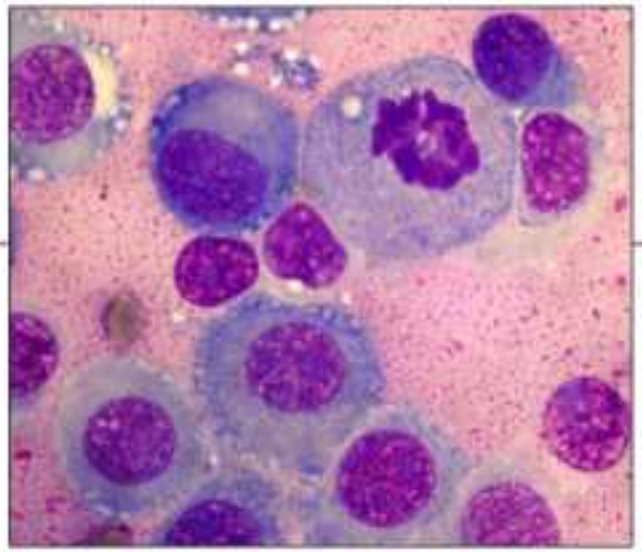
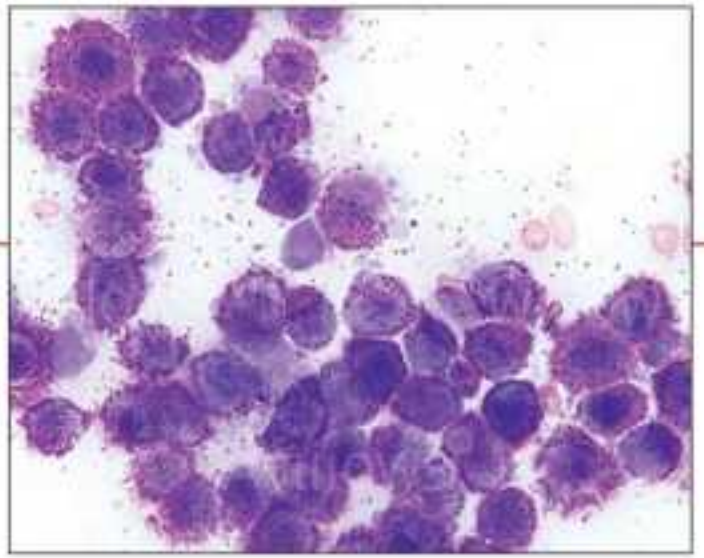
Diagnostic Cytology





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Lecture Outline

1) Introduction to cytology
2) Cytology sample collection and preparation
3) Cytologic interpretation
4) Case examples

Introduction to cytology

What is cytology / cytopathology?

- *The microscopic examination of cells* collected from a patient, in order to determine the underlying pathophysiological process.

The purpose of cytology:

- To provide a diagnostic method which help the clinician to determine if a lesion (solid tissue mass, body cavity fluid, or wash).

Introduction (cont.)

- It differs importantly from histopathology in that cytology examines only discrete cells or small groups of cells, but histopathology examines intact tissue.

Histopathology

- Deals with the form and the structure / architecture of the tissue.
- Evaluation usually begins with a tissue biopsy so it is more invasive and traumatic.

Cytology

- Deals with the changes within the nucleus and cytoplasm of cells. No architecture
- Fine needle with small gauge are usually preferred so it is less invasive and traumatic.

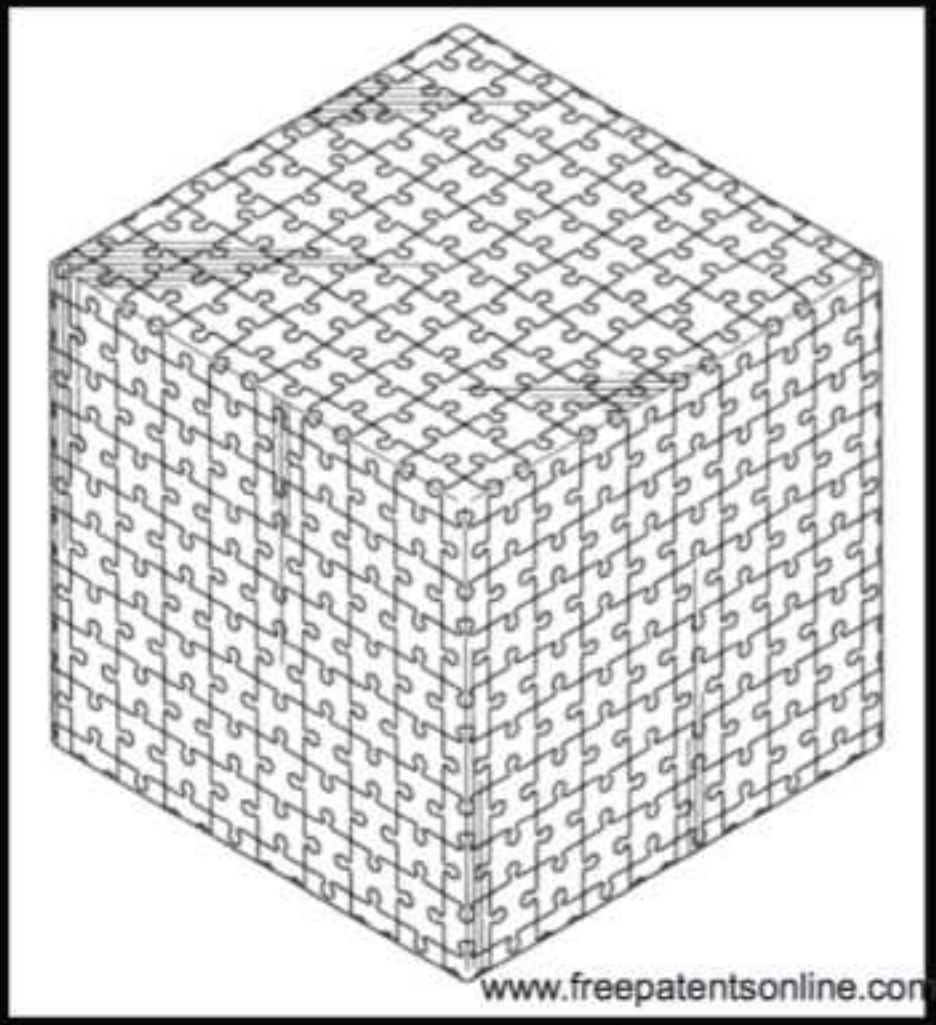
Histopathology

- Diagnosis obtained after days.
- Higher cost
- Basic stain is H&E

Cytology

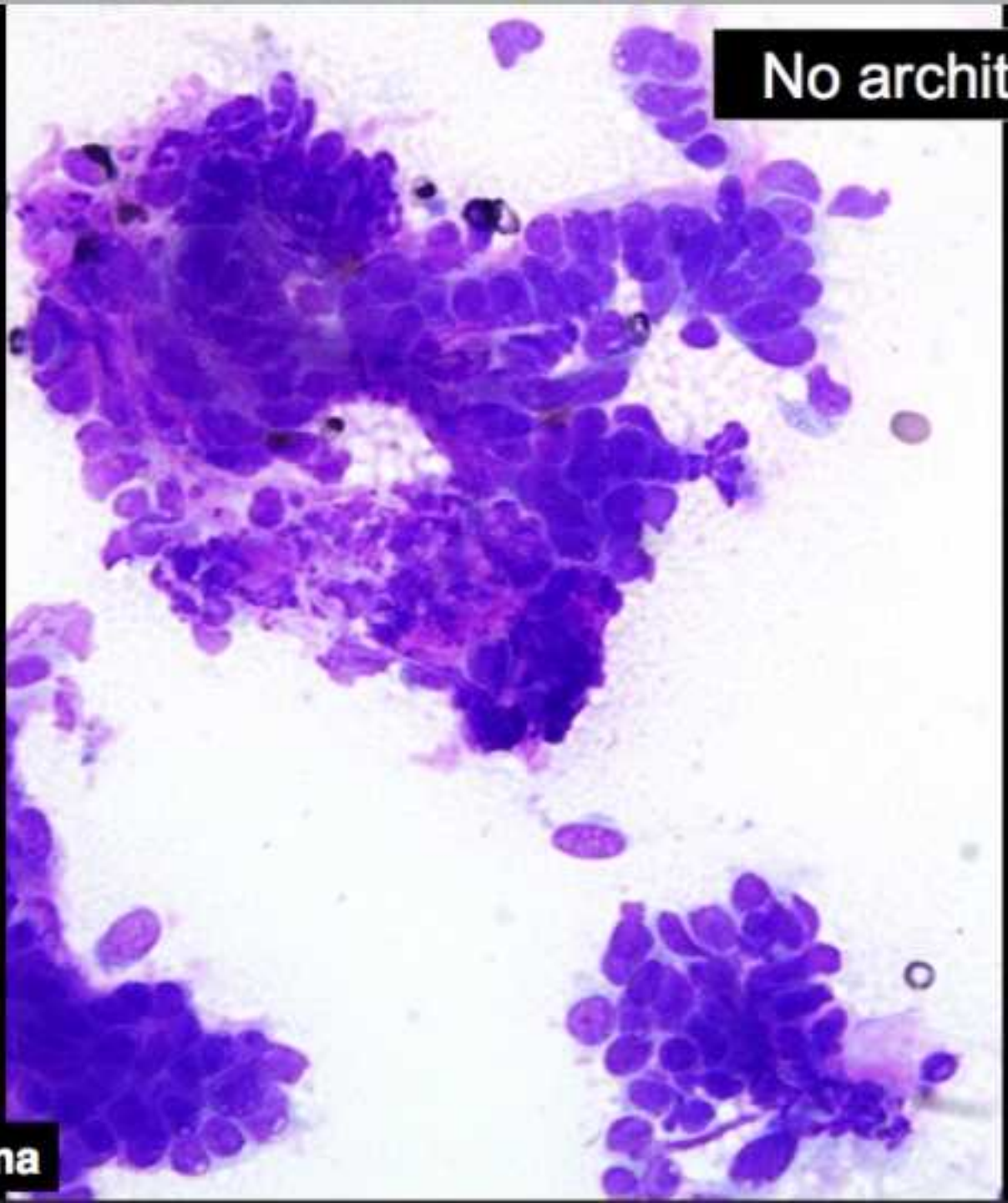
- Rapid diagnosis that could be obtained within minutes.
- Lower cost
- Basic stains are Diff-quick®, Papanicolaou, Giemsa

Architecture!



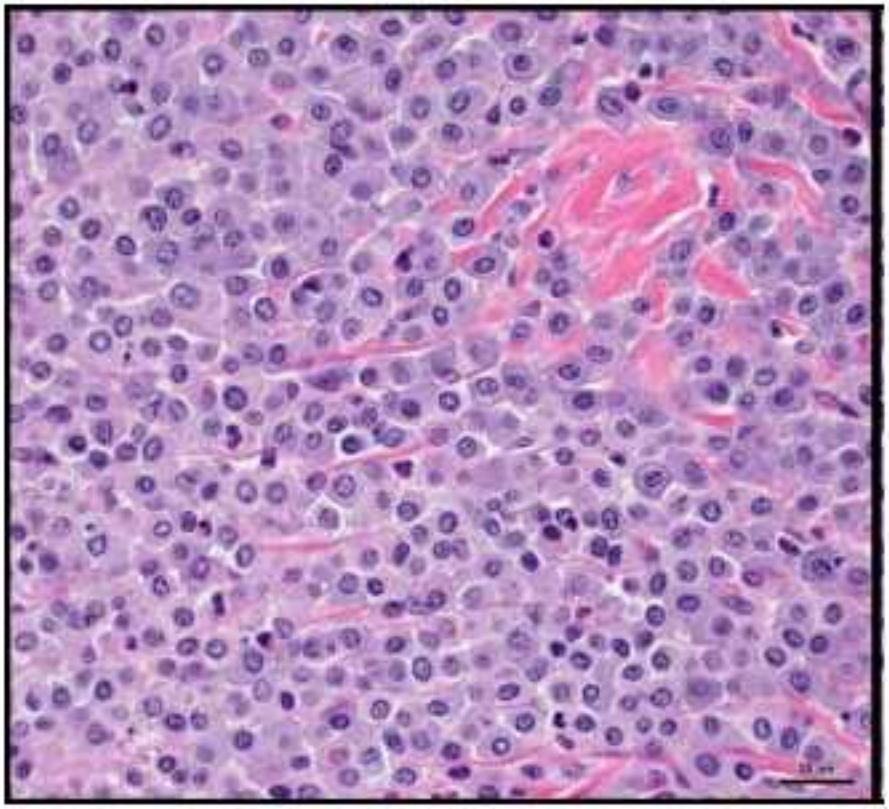
Architecture

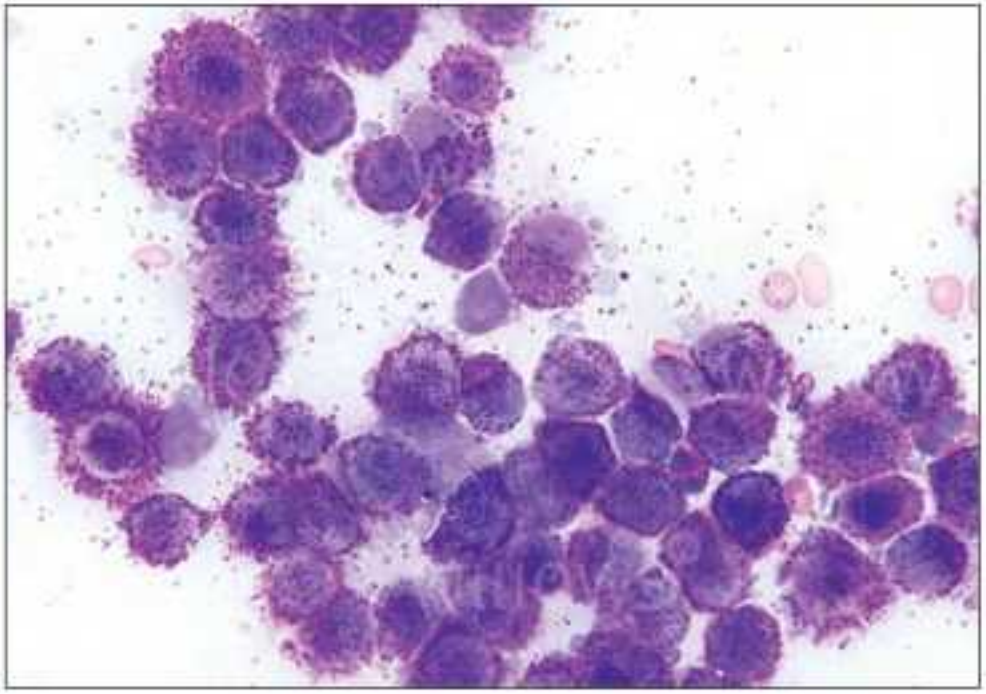
Trichoblastoma



Trichoblastoma

Mast cell tumor (Histopathology vs Cytology)





Advantages of Cytology

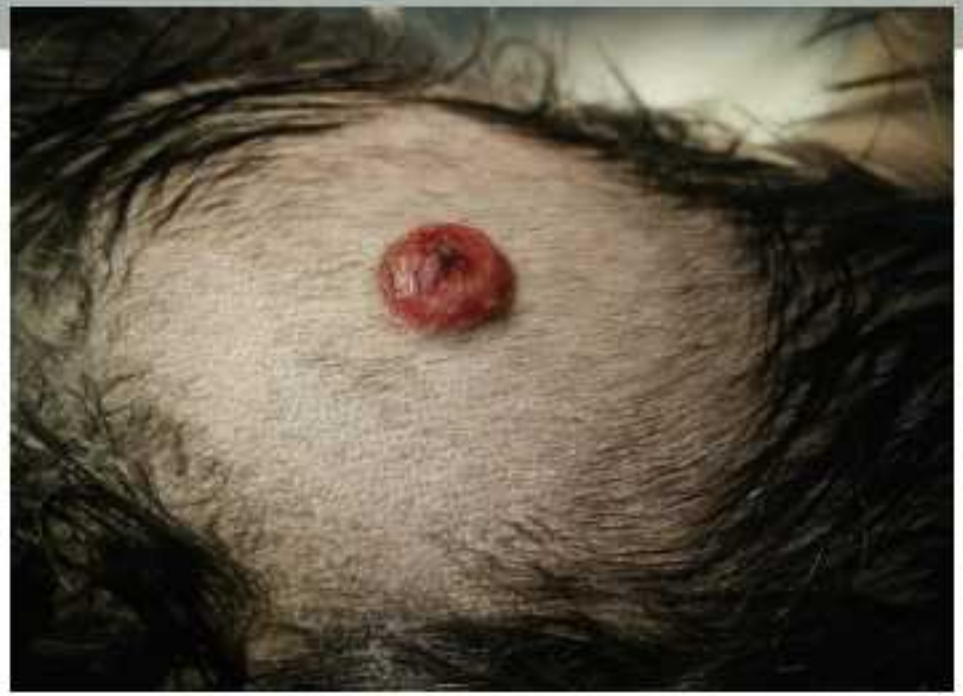
- Simple and rapid diagnosis***
- Inexpensive
- Ability to characterize the cellular components of various fluids.
- Minimally invasive diagnostic procedure.
- Anesthesia not necessary for skin lumps.
- Establish diagnosis and/or treatment plan.
- Basic interpretation can be performed in-clinic.

