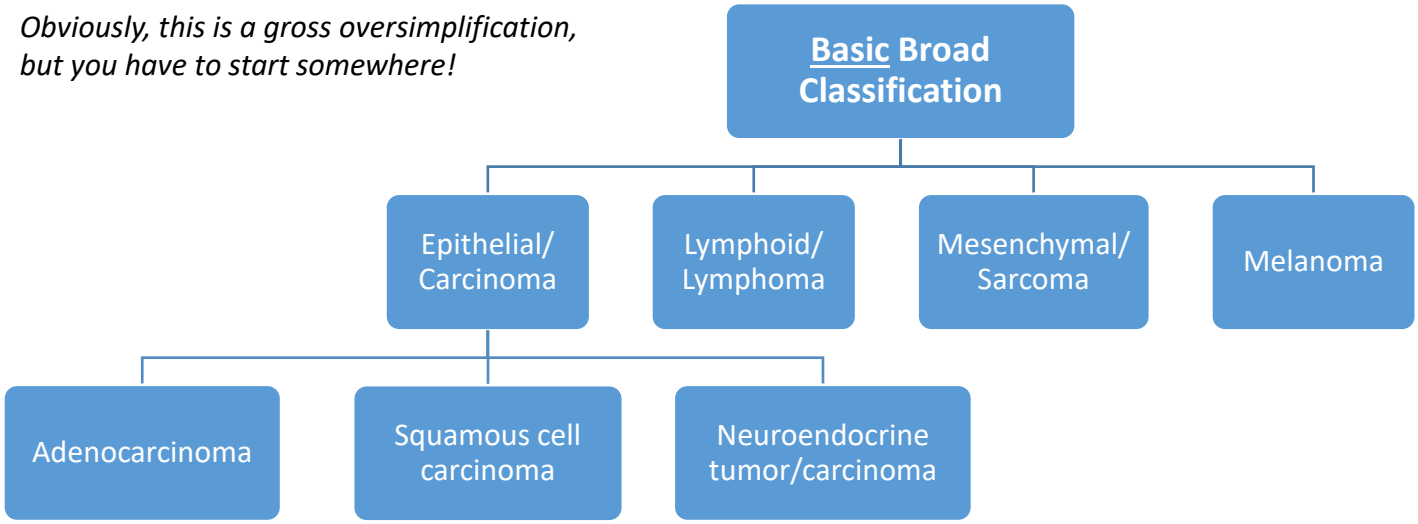
(There are plenty of exceptions to this, for example mucinous carcinomas)



Basic Lines of Differentiation

Always think broadly and first try to put things into a "bucket," then you can get more specific after.

Obviously, this is a gross oversimplification, but you have to start somewhere!



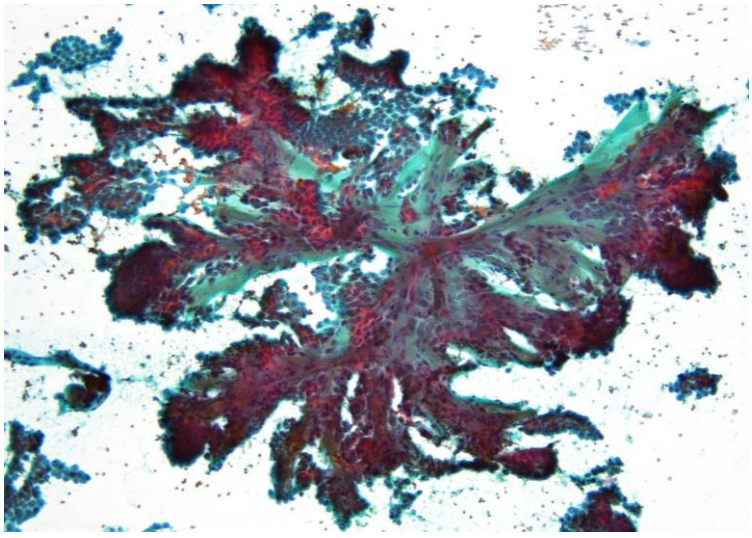
Inspired by the DeMay "Gut coarse" and "Building blocks"