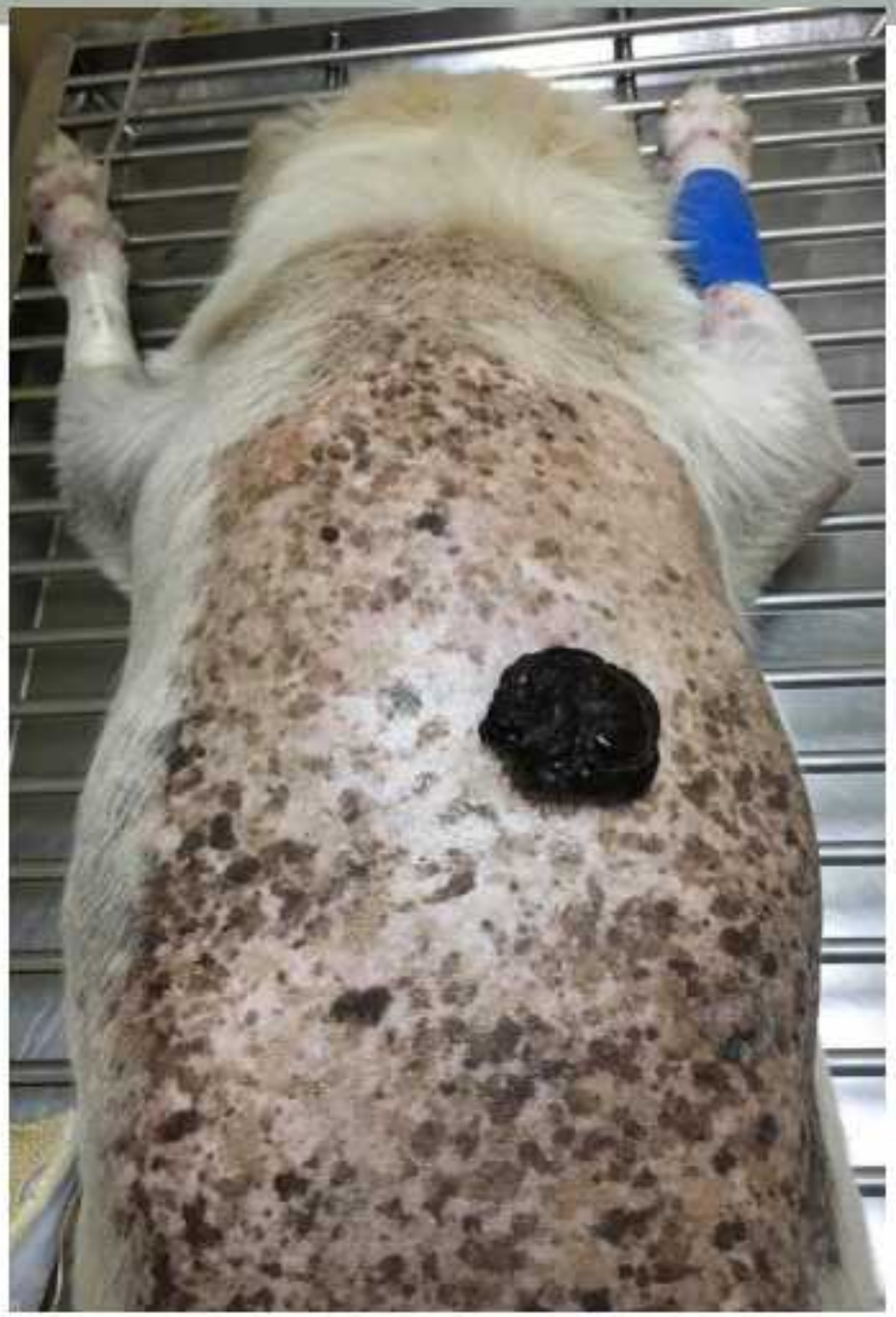
Disadvantages of Diagnostic cytology

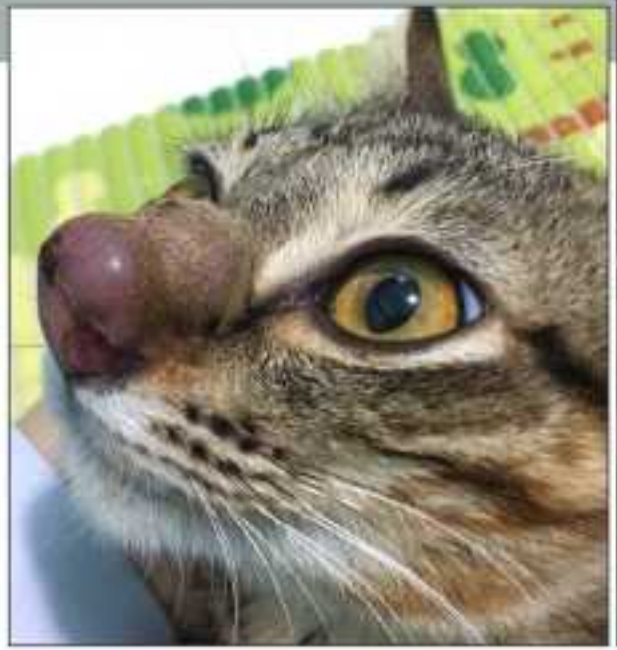
- Tumors cannot be graded.
- Margin cannot be evaluated.
- Limited information about structural arrangement (architecture) of the cells within the lesion.
- Less definitive diagnosis as to the specific tumor type or the distribution of an inflammatory infiltrate.
- Very bad negative test: the possibility that the specimen may not represent the primary lesion.

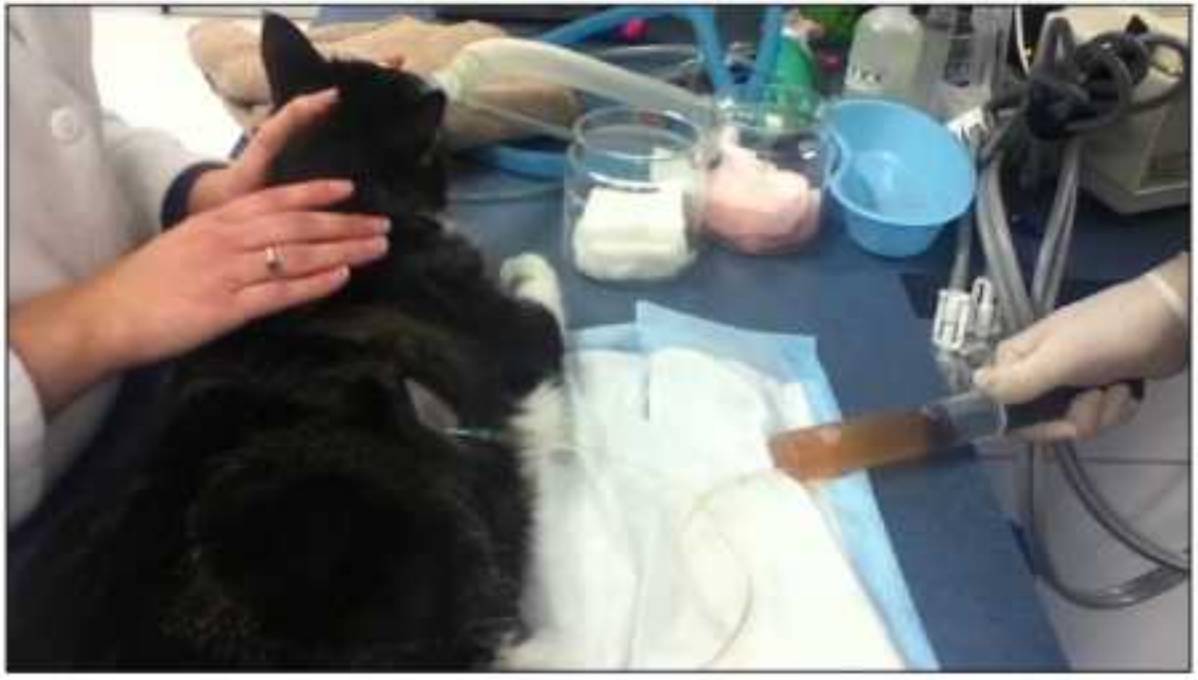
General indication for cytologic use

- Lumps and Bumps lesions
- Cutaneous / subcutaneous mass, ulcerative lesion
- Diffuse organomegaly
- Effusion: thoracic and abdomen
- Urine sediment, Bladder washing
- Respiratory diseases: nasal swab, tracheal wash
- Ocular diseases: conjunctiva
- Estrous detection
- Lymphadenopathies
- Neurological diseases: CSF analysis

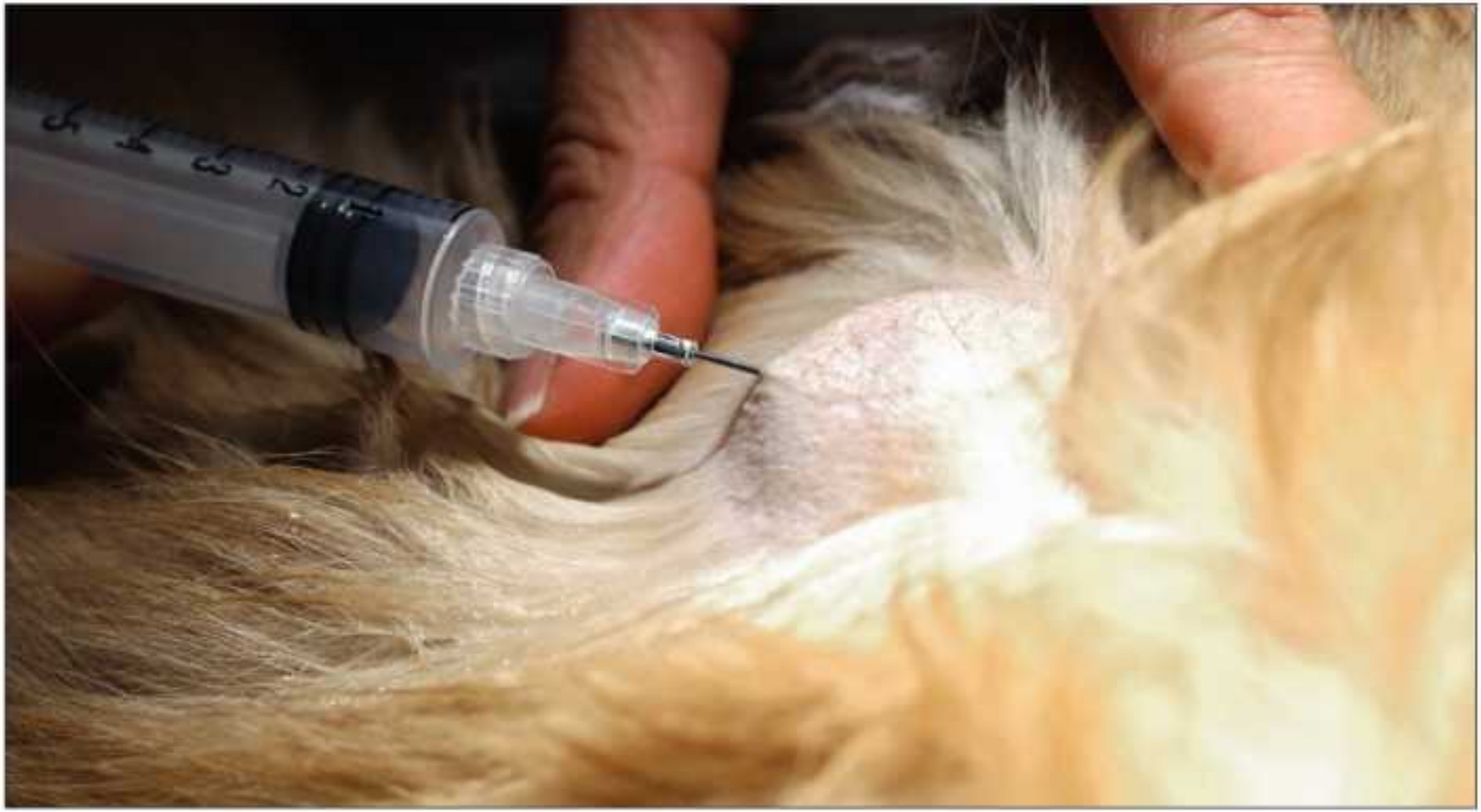








Sample Collection and Preparation



Source: Clinician's brief

Sample Collection Techniques

1. Fine-needle aspiration/biopsy (FNA)
2. Fine-needle capillary sampling
3. Swabbing
4. Scraping / Brushing
5. Impression smear (tissue imprint)
6. Other: body cavity fluid, urine, and wash.

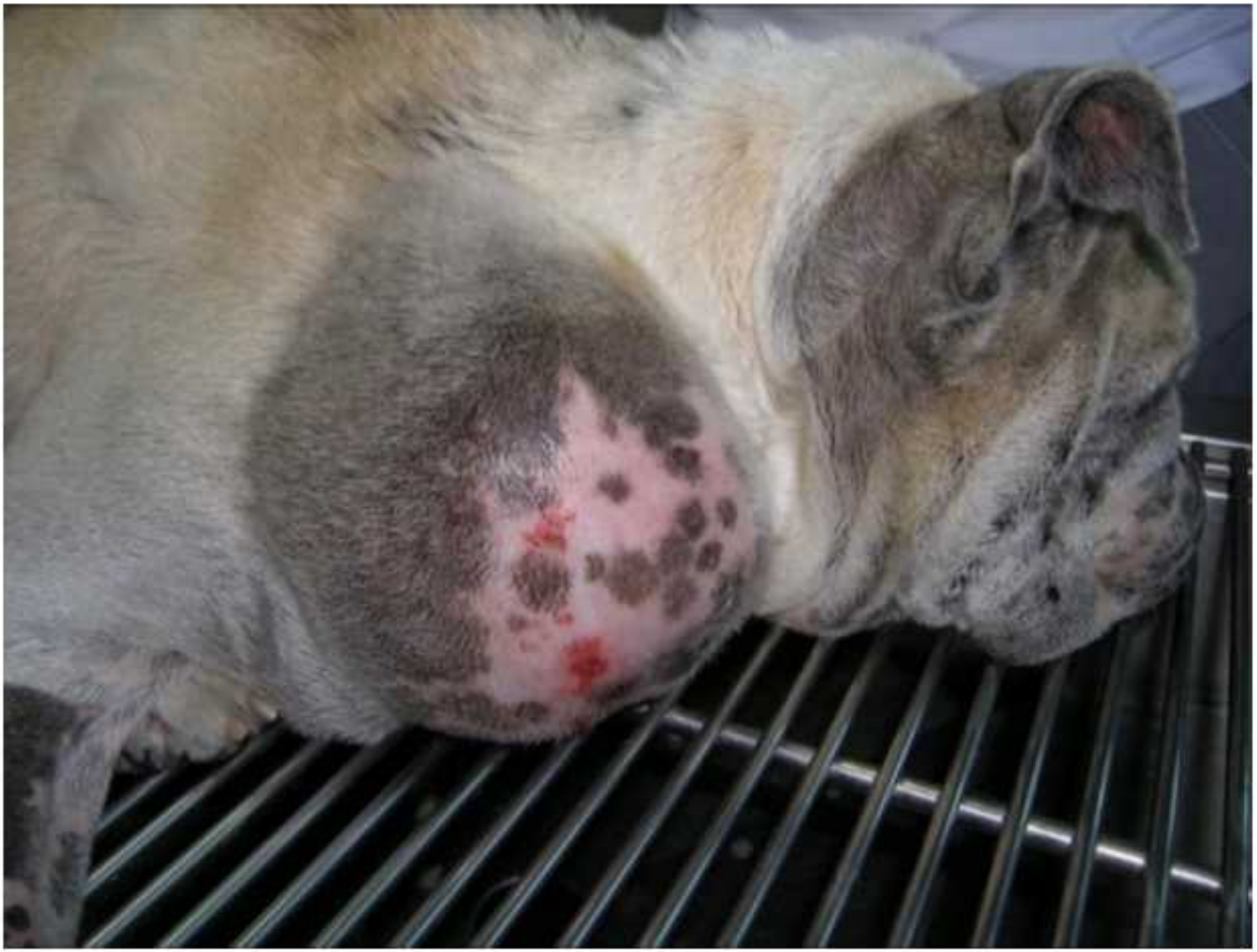
: The technique, used to collect cytological samples and prepare slides, varies, depending on the anatomical location and characteristic of the tissue.

Fine-Needle Aspiration

- Fine-needle biopsy may be performed with a needle attached to a syringe.
- FNA is preferred for tissue with normal or low blood vascularity as well as tissue containing fibrous stroma.
- Recommended technique for collect sample from solid tissue masses, which can be palpable.
- Non-palpable masses, need for ultrasound guided collection.

Fine-Needle Aspiration

- Very little risk to patient
  - Less complications to internal organs than core biopsy techniques.
- Disadvantage: May not get a good sample because using just a small needle.



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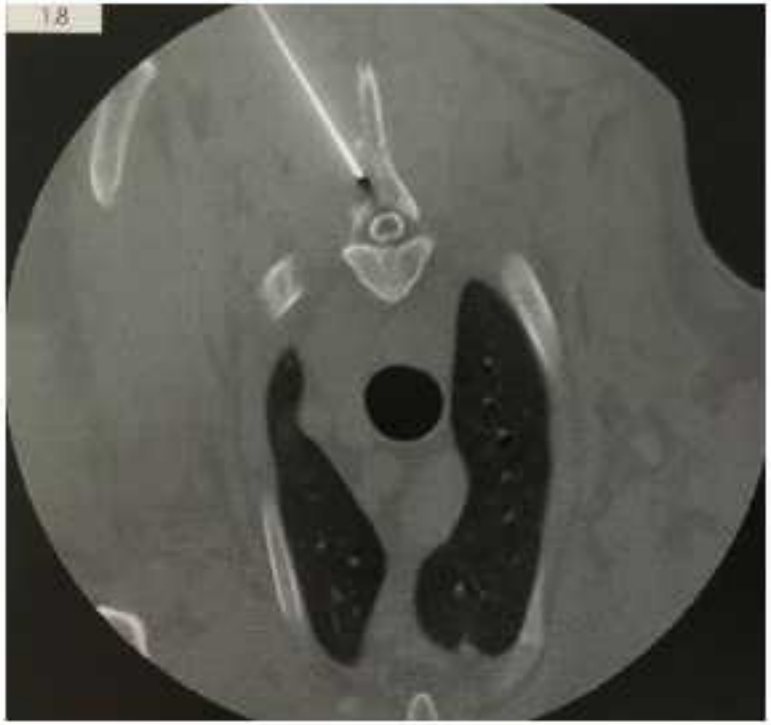


Materials: What you will need

- Gloves
- Cotton balls soaked in 70% isopropyl alcohol
- Syringes (6-12 mL)
- Needles (1-1.5 inches, 20-22 gauge)
- EDTA tube
- 2 to 10 clean glass microscope slides
- Hair dryer (optional)
- Writing utensil (eg, pencil, slide marker)

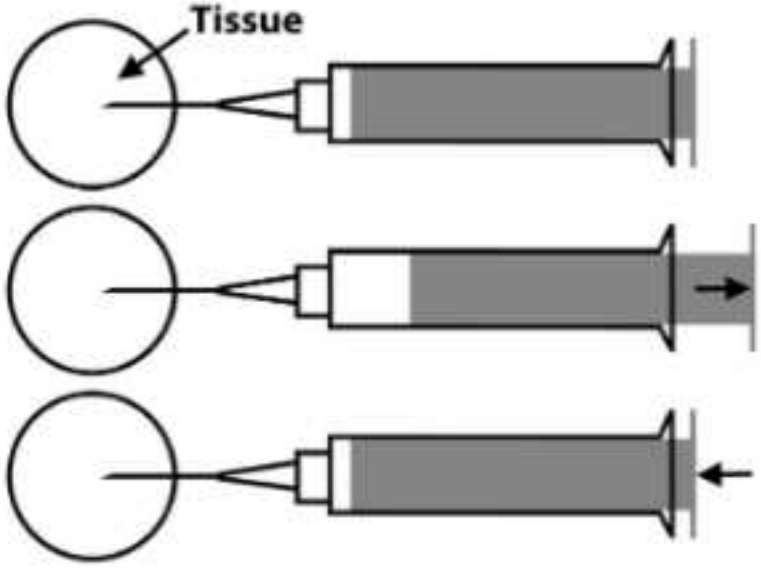
Required Materials

Gather the following materials for collecting cytologic samples: alcohol-soaked cotton balls, clippers, EDTA blood tubes, syringes 6-12 mL, 20- or 22-gauge, 1- to 1.5-inch needles, new glass slides, and slide marker.

Methods

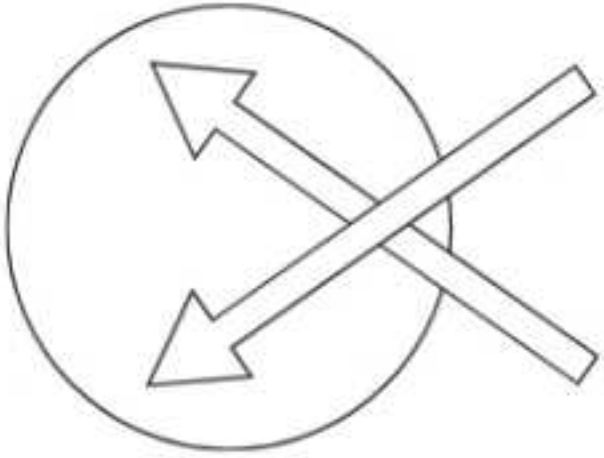
STEP 1 & 2

Stabilize the tissue of interest with one hand. With the other hand, insert the needle tip (bevel side up) into the tissue, then retract and release the syringe plunger. The amount of negative pressure may vary depending on the consistency of the tissue; softer tissues may require less pressure, whereas firmer tissues may require three-fourths of the syringe volume. To avoid sample hemodilution, apply negative pressure for no more than a few seconds in any given area.



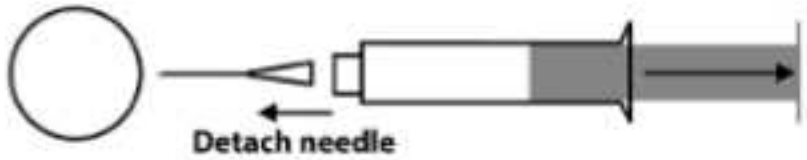
STEP 3

Without completely removing the needle from the tissue of interest, retract and redirect the needle tip into a different area. Retract and release the syringe plunger as described in ***Step 2***. Repeat as many as 4 times, depending on lesion size.



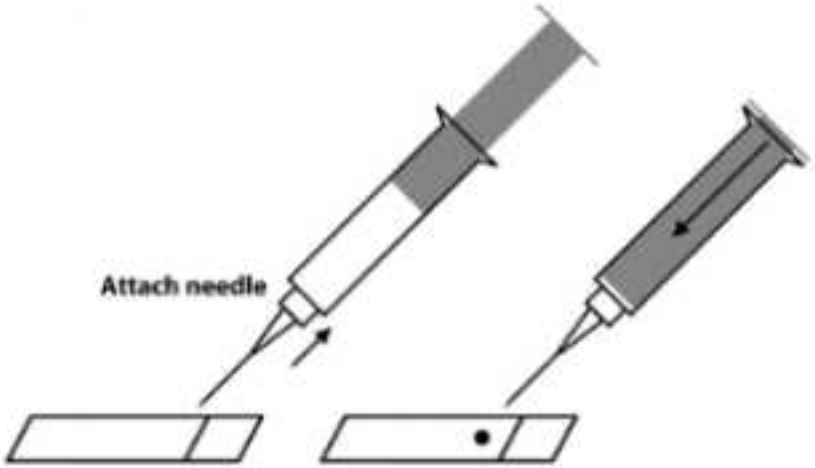
STEP 4

Completely remove the needle from the tissue. The sampled tissue should be within the needle shaft and hub. Detach the needle from the syringe, then retract the plunger to fill the syringe with air.



STEP 5

Reattach the needle to the syringe. Direct the needle tip (bevel side down) onto the surface of a glass slide, and force a small amount of the aspirated tissue onto the glass surface ≈0.5 inches from the frosted edge by quickly and firmly compressing the plunger. Repeat with additional glass slides as necessary.



Source: Clinician brief

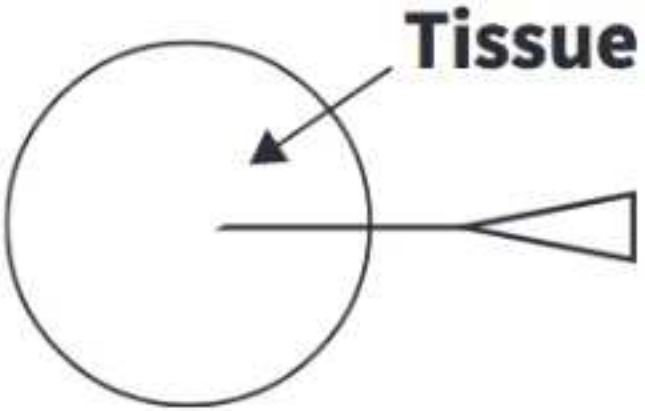
Fine-Needle Capillary Sampling

- Non-aspiration technique
- Used to reduce blood contamination when the lesion is suspected to be highly vascularized.
- eg, Thyroid gland, Hemangiosarcoma.
- or when the aspiration pressure results in ruptured cells (eg, cells of the thyroid gland and some lymphomas).
- Materials same as FNA.

Methods

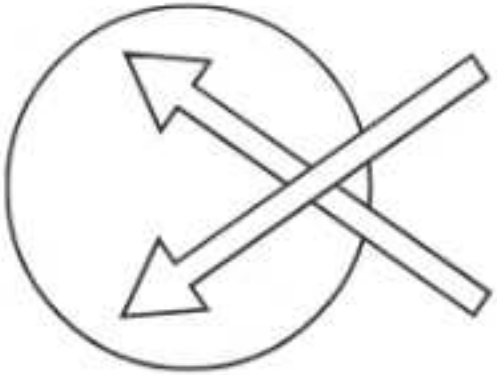
STEP 1

Stabilize the tissue of interest with one hand. With the other hand, insert the needle tip (bevel side up) into the tissue of interest.



STEP 2

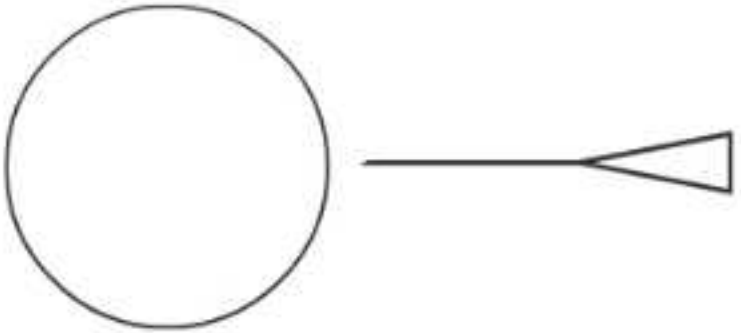
Without completely removing the needle from the tissue of interest, retract and redirect the needle tip into a different area. Repeat as many as 4 times, depending on lesion size.



Source: Clinician brief

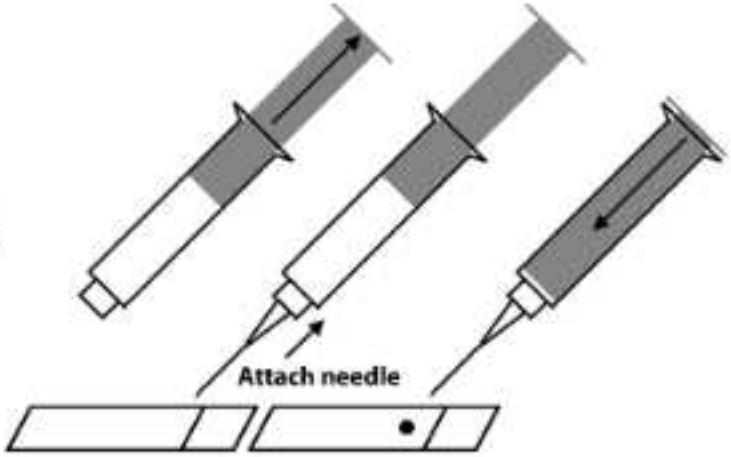
STEP 3

Completely remove the needle from the tissue. The sampled tissue should be within the needle shaft and hub.



STEP 4

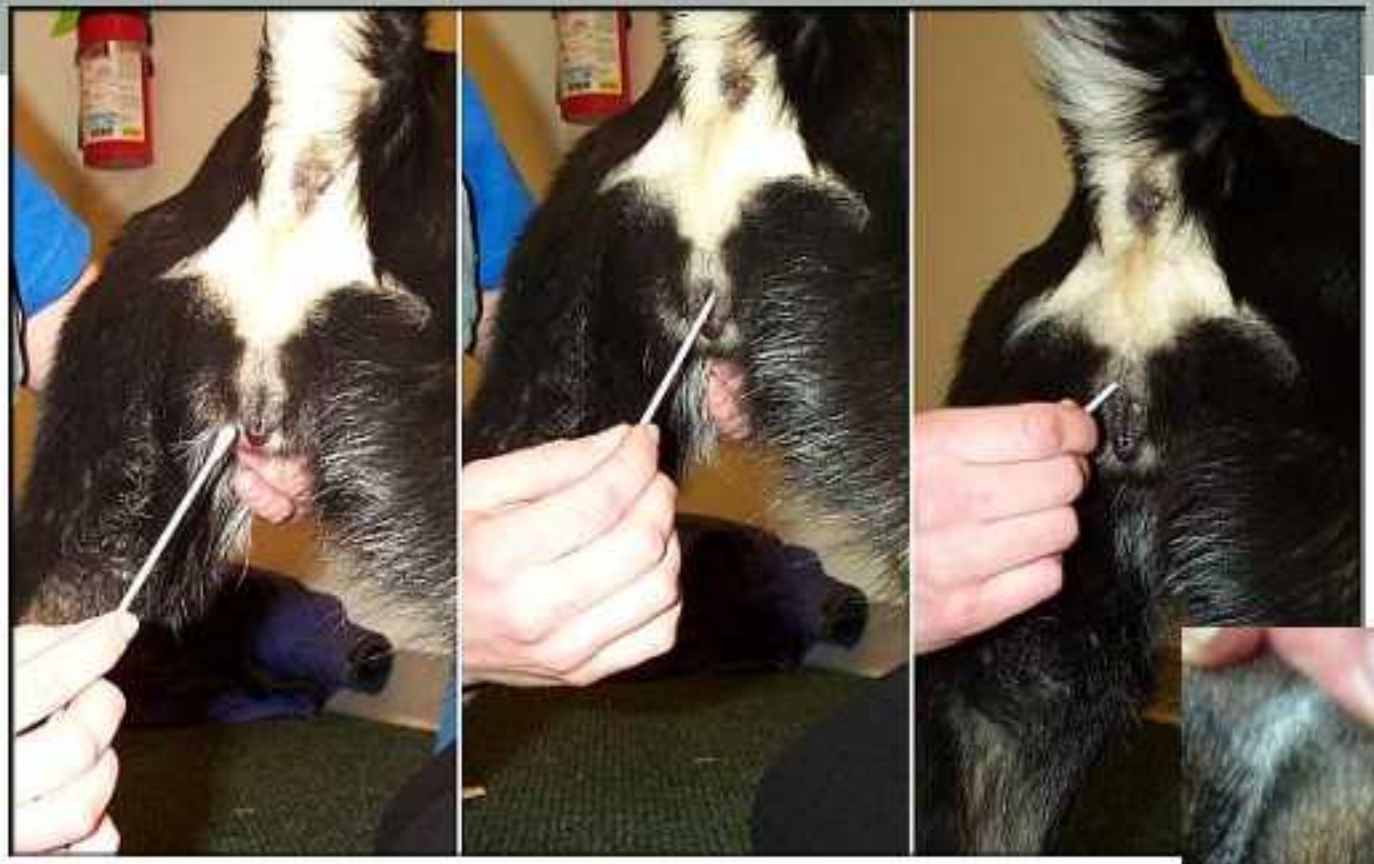
Retract the syringe plunger to fill the syringe with air, then attach the needle and syringe. Direct the needle tip (bevel side down) onto the surface of a glass slide, and force a small amount of the aspirated tissue onto the glass surface ≈0.5 inches from the frosted edge by quickly and firmly compressing the plunger. Repeat with additional glass slides as necessary.

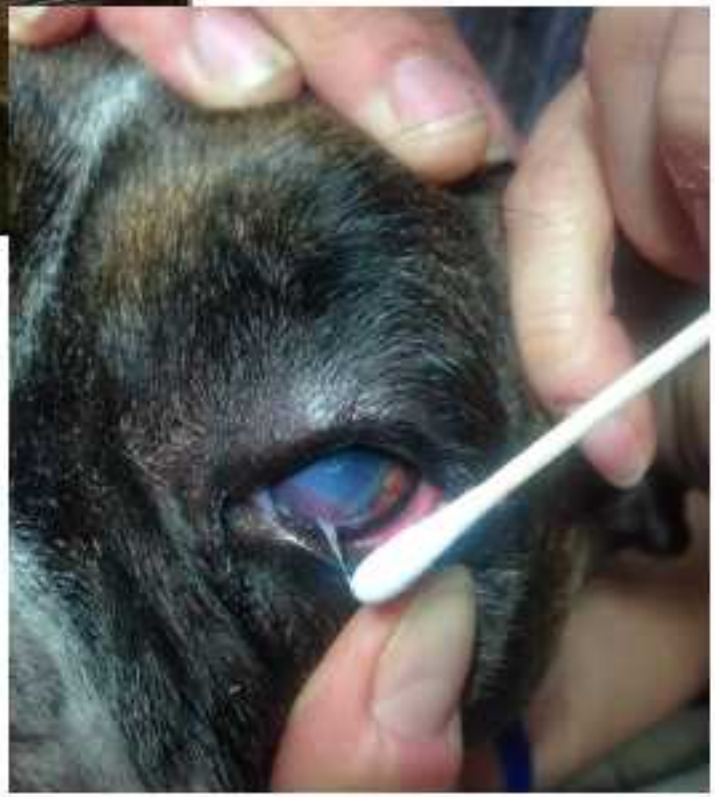


Source: Clinician brief

Swabbing

- A swab or cotton bud is used to collect samples from fistulous tracts, ears, conjunctiva and the vagina.
- Recommened for exfoliative cells, round cell tumor of mucosa, inflammatory cells.
- The swab must first be moistened in isotonic saline.
- The swab is rubbed against the surface of the lesion and then gently rolled along a slide.





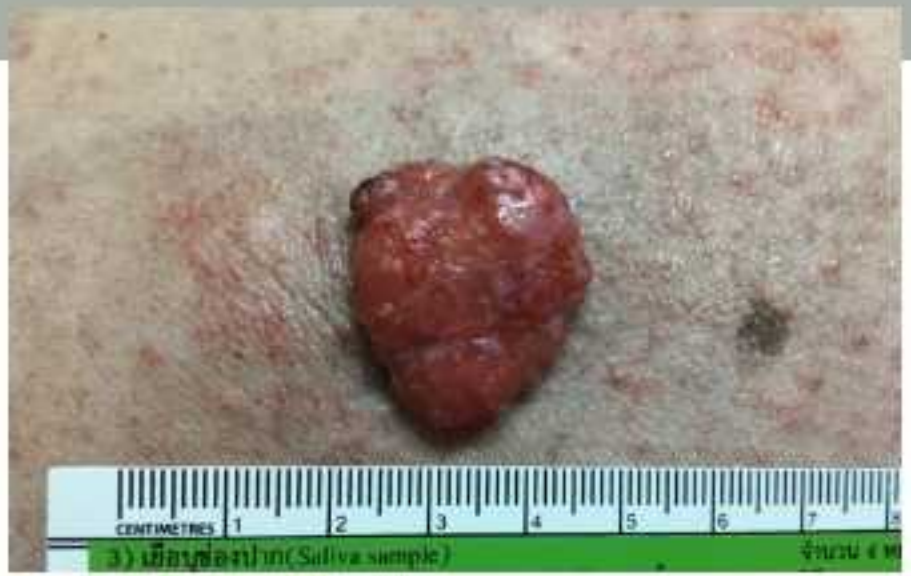
Scraping and Brushing

- Collection sample from only external lesion (skin lesion, oronasal cavity, and conjunctiva.
- Obtaining material from ulcerated cutaneous masses.
- Advantage:
  - Can collect many cells from firm lesions and yield few cells.
- Disadvantage:
  - Collected only superficial sample.
  - Often reflect a secondary infection (scraping may harvest only the superficial inflammatory cells and not the underlying neoplastic cells.)