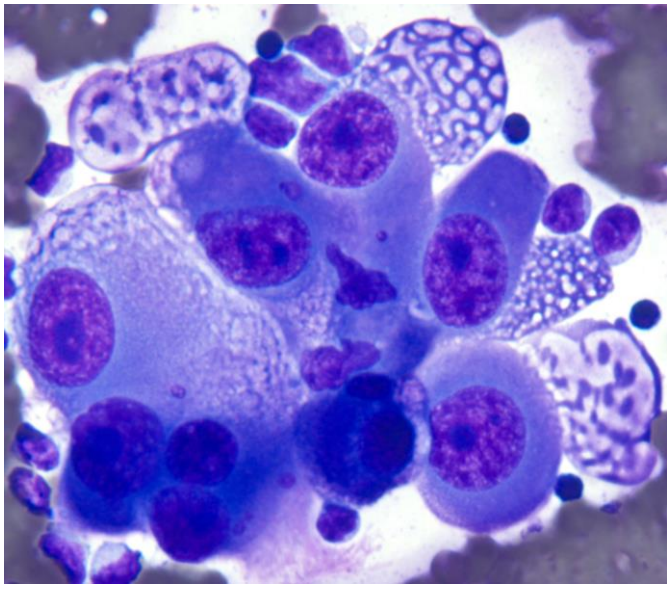
Epithelial cells/Carcinoma

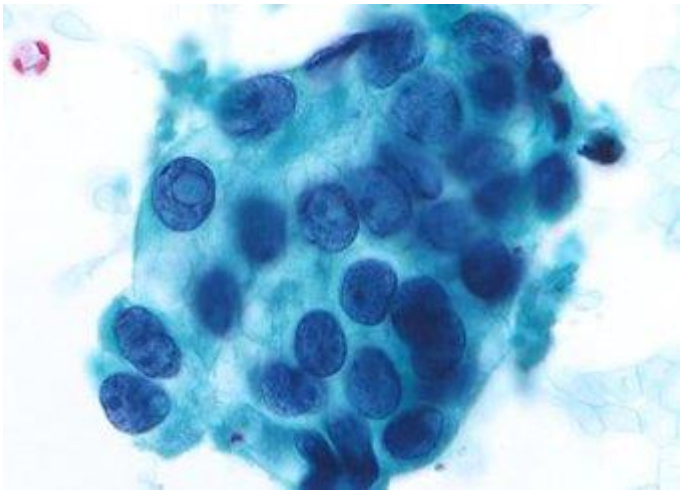
Epithelial cells form structures, so even when smeared, they remain in **cohesive clusters**

Compared to blood cells, they are also **relatively large in size**

They often have moderate to **abundant cytoplasm** and therefore appear "*epithelioid.* (obviously not true of all carcinomas though… I'm looking at you small cell carcinoma!)"







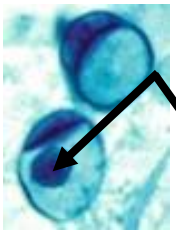
Glandular Cells/ Adenocarcinoma

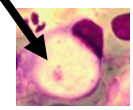
May **form glands** or papillae

Characteristically **produce mucin**, which may be visible in cytoplasm.

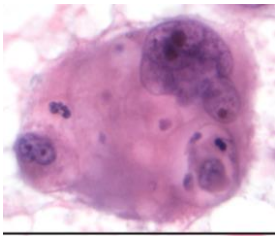
Cytoplasm often appears "**delicate**" (fluffy to granular) with **less distinct cell borders**. Blueish cytoplasm on Pap usually.

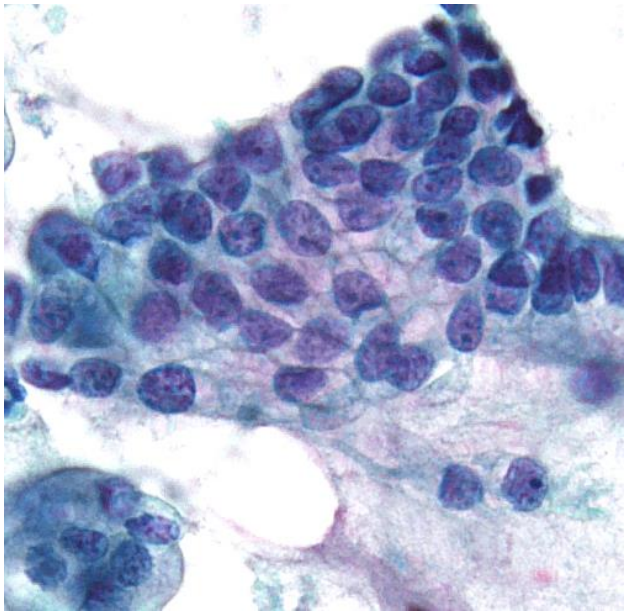
Often columnar with nucleus **polarized** at one end.
Can see **Signet ring cells**.