Source: Clinician brief

Impression Smears

- Recommended for:
  - Exfoliative cells of mucosa
  - Cut surfaces from excised lesions (surgery / postmortem).
- Ulcerated mass should be imprinted after cleaning off necrotic debris.
- Blood and tissue fluid should first be removed from the surface of clean lesion.
- *Advantage*: easy to collection and minimal restrain.
- *Disadvantage*: collect fewer cells and greater contamination.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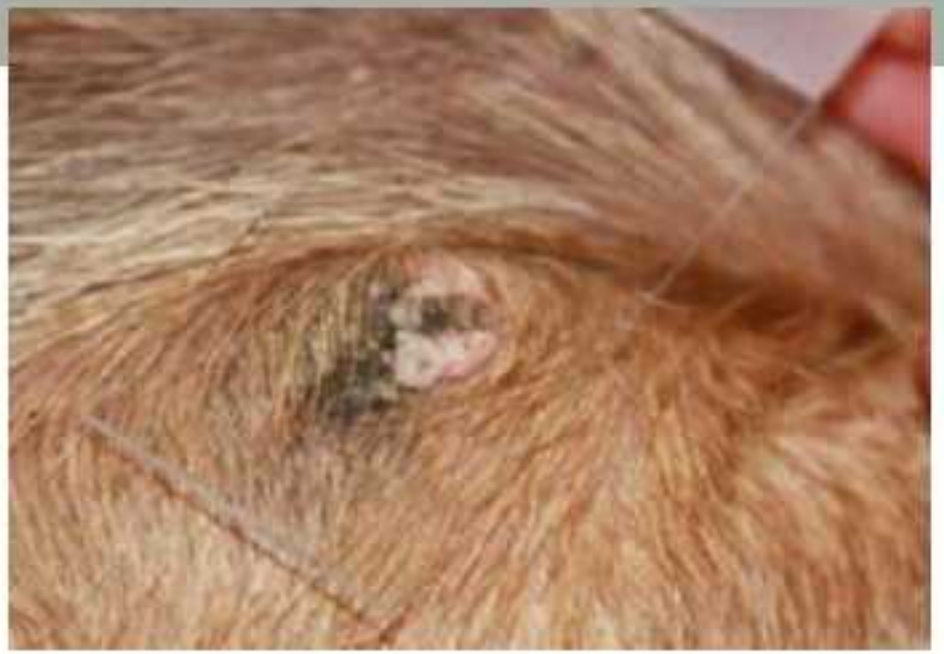
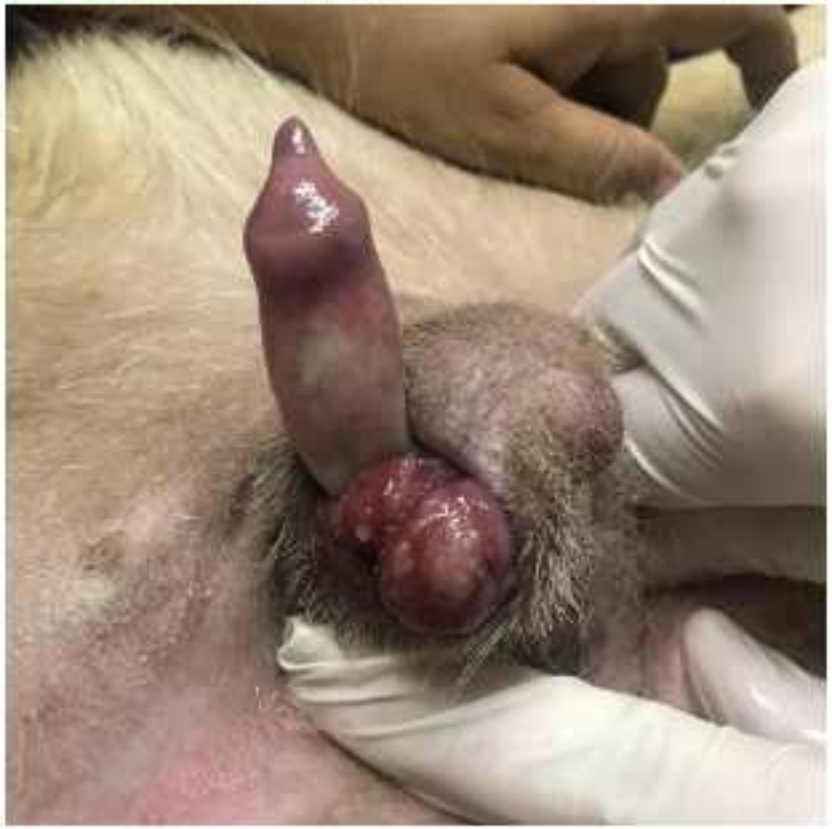
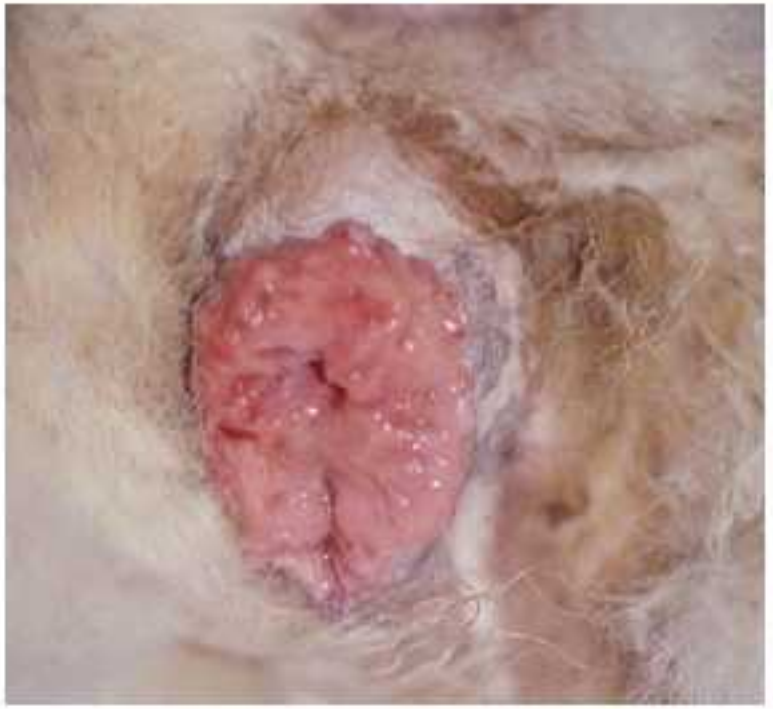






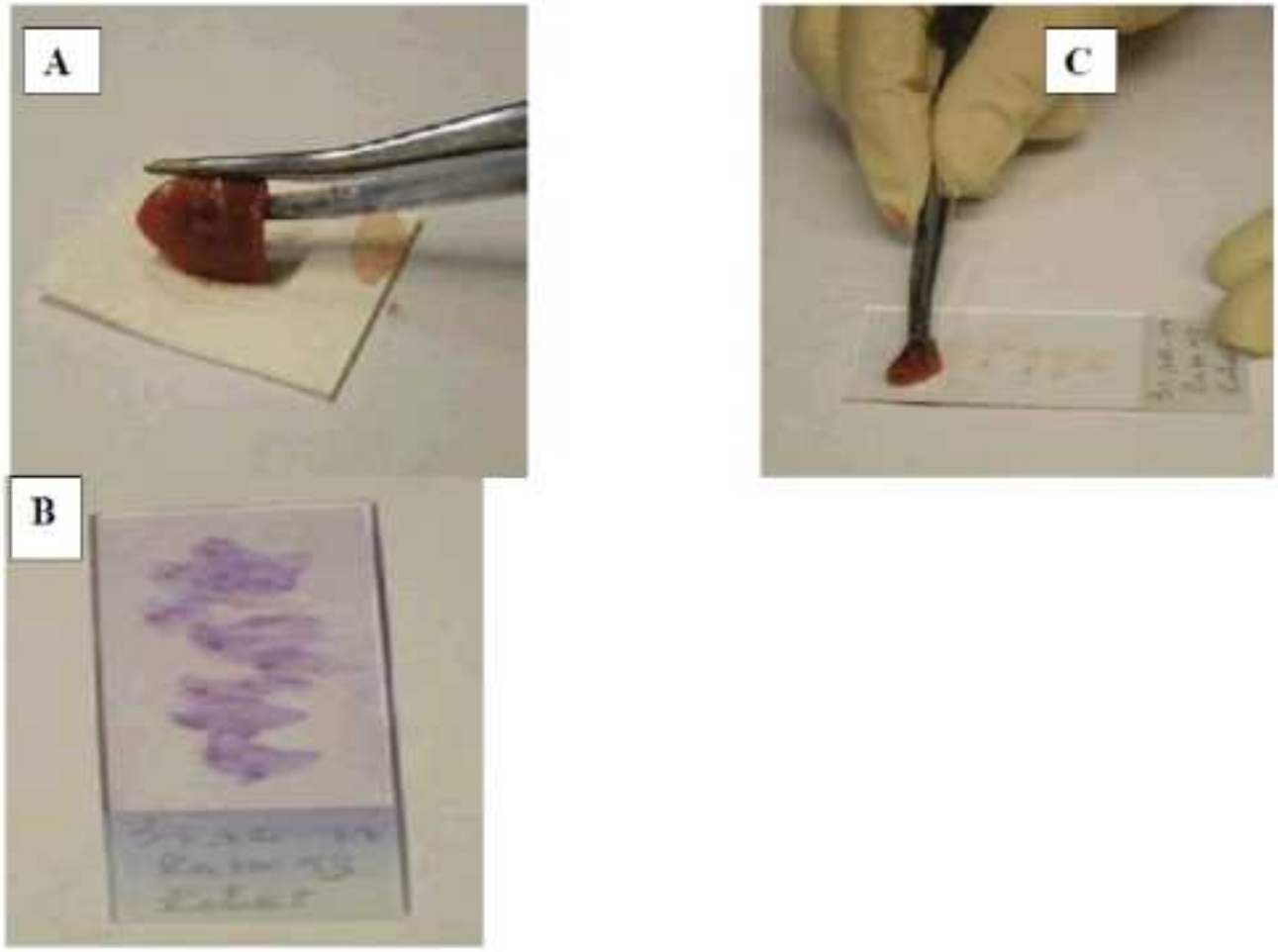


Figure 3: Impression smear of a liver biopsy. (A) Blotting gently on a filter paper. (B and C) The imprint smear is prepared by touching the slide with the surface of the biopsy in several areas [21].

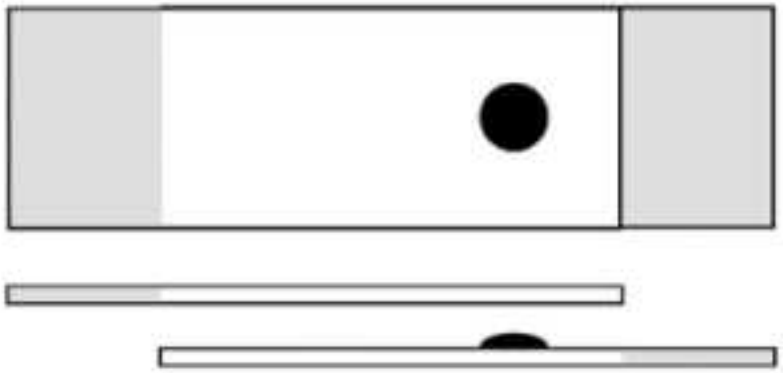
Smear preparation techniques

- Compression (squash) preparation
- Direct smear preparation
- Modified squash preparation
- Needles spread or "starfish" preparation

Compression (squash) technique

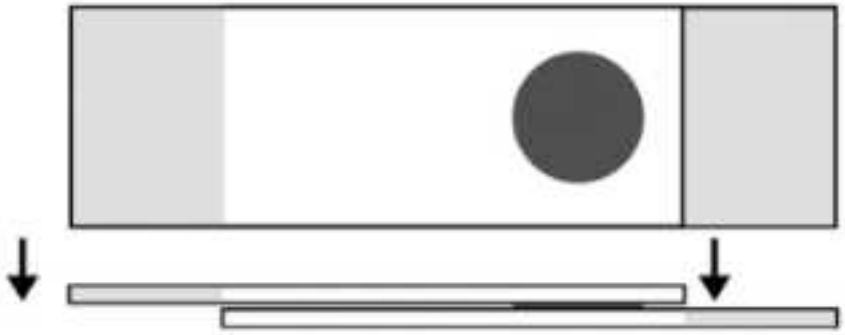
STEP 1

Using the thumb and forefinger, firmly grasp the frosted edge of the slide with the sample facing upward. Using the opposite hand, hold a second clean glass slide above the sample.



STEP 2

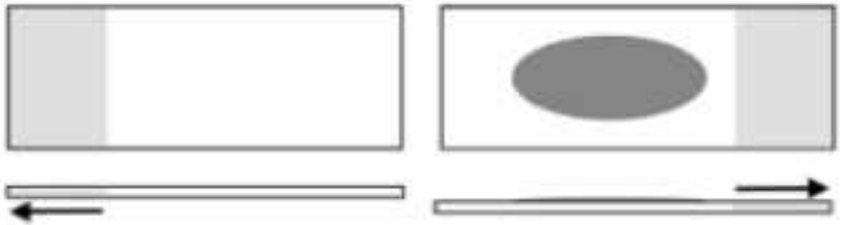
Gently lower the top glass slide onto the slide with the sample, allowing the weight of the top glass slide to disperse the sample. If the sample is excessively thick and does not allow the top slide to lay flat, apply gentle compression until the sample disperses.



Source: Clinician brief

STEP 3

Pull the top and bottom slides apart in opposite directions while keeping the surfaces of the slides in contact and parallel. Most samples can be spread with just the weight of the slides. Thicker preparations may require gentle compression while the slides are pulled apart.



STEP 4

Allow the slides to air dry, or use a hair dryer on a cool setting to speed the drying process. Label the slides with patient and tissue identifying information.

Source: Clinician brief

Direct smear preparation

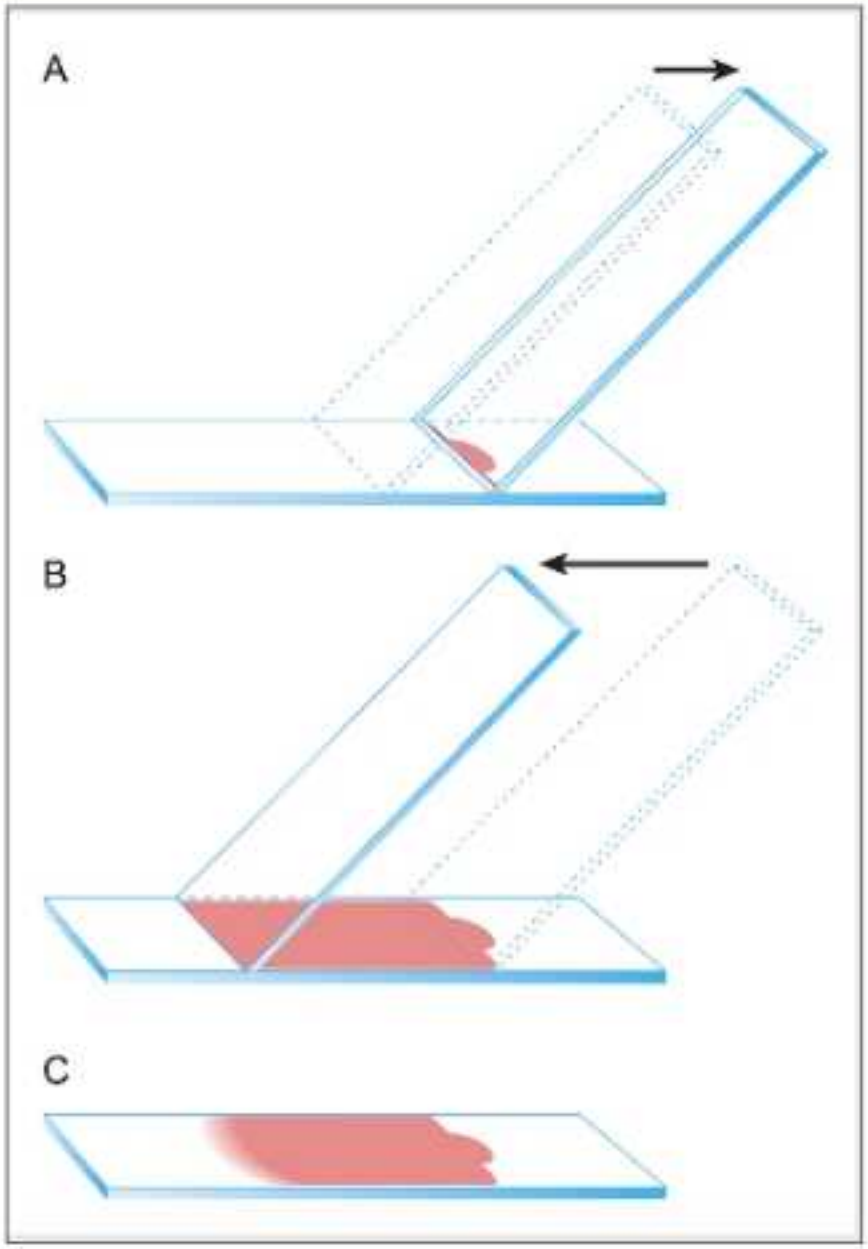
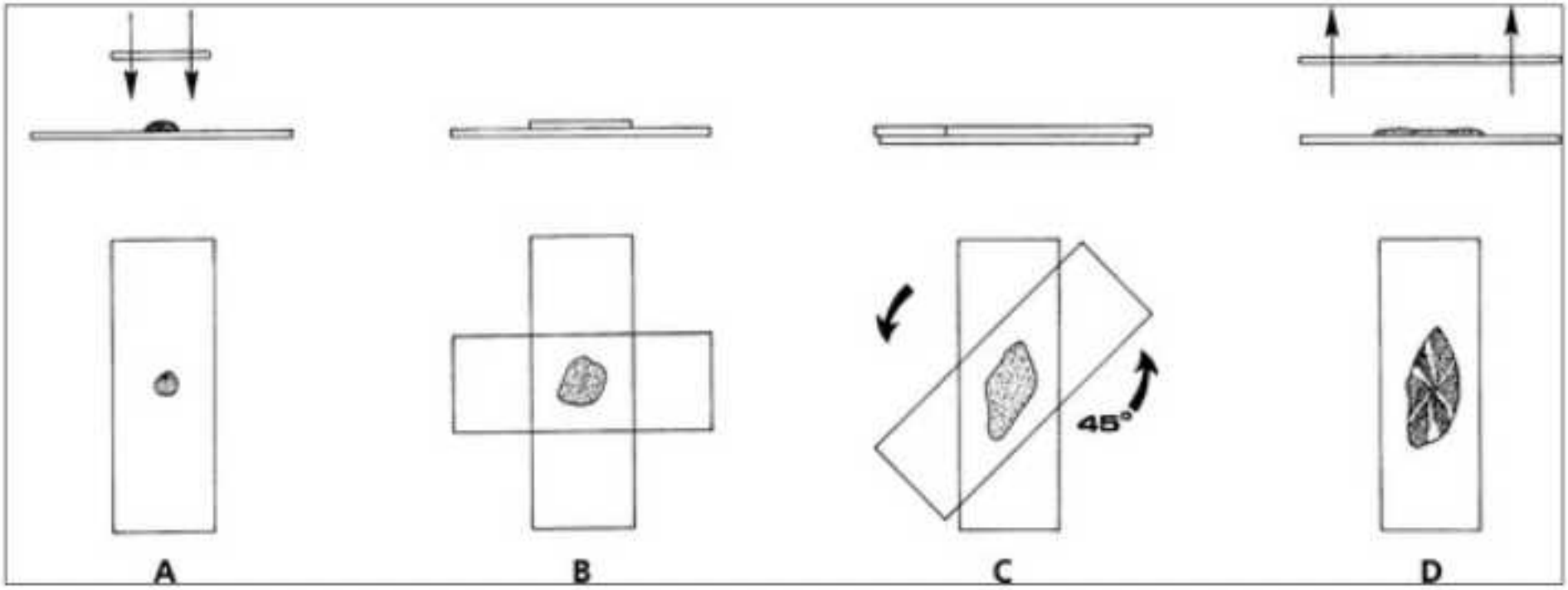


Illustration of the direct smear technique. (A) Apply a small drop of fluid 1 cm from the frosted end of the slide. Gently slide back a second spreader slide toward the fluid drop so that the fluid spreads along the spreader slide edge. (B) Gently advance the spreader slide smoothly and quickly at an approximately 45-degree angle along the slide to create a smear. (C) An appropriately prepared fluid cytology sample will appear similar to this image with a visible feathered edge. Be sure not to run the sample off the end of the slide.

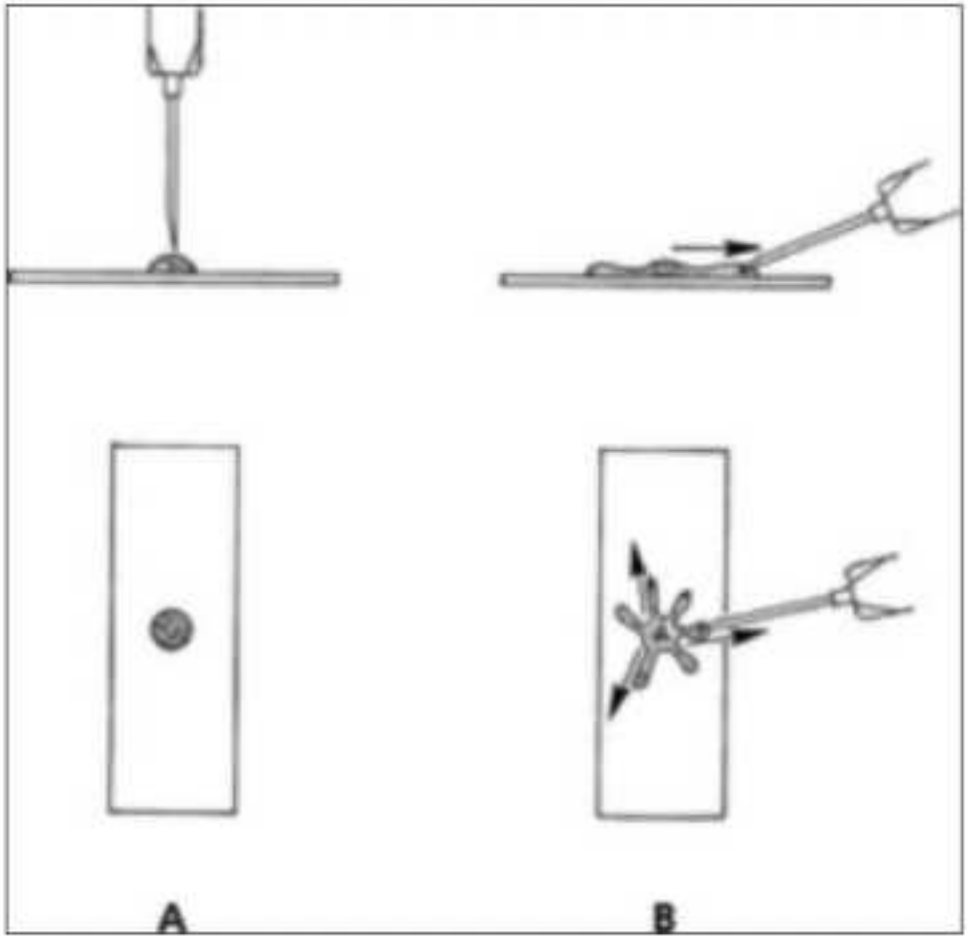
Source: Clinician brief

Modified squash preparation



Modification of squash preparation. A, Expel portion of aspirate onto glass microscope slide and place another slide over sample. B, This spreads sample. Take care not to place excessive pressure on slide, causing cells to rupture. C, Rotate top slide about 45 degrees and lift directly upward, producing spread preparation with subtle ridges and valleys of cell (D).

Needle spread (starfish) preparation



Starfish preparation: This technique for spreading aspirate is to drag the aspirate peripherally in several directions with the point of syringe needle, producing a starfish shape.

Slides fixation and staining

- Types of fixative:

1. ***Dry fixation:***
   - The slide is dry by air quickly after the material is spread on the slide.
   - Followed by hematological stains like Wright-Giemsa or Diff-Quick.
2. ***Wet fixation:***
   - Ethanol 95% is the best wet fixative
   - Wet fixation with subsequent Air Drying: used for transfer slides to the laboratory.

Slides fixation and staining

- Staining slides

1. *Diff-Quick*:
   - Great cytoplasmic detail.
   - Mast cell granules may not stain well.
   - Frequent use → contamination
2. *Wright stain*:
   - Blood smears
3. *Papanicolau stains:*
   - Used in human Ob'gyn exams, stain nucleus and nuclear material better.

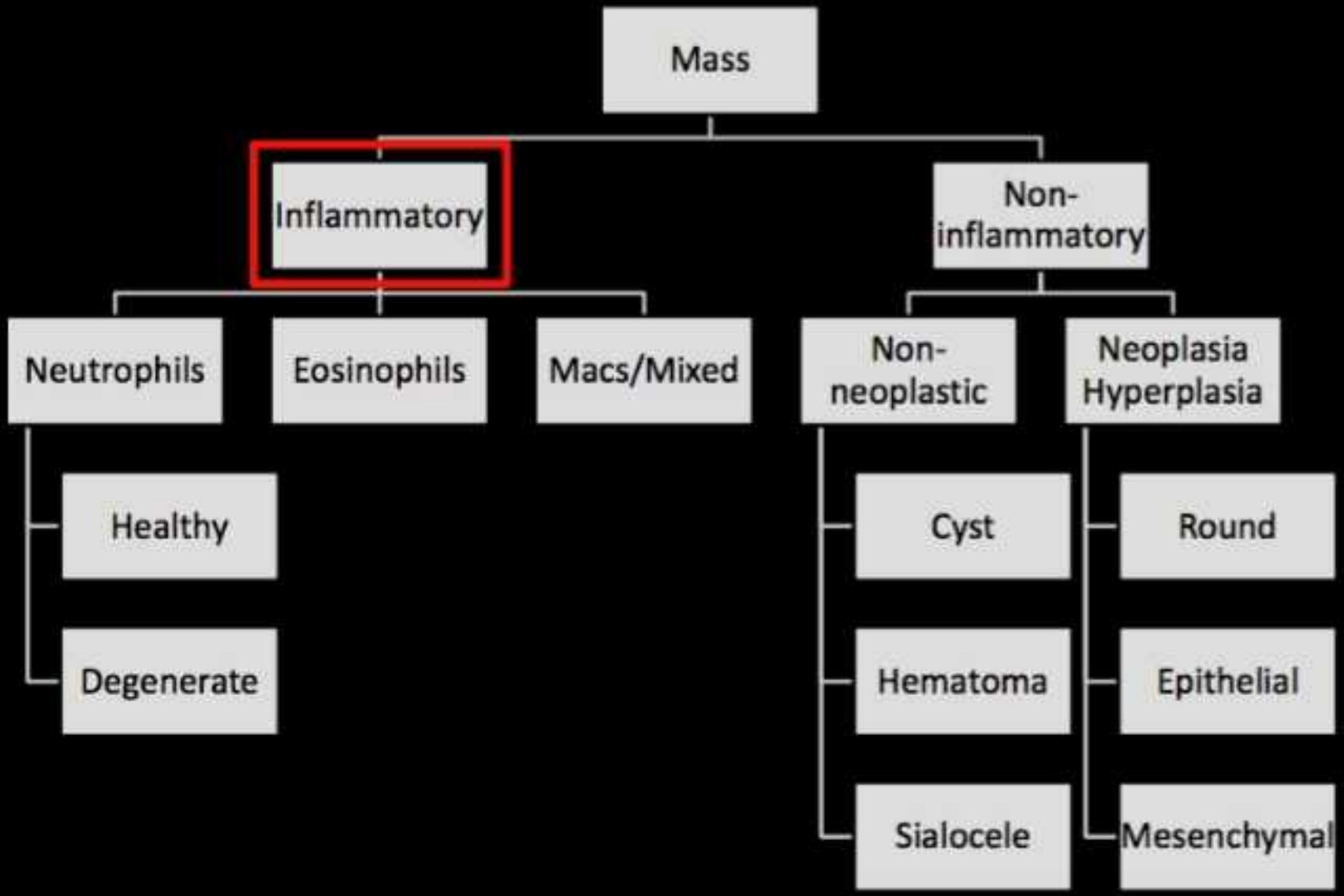
The requirement for a good cytological preparation

- Adequate cellularity
- Monolayer
- Intact cells
- Minimal blood contamination
- Submit several smears
- The sample is representative of the lesion

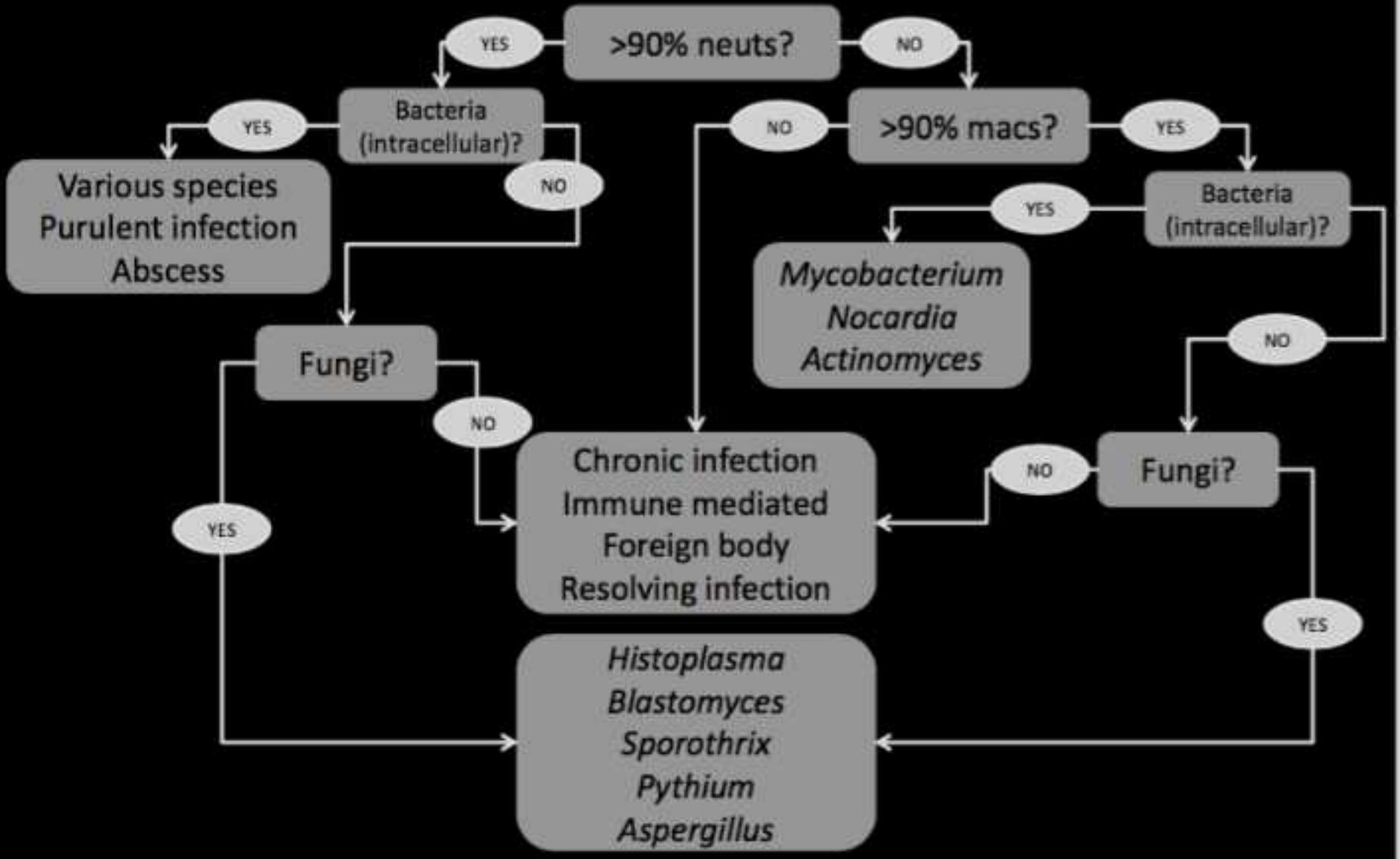
PRINCIPLES OF CYTOLOGICAL INTERPRETATION

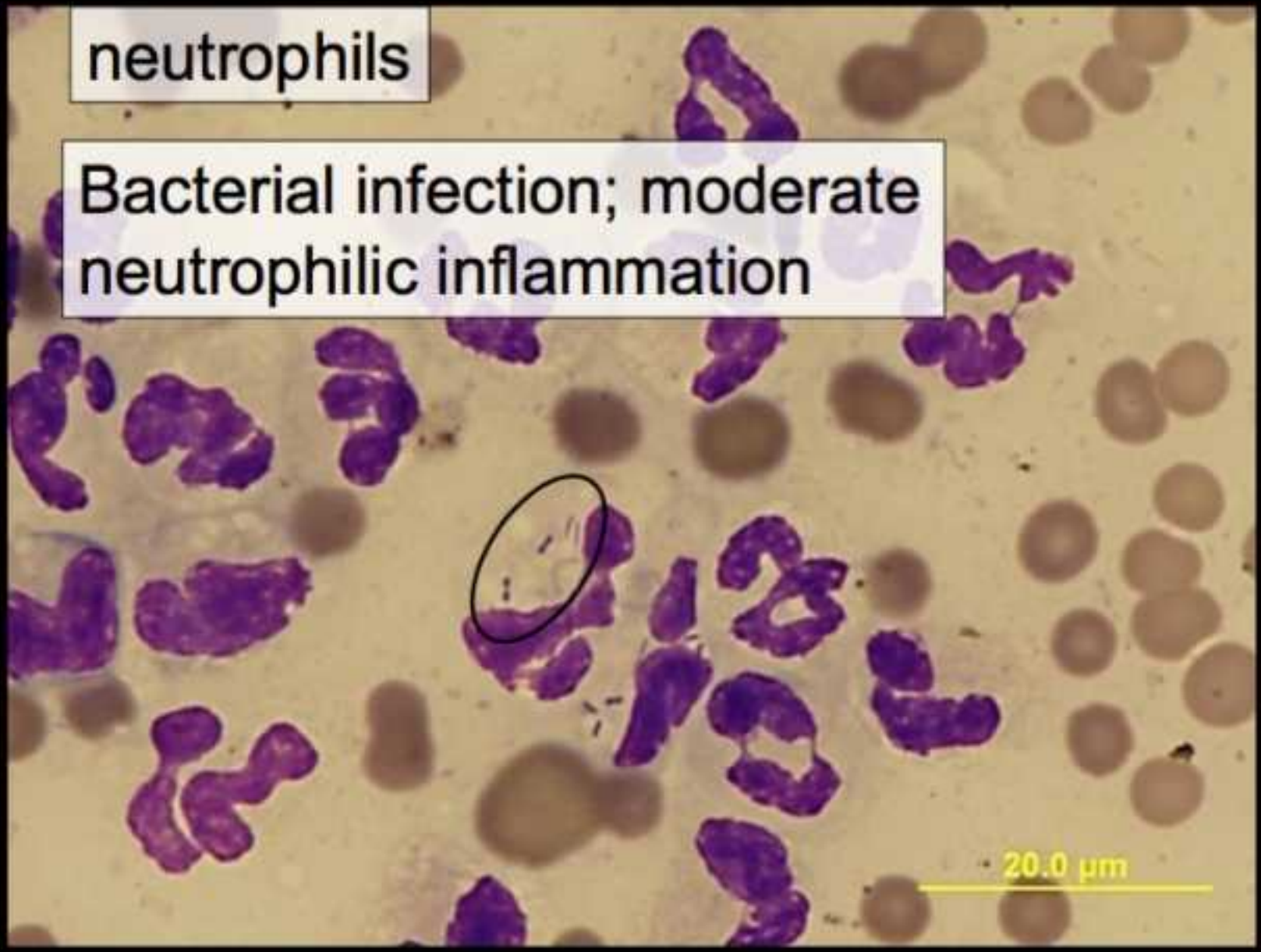
Source: Clinician brief

Interpretation: Diagnostic Tree



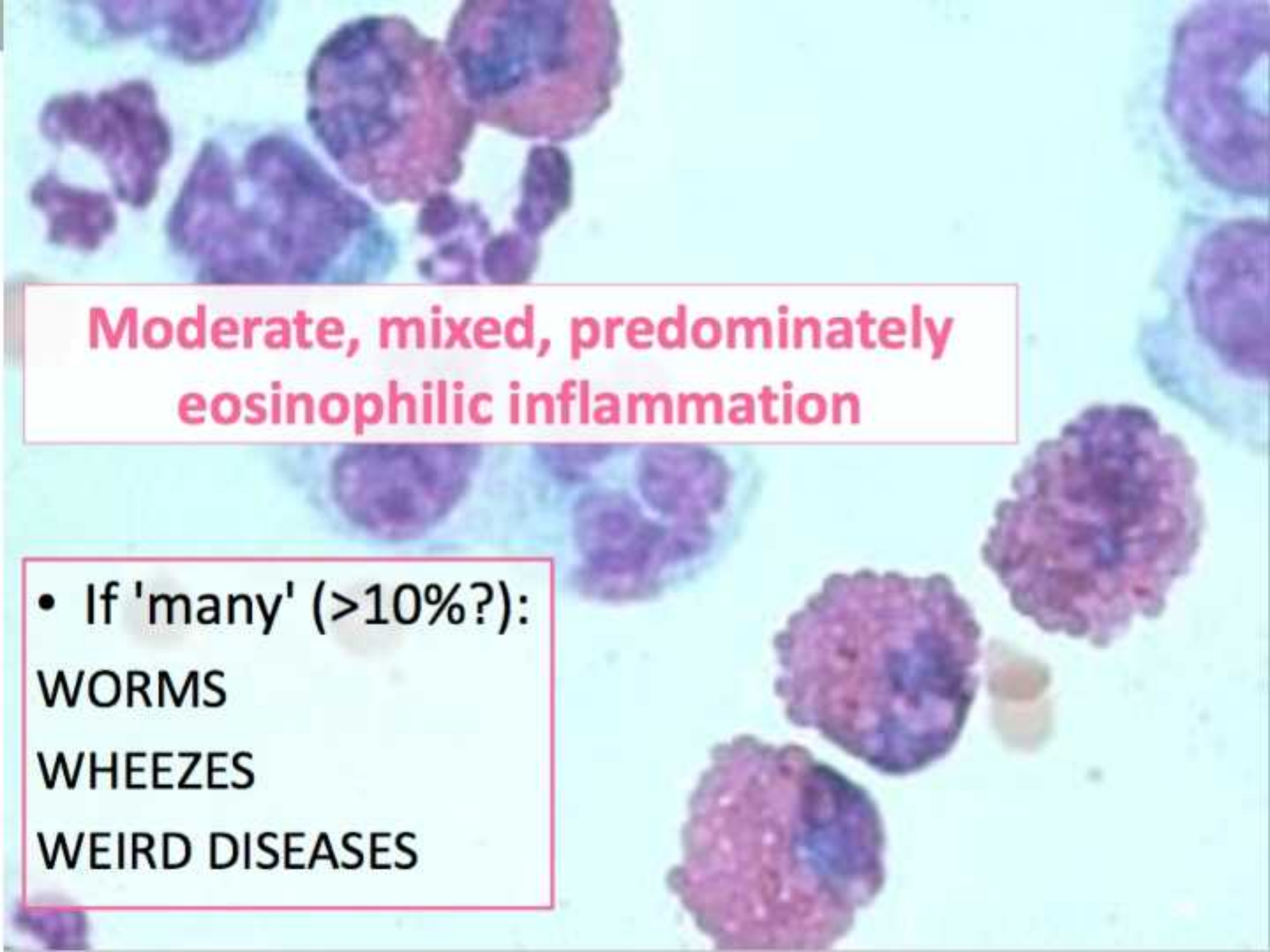
Inflammation





Which cells predominate?