Intracytoplasmic lumina: Targetoid secretory vacuoles associated with breast cancer

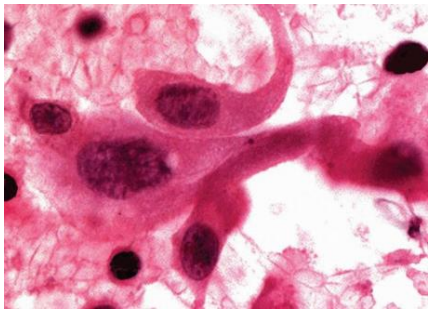




Squamous cell cells/carcinoma

Produce keratin → **Bright orangish on Pap stains**. Can see keratin pearls

Cytoplasm appears "**dense**" with **distinct cell borders**

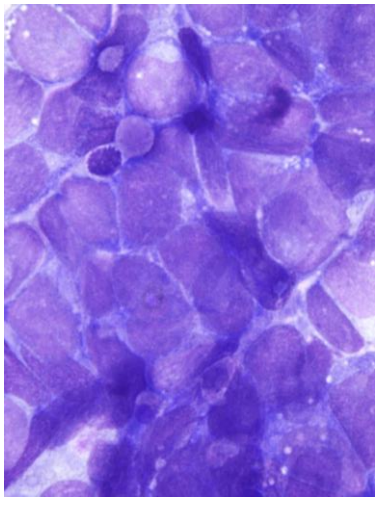


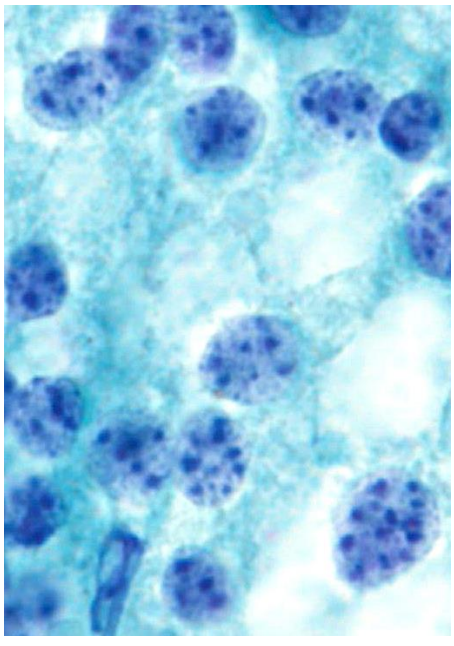
Neuroendocrine Cells/ Tumors

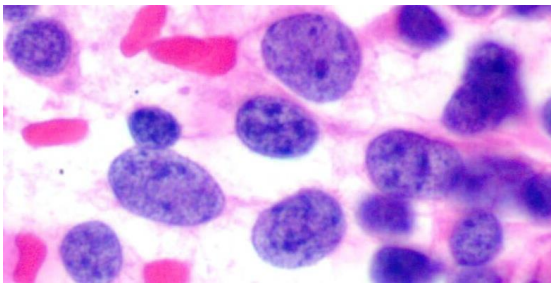
Nuclear **chromatin appears stippled** like "**Salt and Pepper**"

Cells are often **discohesive**

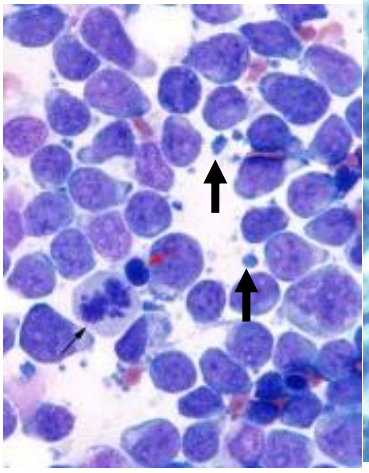
May have granular cytoplasm with secretory granules.

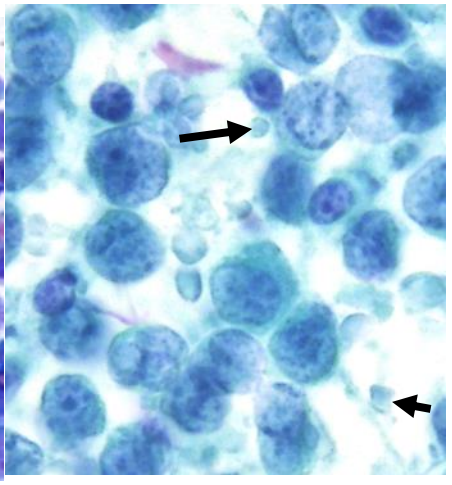






Lymphocytes/Lymphoma





Discohesive small cells (remember, they must circulate through vessels, so they have to be small and loose)

Scant cytoplasm

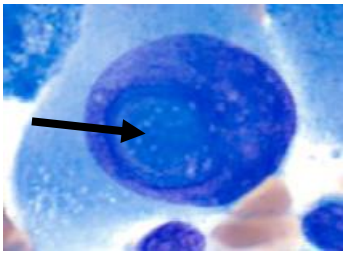
Lymphoglandular bodies (pieces of lymphocyte cytoplasm that peel off during smearing →)

Consider **sending for flow cytometry** at time of adequacy to evaluate for lymphoma