Look for etiology?



Moderate, mixed, predominately eosinophilic inflammation

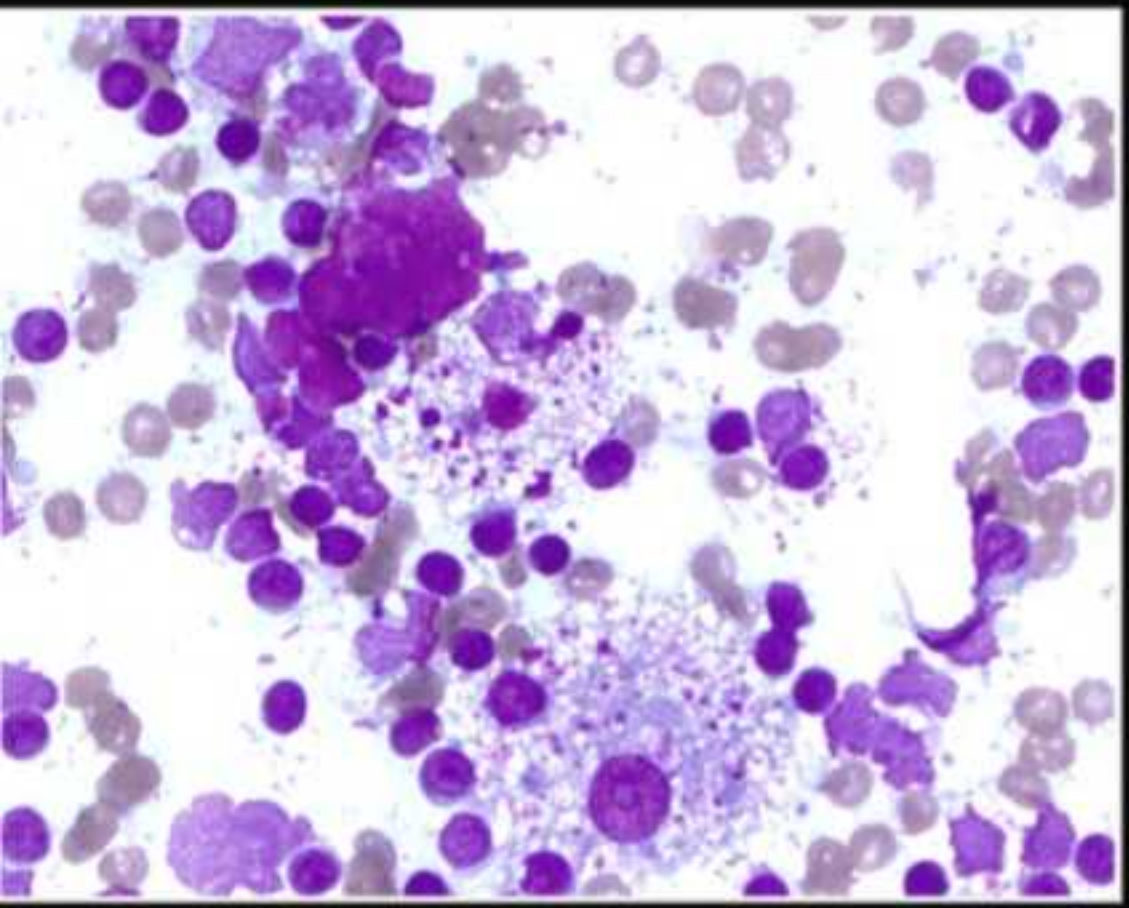
- If 'many' (>10%?):

WORMS

WHEEZES

WEIRD DISEASES

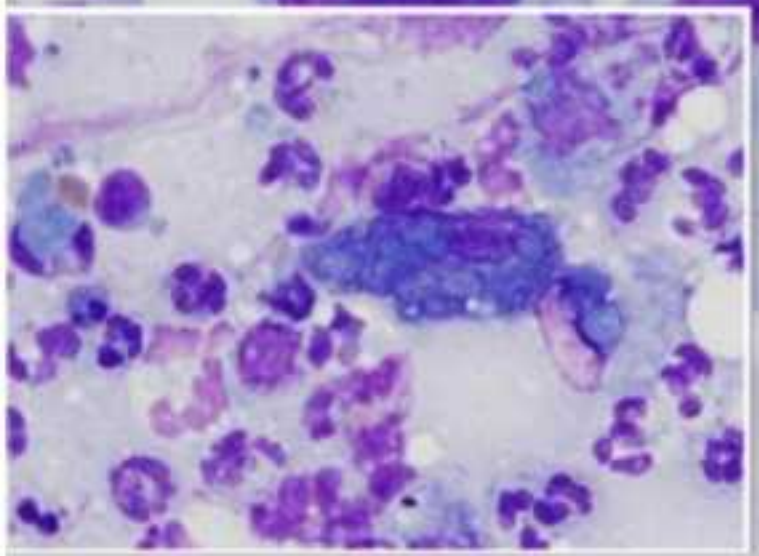
SQ mass, right hip, canine.



- Mixed Inflammation
- lymphs predominate
- Magenta, globular material

Vaccine reaction

"Blue" vaccine reactions



ORGANISMS

Characteristic of microrganisms

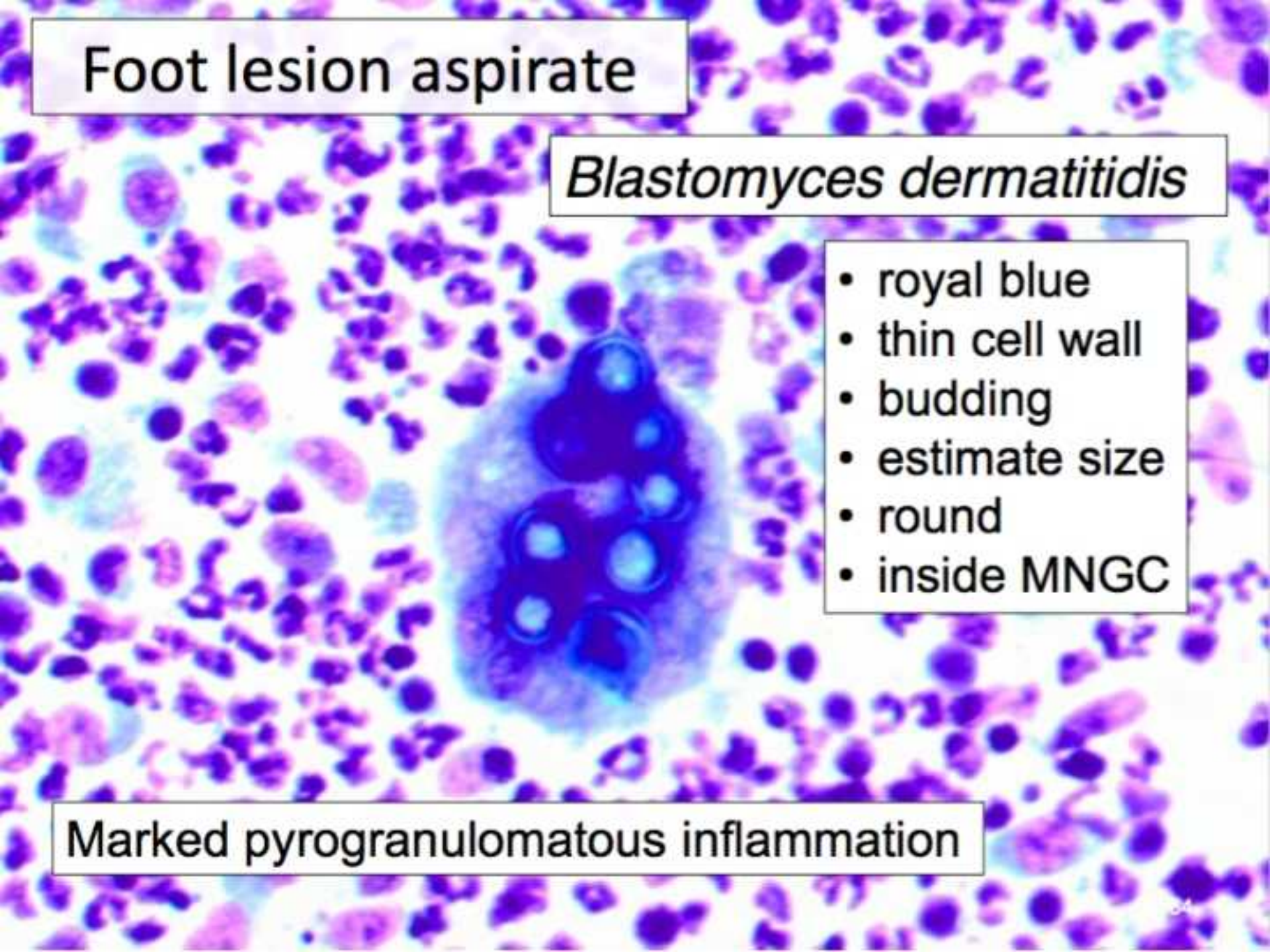
- Color?
- Size?
- Cell walls
- Intra and/or extracellular?
- In what cell types?
- Character of cytoplasm?
- Nuclear shape?
- Nuclear location? color?

RBC (7µm) & Smaller

- *Histoplasma*
- *Toxoplasma*
- *Leishmania*
- *Trypanosoma*
- *Malassezia*
- *Sporotrix*

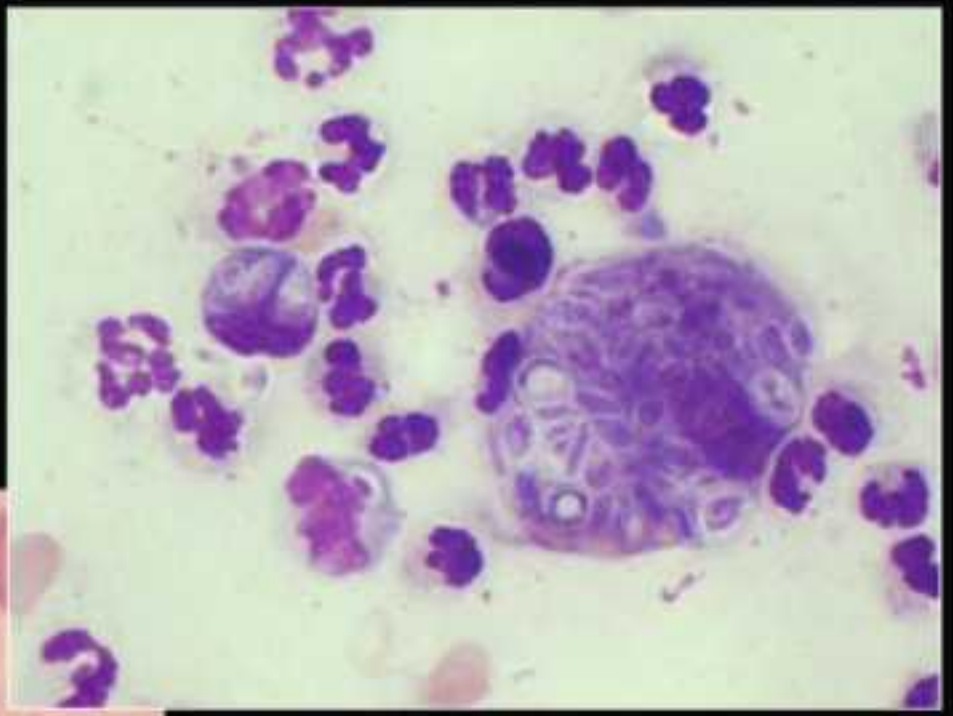
Neutrophils (15µm) & Up

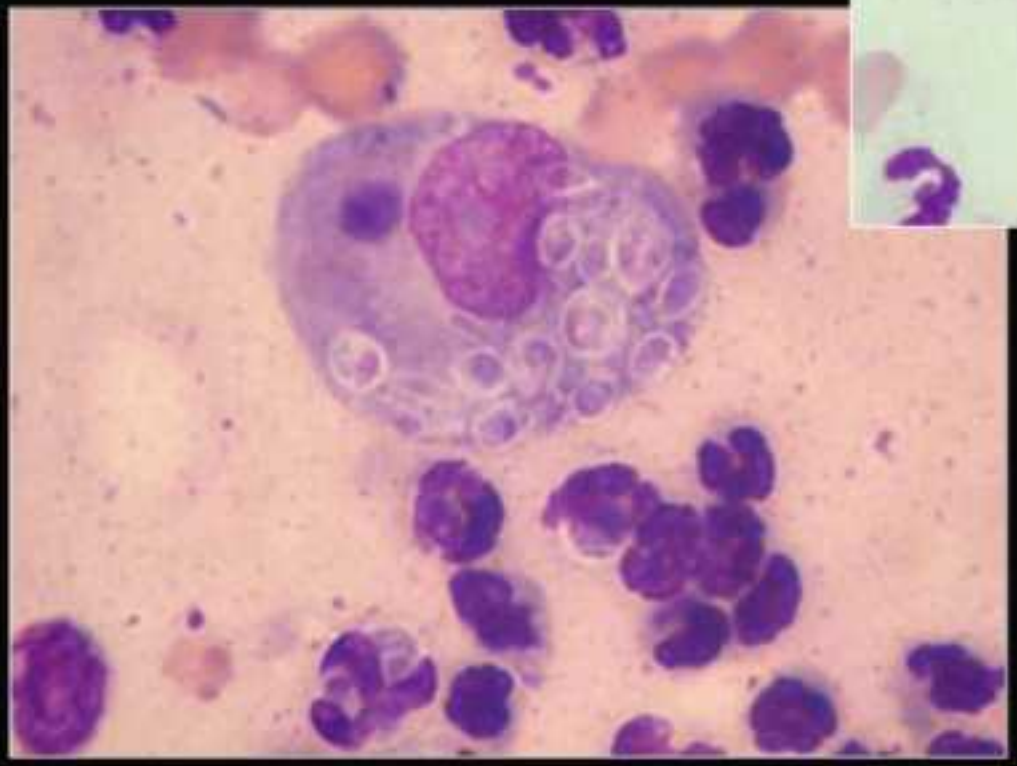
- *Cryptococcus*
- *Blastomyces*
- *Rhinosporidium*
- *Coccidioides*
- Parasites
- Hyphae
  - *Aspergillus*
  - *Mucor*


Foot lesion aspirate
Blastomyces dermatitidis
• royal blue
• thin cell wall
• budding
• estimate size
• round
• inside MNGC
Marked pyrogranulomatous inflammation