Melanocytes/Melanoma

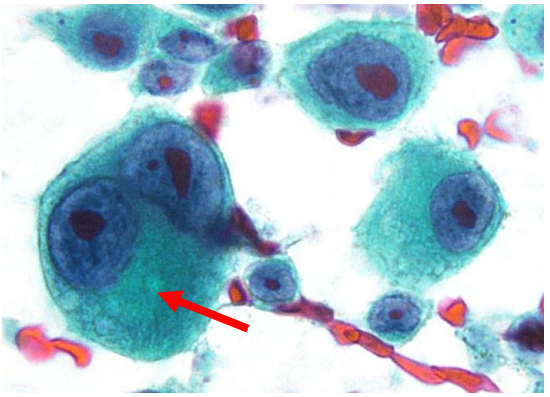
Large, discohesive cells. Often **very cellular** aspirates.

Frequently prominent nucleoli
Double mirror image nuclei (DMIN)
("bug-eyed demons")



Cytoplasmic melanin pigment (→)

Intranuclear pseudoinclusions (→)



Mesenchymal/Sarcoma

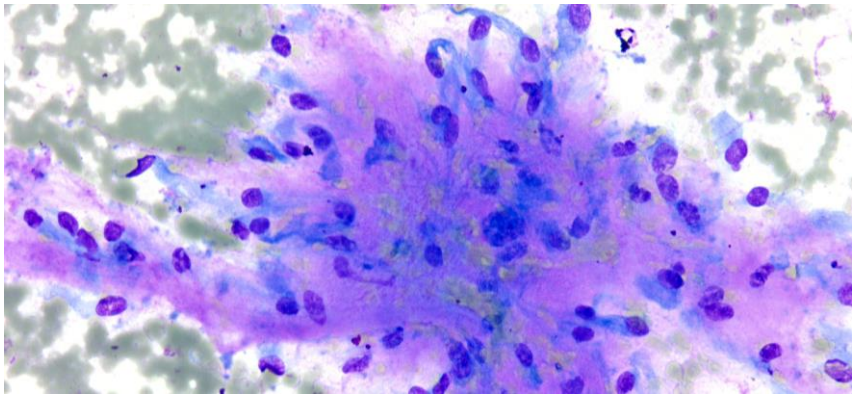
Classically, **Spindled cells** = long, narrow cells with relatively scant cytoplasm and cigar-like nuclei, but can have round or epithelioid cells.

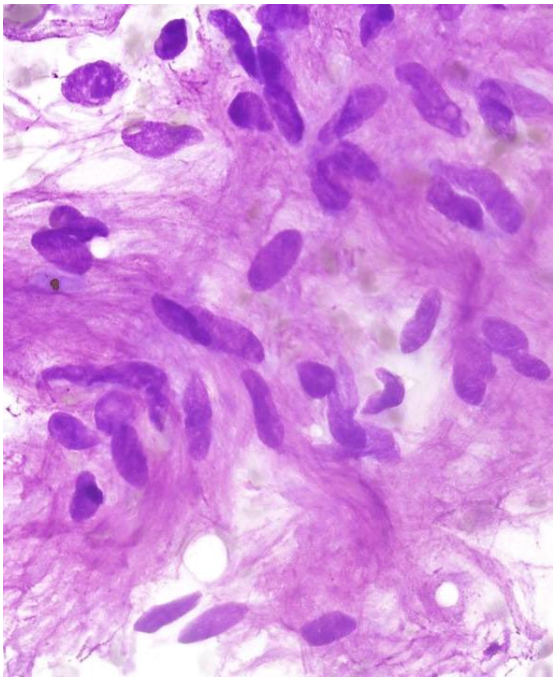
Frequent **extracellular matrix**

Neural tumors often have "buckled" or "fishhook" nuclei.

Very variable pleomorphism

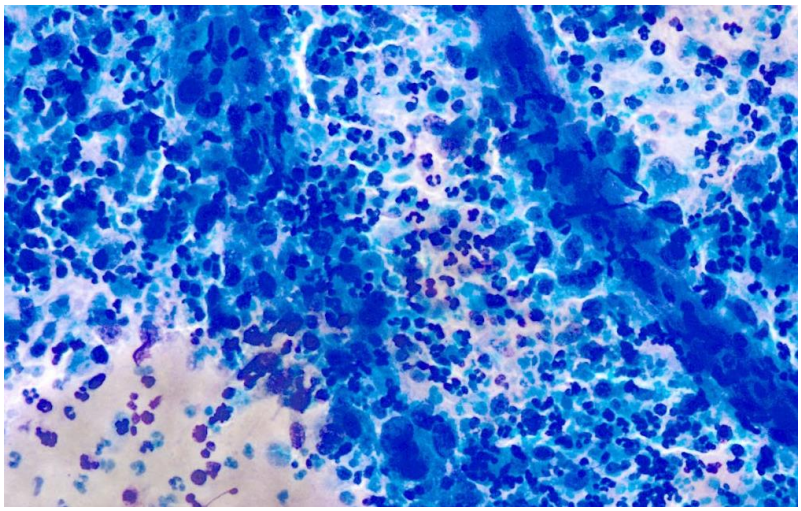
Often paucicellular aspirates due to dense extracellular fibrous stroma





Common Findings to Recognize

Abscess



Abundant Neutrophils (some of which may be degenerating)

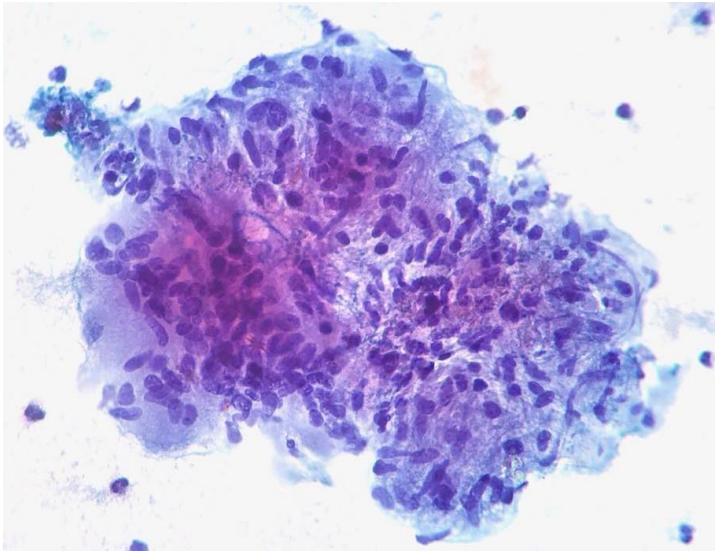
Necrosis and fibrin

Macrophages, bacteria, foreign material

At time of adequacy assessment, one will see frank pus. If this happens, remember to **culture it!**

Clinically: Warm, Red, Tender

Granulomas

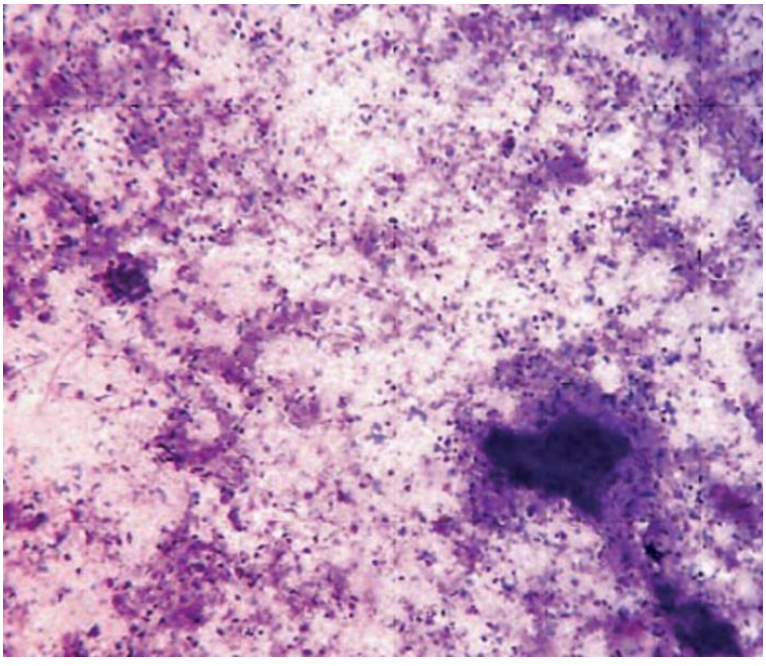


Nodular collections of **epithelioid histiocytes**
Often in loose **syncytial aggregates**
Can resemble a swirling school of fish

Histiocytes may be spindled or epithelioid with elongated nuclei resembling bananas or boomerangs
Can see **Multinucleated Giant Cells**

DDX: Infection (esp. TB & Fungi), Sarcoidosis, foreign material→ so try to do **cultures** or at least **bug stains**!

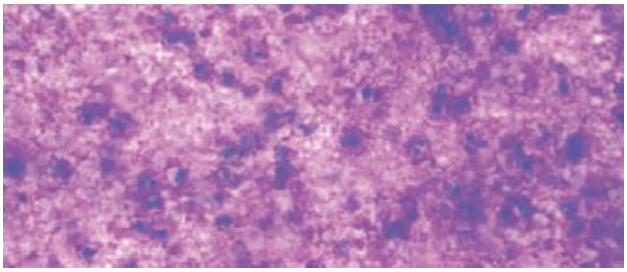
Necrosis



Lots of "**grungy" particle fragments, degenerated blood, and fibrin** without any nucleated cells.