Sporothrix schenckii

Moderate pyogranulomatous inflammation

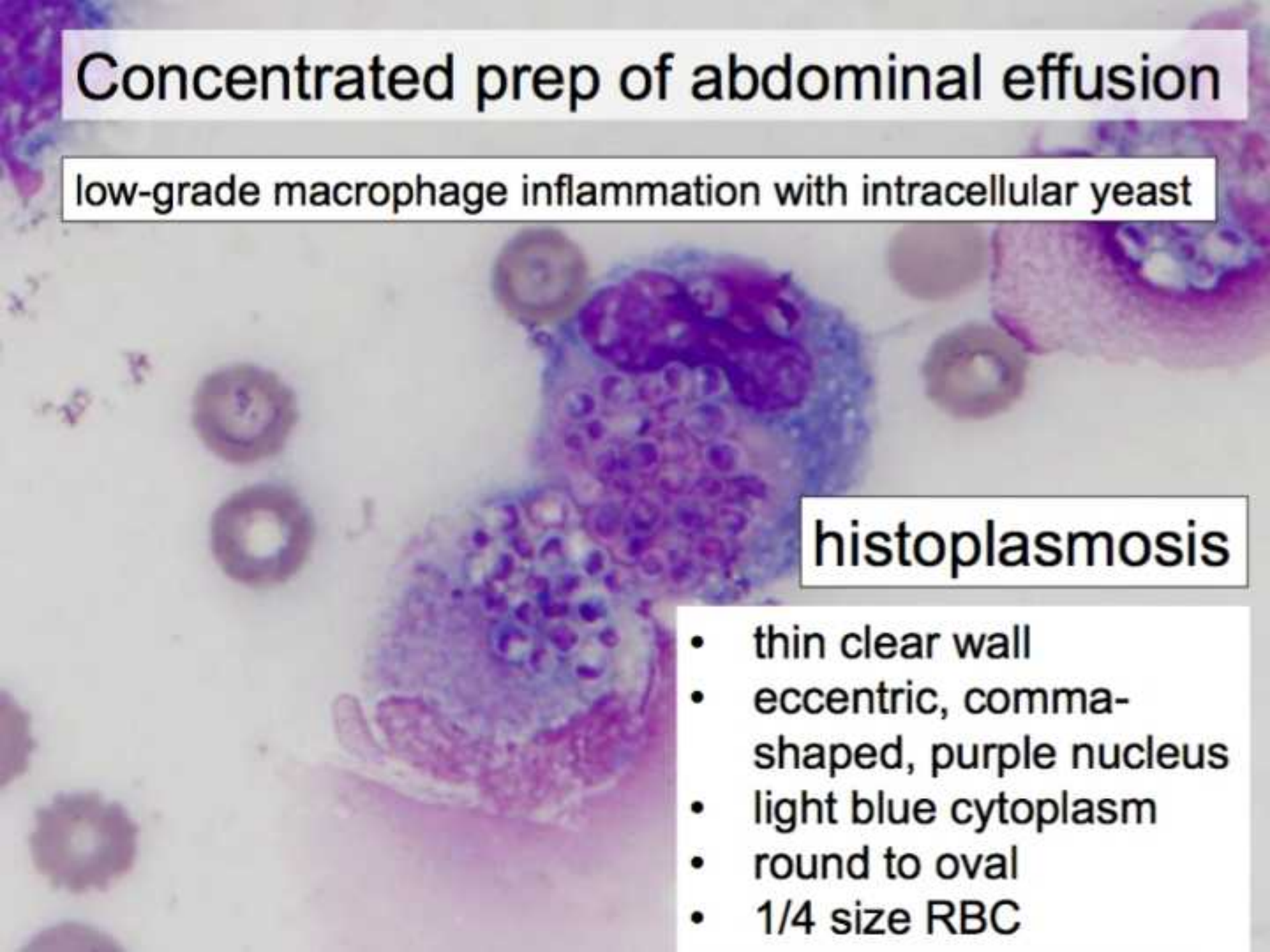




- within in macs
- 2-3 um
- round to cigar shaped
- thin, clear, cell wall
- eccentric, purple nucleus
- light blue cytoplasm

Concentrated prep of abdominal effusion

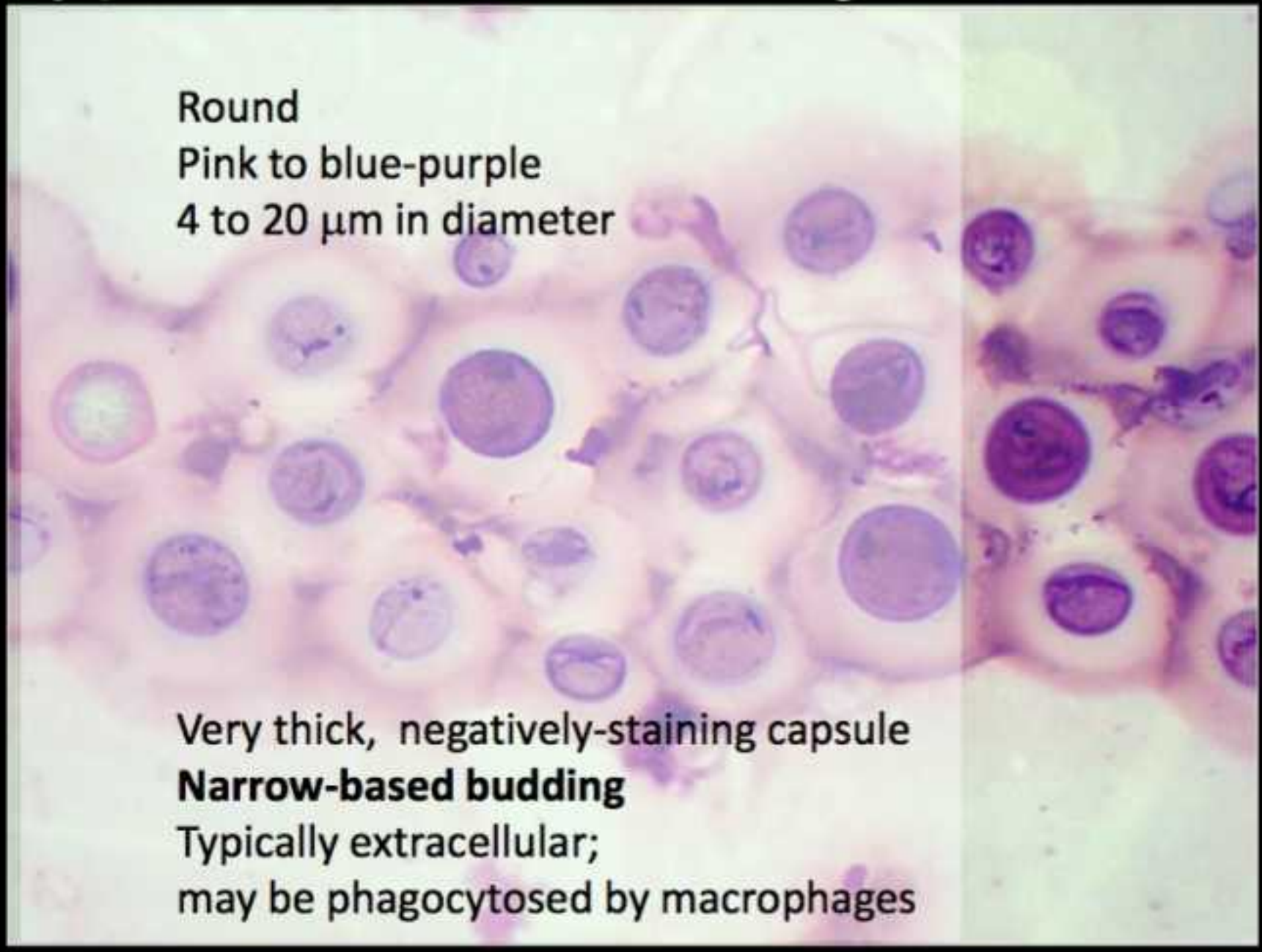
low-grade macrophage inflammation with intracellular yeast



histoplasmosis

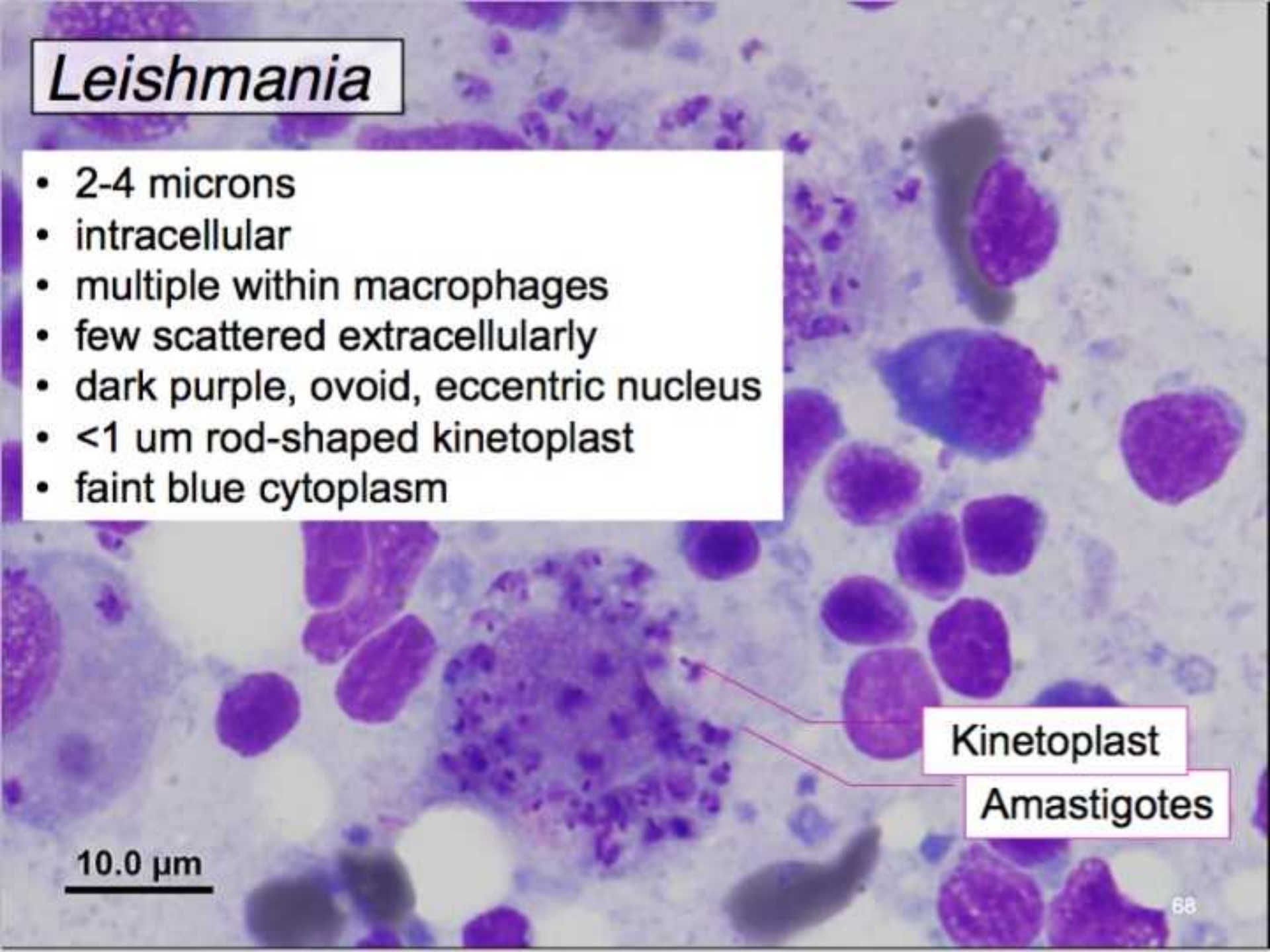
- thin clear wall
- eccentric, comma-shaped, purple nucleus
- light blue cytoplasm
- round to oval
- 1/4 size RBC

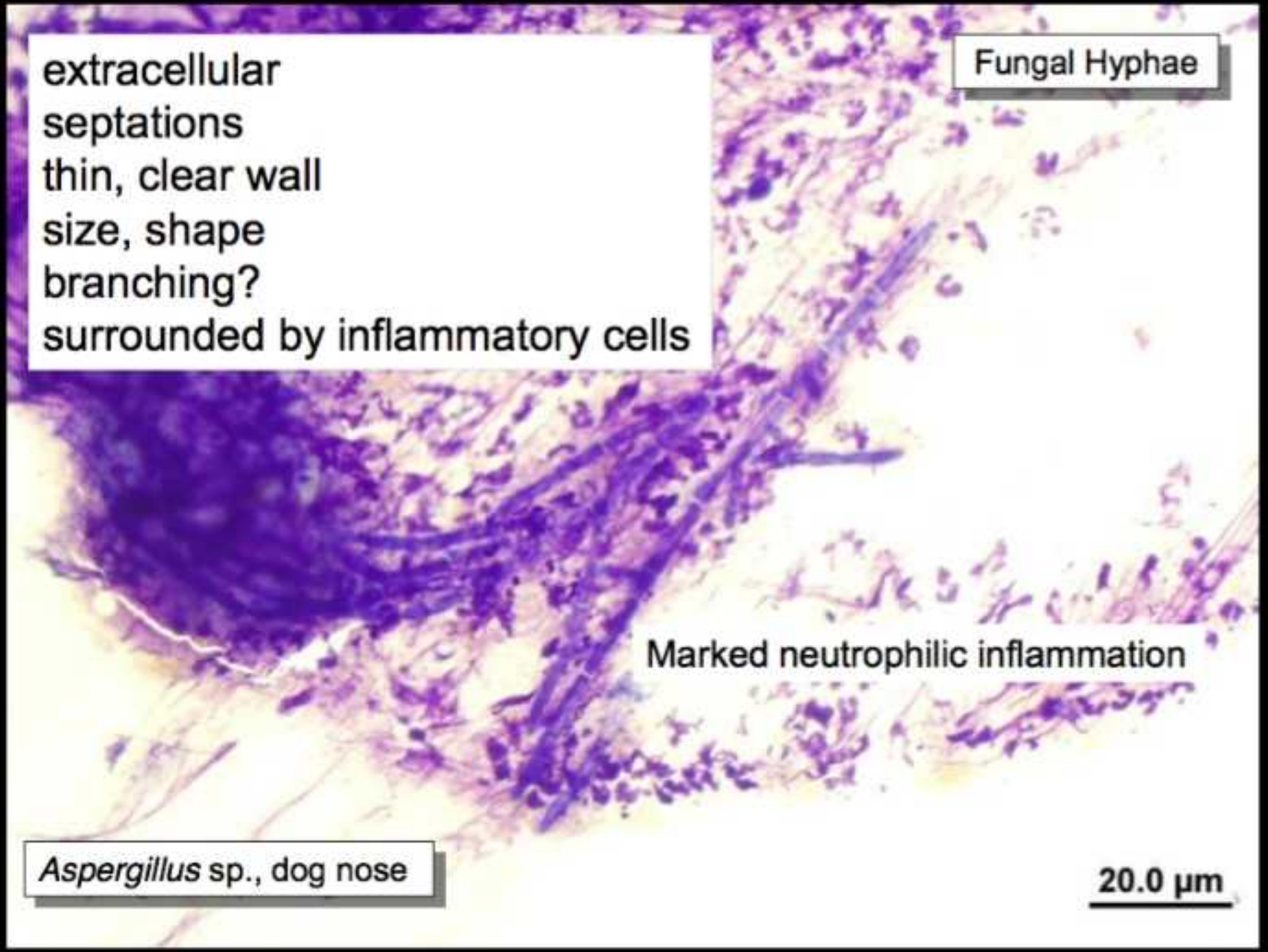
Crytpococcus neoformans, C. gattii



Leishmania

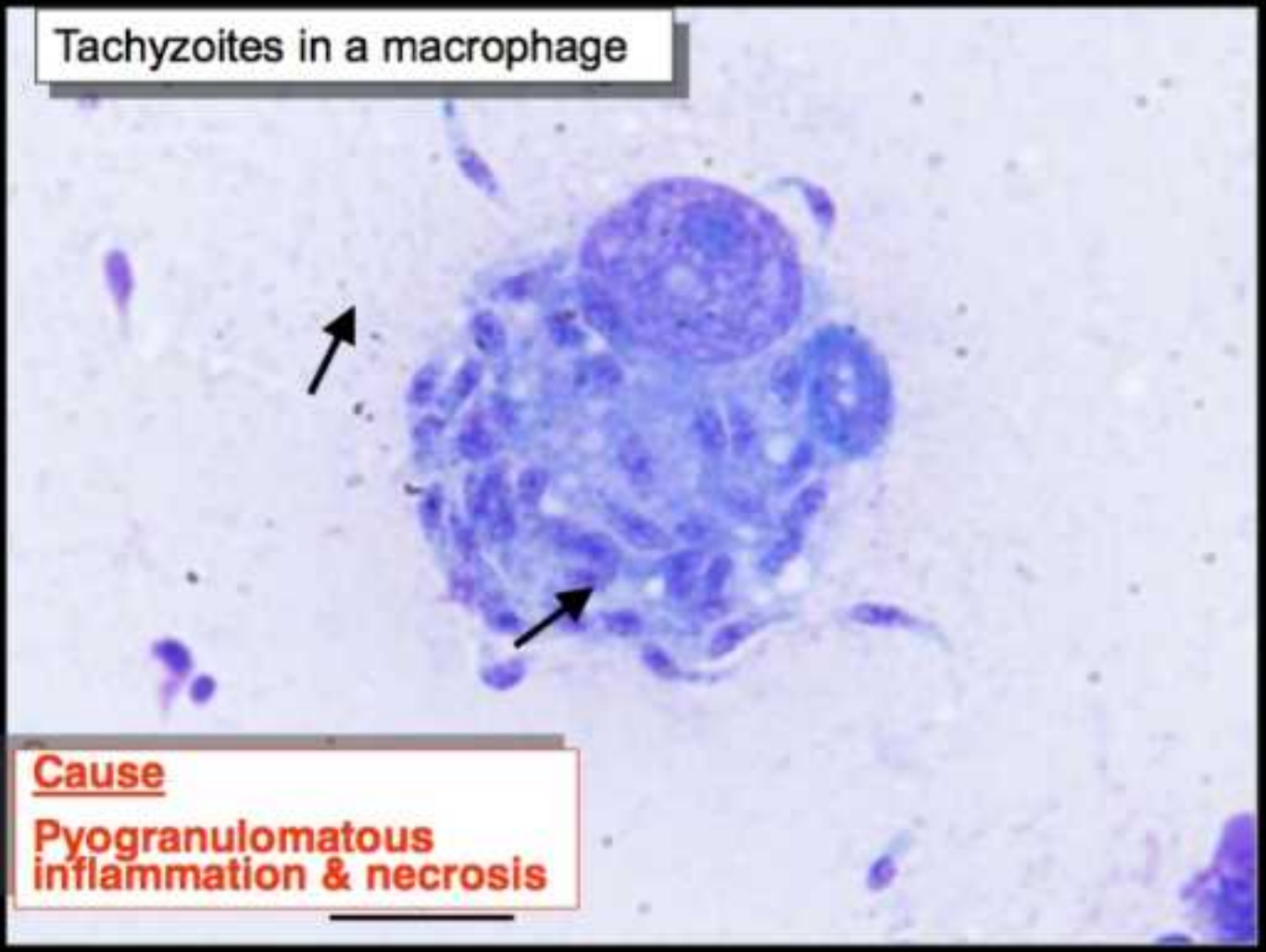
- 2-4 microns
- intracellular
- multiple within macrophages
- few scattered extracellularly
- dark purple, ovoid, eccentric nucleus
- <1 um rod-shaped kinetoplast
- faint blue cytoplasm




extracellular
septations
thin, clear wall
size, shape
branching?
surrounded by inflammatory cells
Fungal Hyphae
Marked neutrophilic inflammation
Aspergillus sp., dog nose
20.0 µm