Can be seen in non-neoplastic processes and neoplastic processes (so look around for viable cells to suggest what might have caused it).

May see **macrophages** trying to clean up.

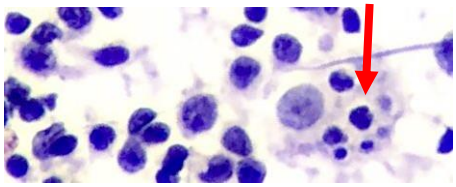


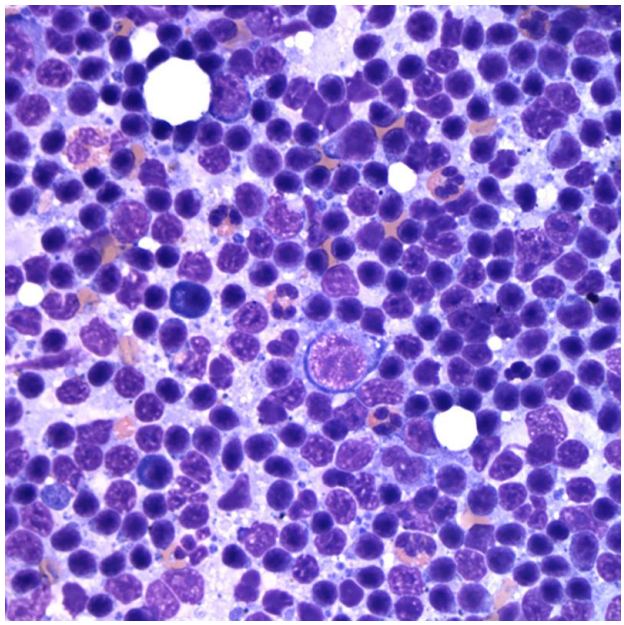
Reactive Lymphoid Hyperplasia

Often very cellular aspirate.
Mixture of small and large lymphocytes (range of maturation) with a **predominance of small lymphocytes**.
Frequently plasma cells and **tingible macrophages** (↓)

May see mitoses. Lymphoglandular bodies.
No malignant cells present!

Consider sending for **flow cytometry** at time of adequacy to evaluate for lymphoma if that is a possibility.





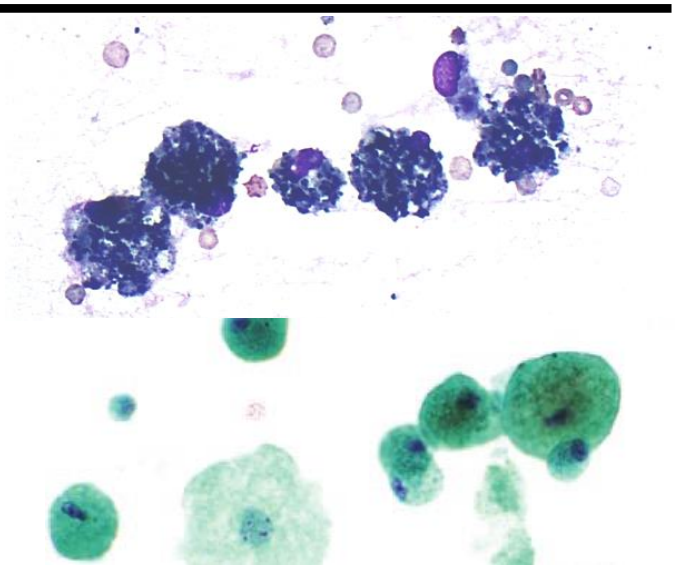
Cyst Fluid

Paucicellular with **scattered macrophages**, which may contain **hemosiderin pigment**

May see scattered debris.

These elements are often non-specific and don't indicate the composition of the cyst lining/wall, so may be "unsatisfactory" for diagnosis.

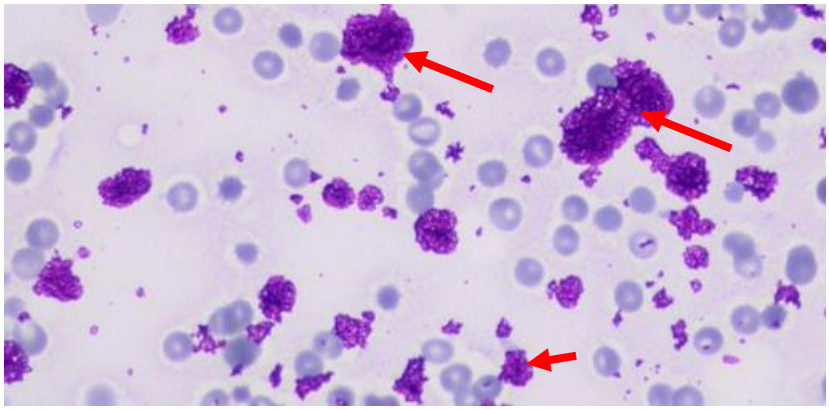
If possible, drain the cyst and then reaspirate the area in attempt to sample the cyst wall.



Ultrasound Gel

Coarsely granular metachromatic material on Romanowsky stains.

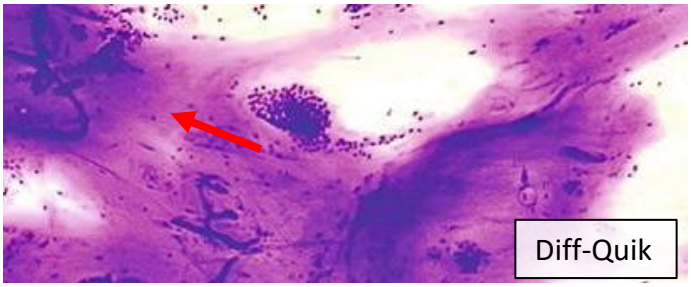
Can obscure diagnostic material.

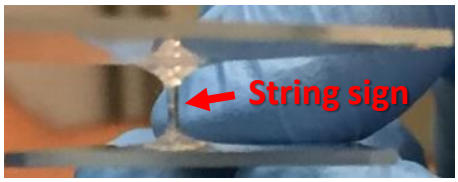


Mucin

Appears as **amorphous wispy material.**
Bright magenta on Diff-Quik. Blueish on Pap.
Can grossly see "string sign" when smearing slides (see below: String of mucin connecting slides).

Can see with mucinous neoplasms *and* mucoceles.

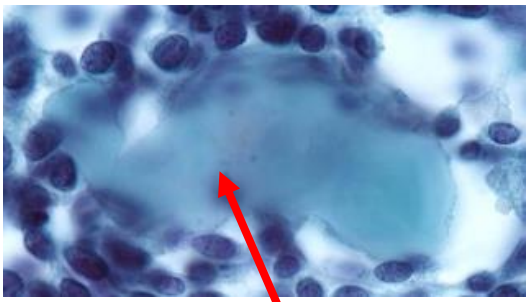




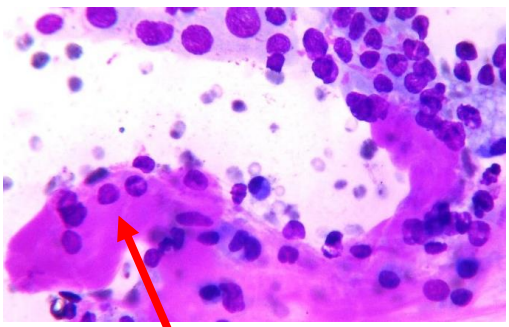
(other) Extracellular material

Examples: Colloid, Basement membrane, Amyloid

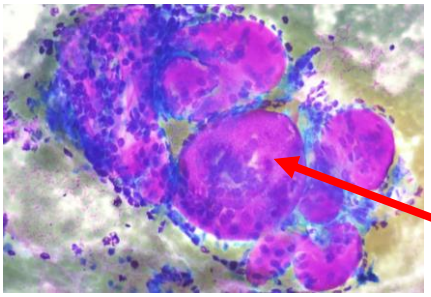
Often has a glassy, smudged look and is brightly colored on Diff-Quik



Amyloid in Medullary thyroid carcinoma



"Bubble gum" colloid in Papillary thyroid carcinoma

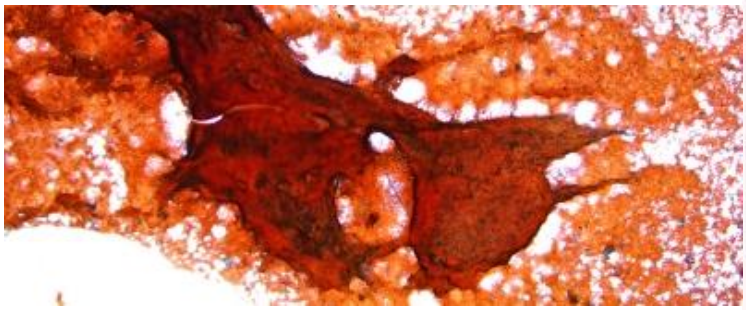


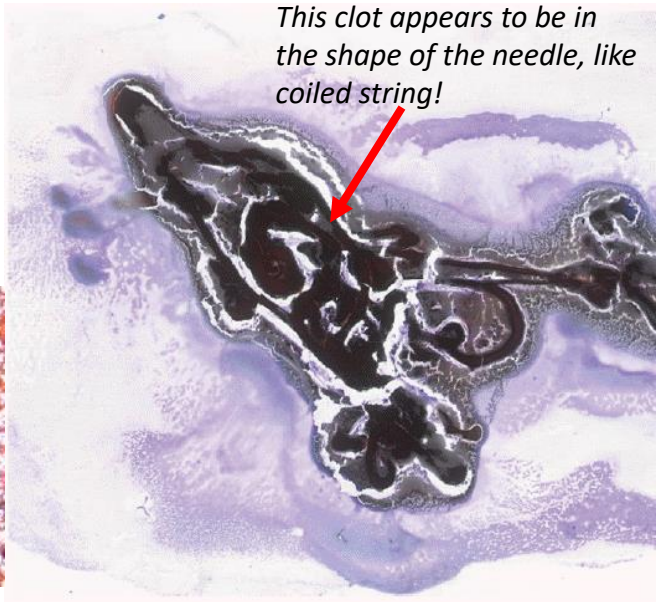
Basement membrane material in Adenoid cystic carcinoma

Blood clot

Common in bloody specimens, obscuring lesional cells, limiting interpretation.

Can be minimized by doing short, fast aspirations with a smaller needle.



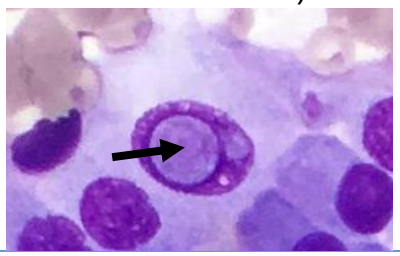


(some) Differential Diagnoses

Intranuclear Pseudoinclusions

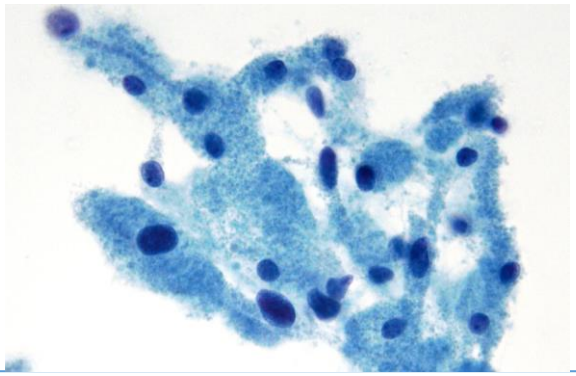
Develop when the cytoplasm pushes into the nucleus (think: a balloon within a balloon)

- Papillary thyroid carcinoma
- Medullary thyroid carcinoma
- Melanoma
- Liver (benign ***and*** malignant hepatocytes)
- Meningioma
- Lung adenocarcinoma



Very Granular Cytoplasm

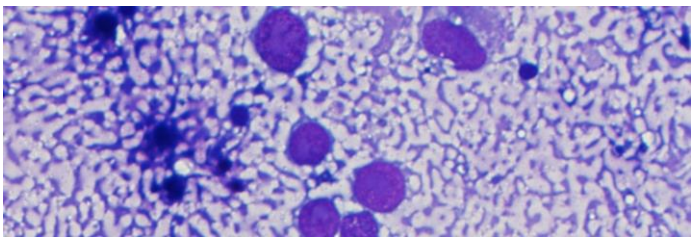
- Granular cell tumor (lysosomes)
- Acinar cell carcinoma (zymogens)
- Oncocytic/Hürthle cell neoplasms (mitochondria)
- Neuroendocrine tumors (neurosecretory granules)
- Hepatocytes/tumors
- Melanoma (melanosomes)
- Adrenal cortical/tumors
- Leydig cells/tumors



Tigroid Background

Seen with glycogen-rich lesions

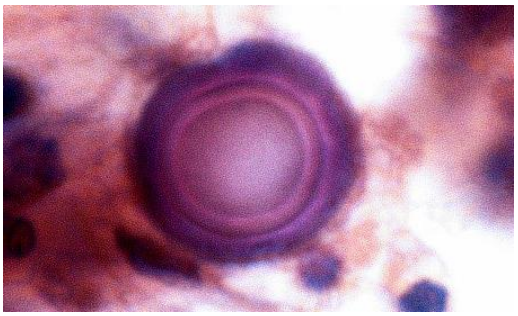
- Seminoma/Dysgerminoma (most classic!)
- Clear cell renal cell carcinoma
- Ewing sarcoma/PNET
- Other glycogen-rich tumors



Psammoma Bodies

Concentric calcifications.
Frequently seen in papillary tumors.

- Papillary thyroid carcinoma
- Serous ovarian tumors
- Mesothelioma
- Papillary renal cell carcinoma
- Meningioma
- Somatostatinoma (duodenum)
- Prolactinoma (pituitary)
- Lung micropapillary adenocarcinoma



If in doubt, you can actually press on these with a pen and radially crack them!

Based on: The Book of Cells: A Breviary of Cytopathology, by Dr. Richard Mac DeMay