Toxoplasma / Neospora / Sarcocystis

2007 ASVCP Meeting Chemistry Cases, #3

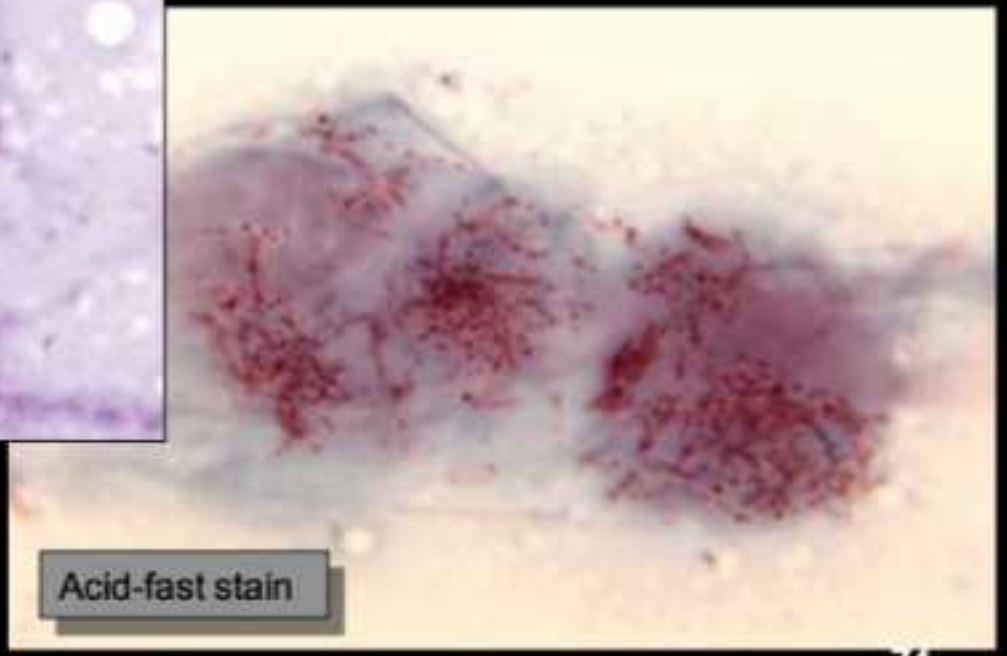


- crescent shaped
- intra/extra cellular
- basophilic cytoplasm
- central purple ovoid nucleus
- 5-7um

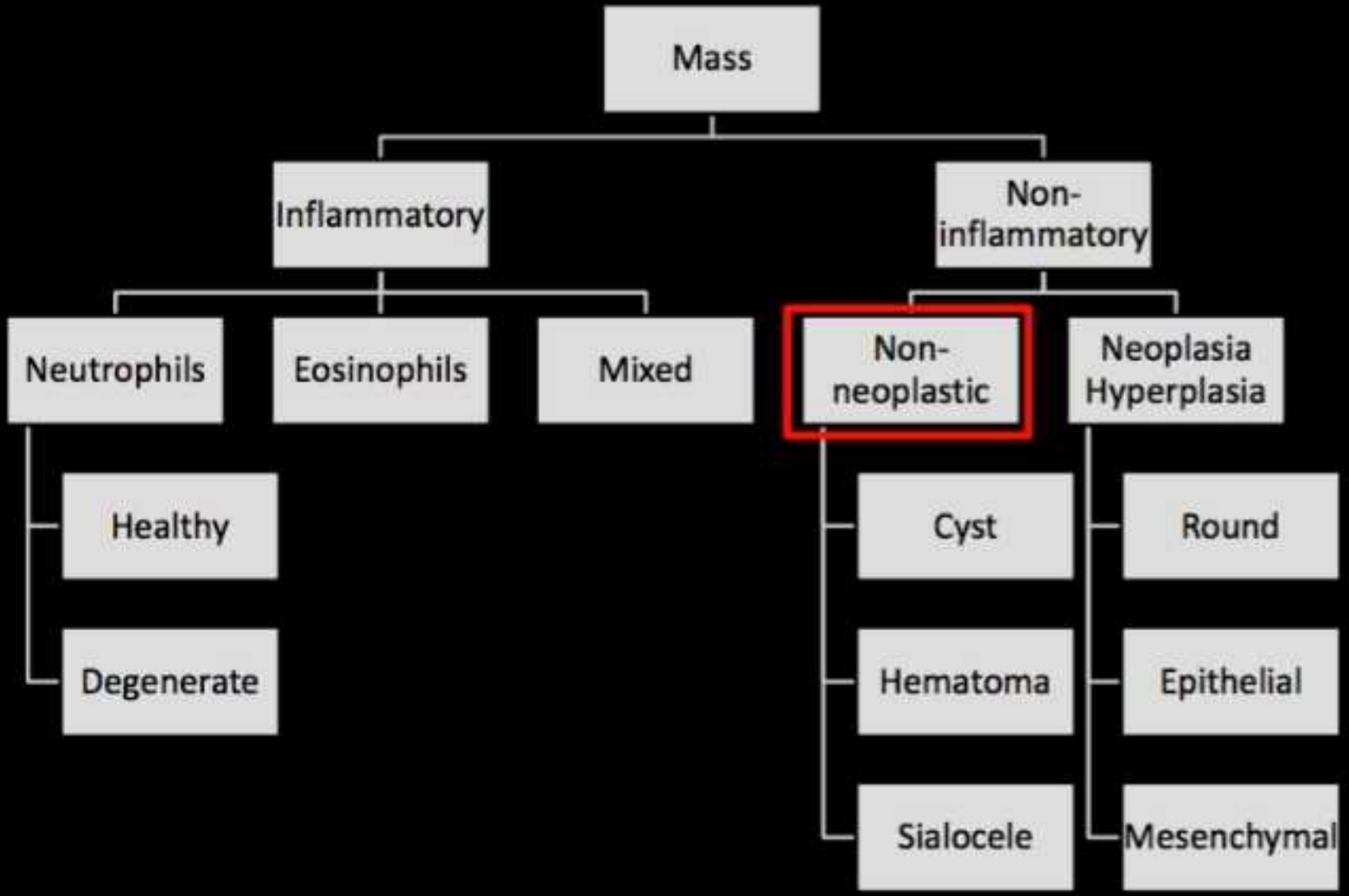
Mycobacterium sp.

VCP 2005; 34(2):161-163.

SQ lesion from a dog



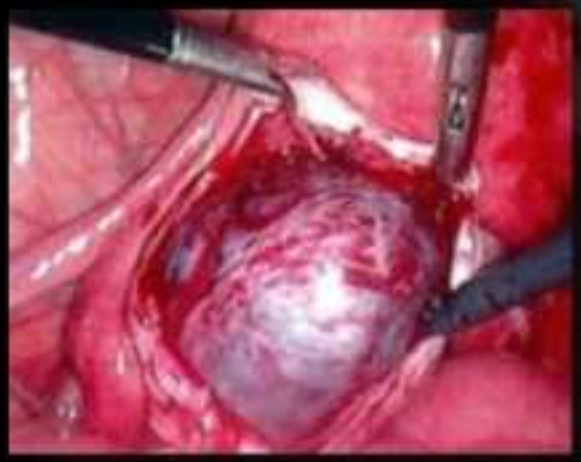
Diagnostic Tree

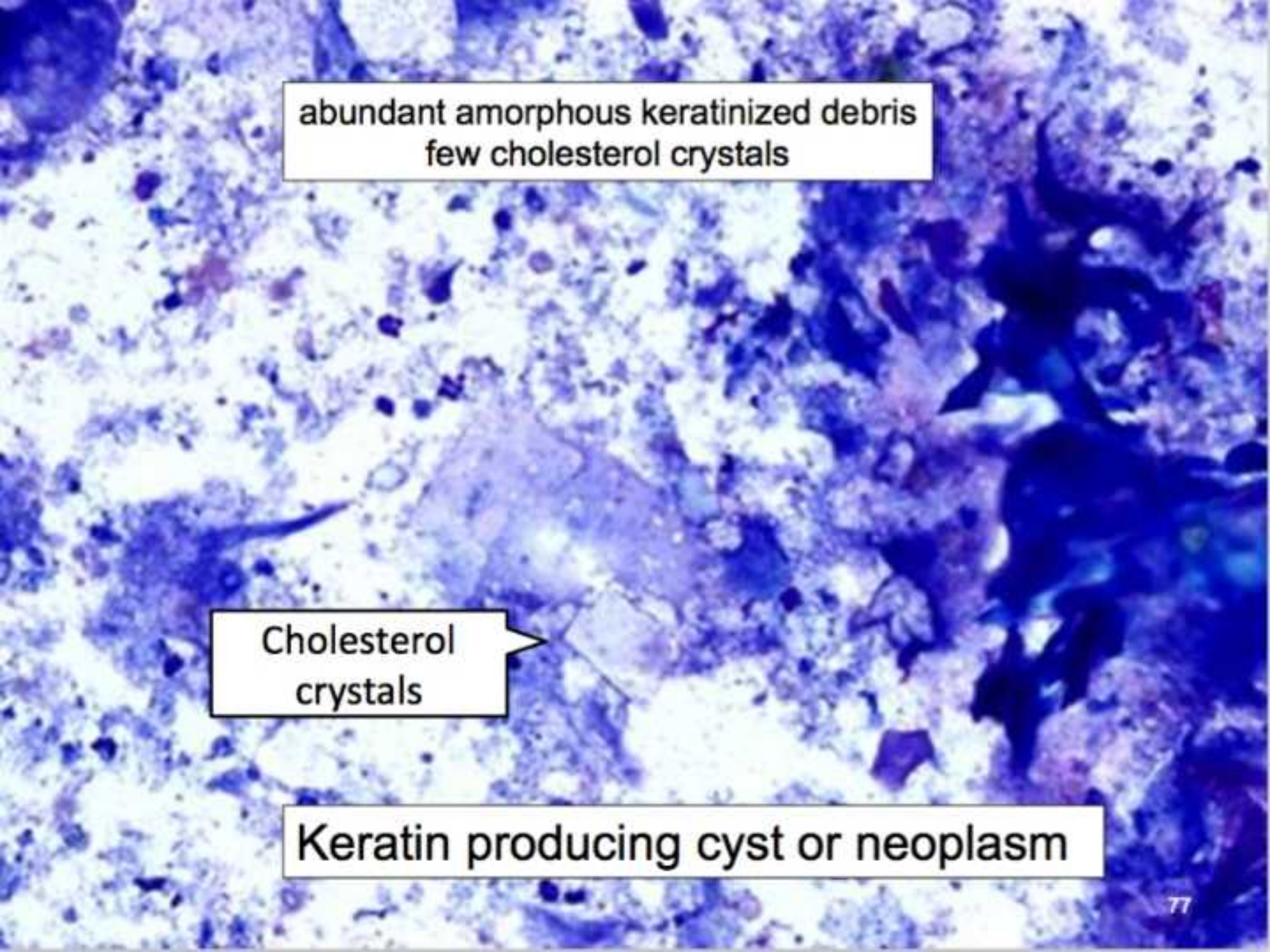


Non-inflammatory / Non-neoplastic Lesions

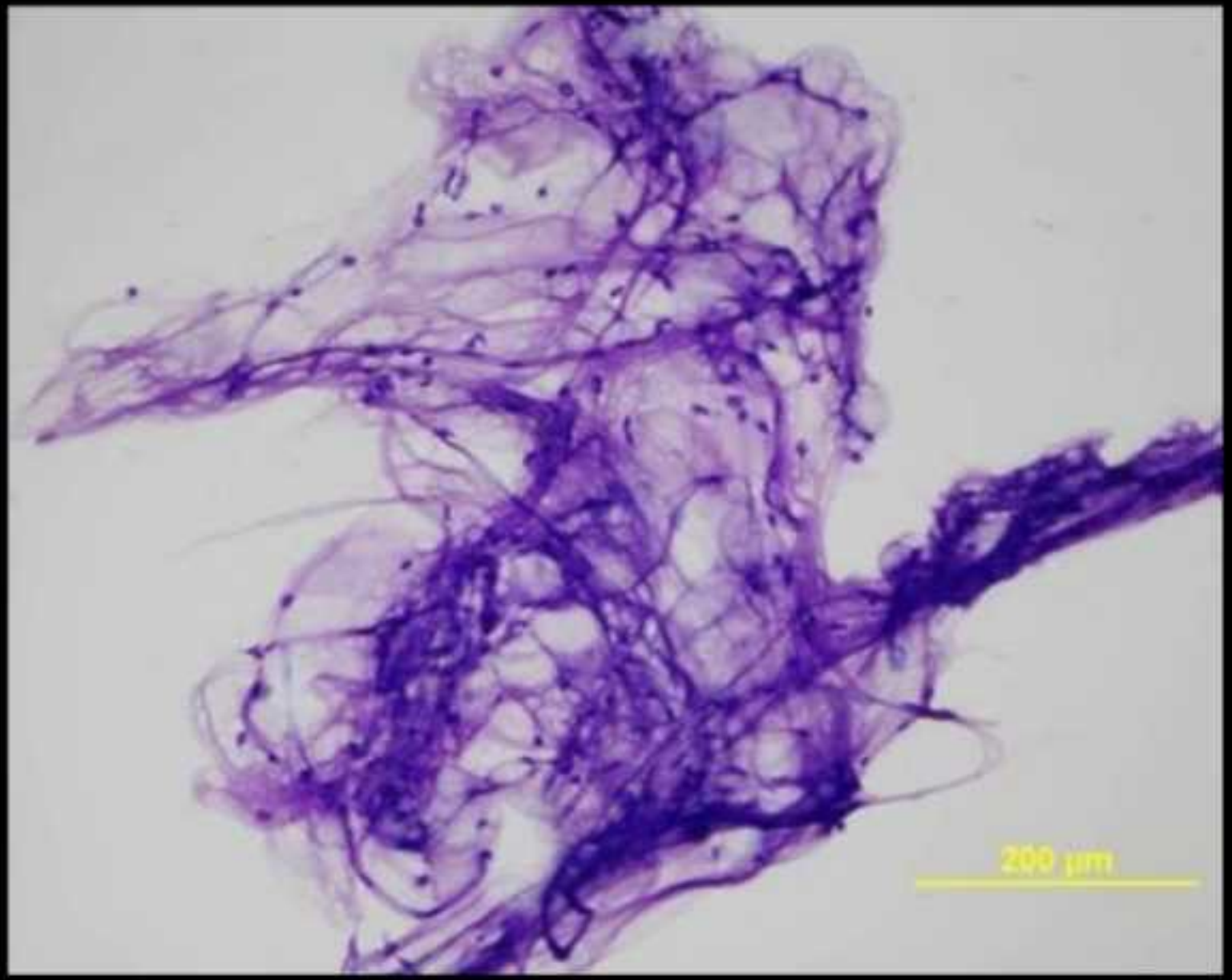
Usually (not always) few to no inflammatory cells:

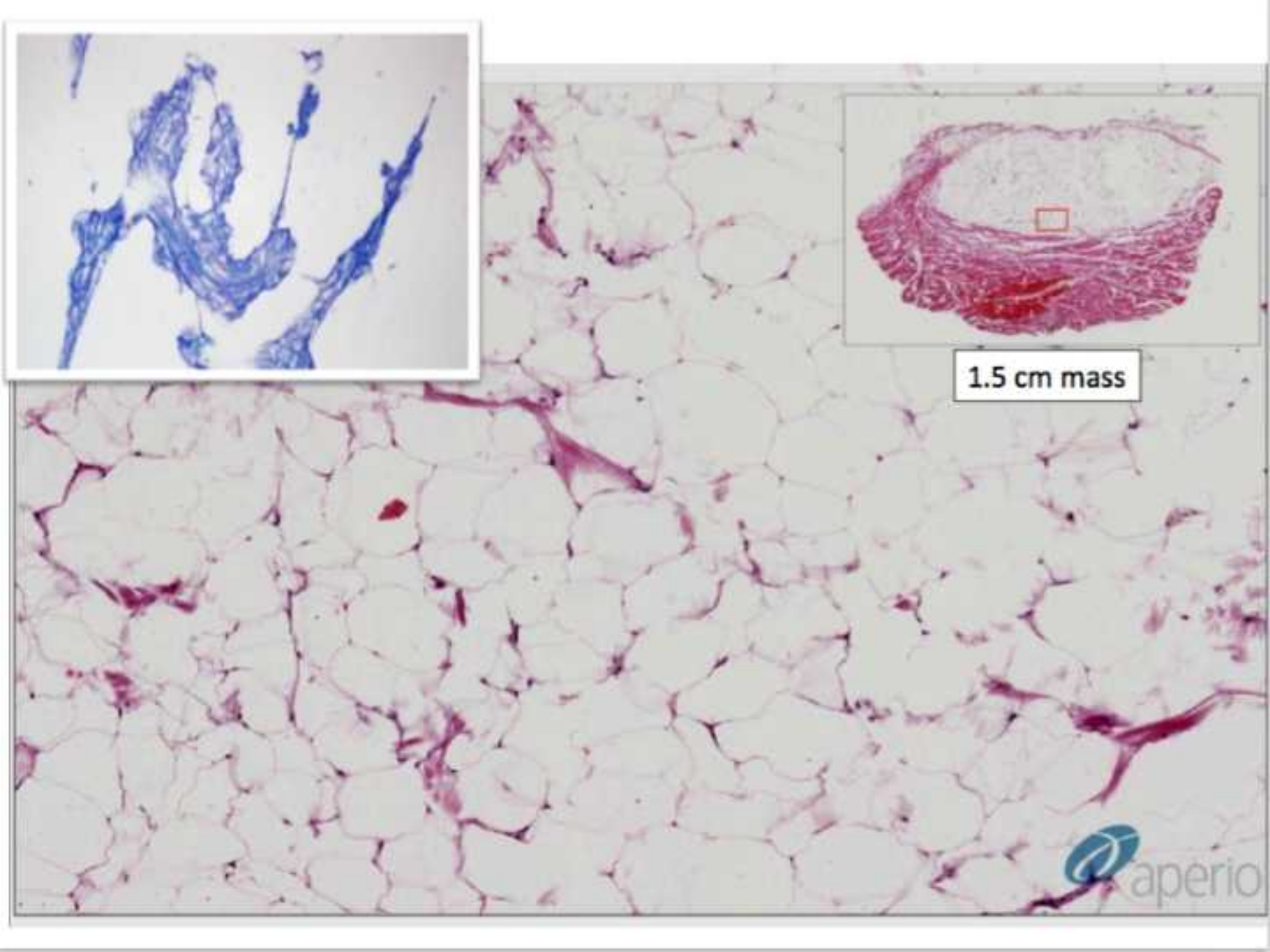
- Cyst
- Seroma/Hygroma
- Hematoma
- Sialocoele
- Neoplasm



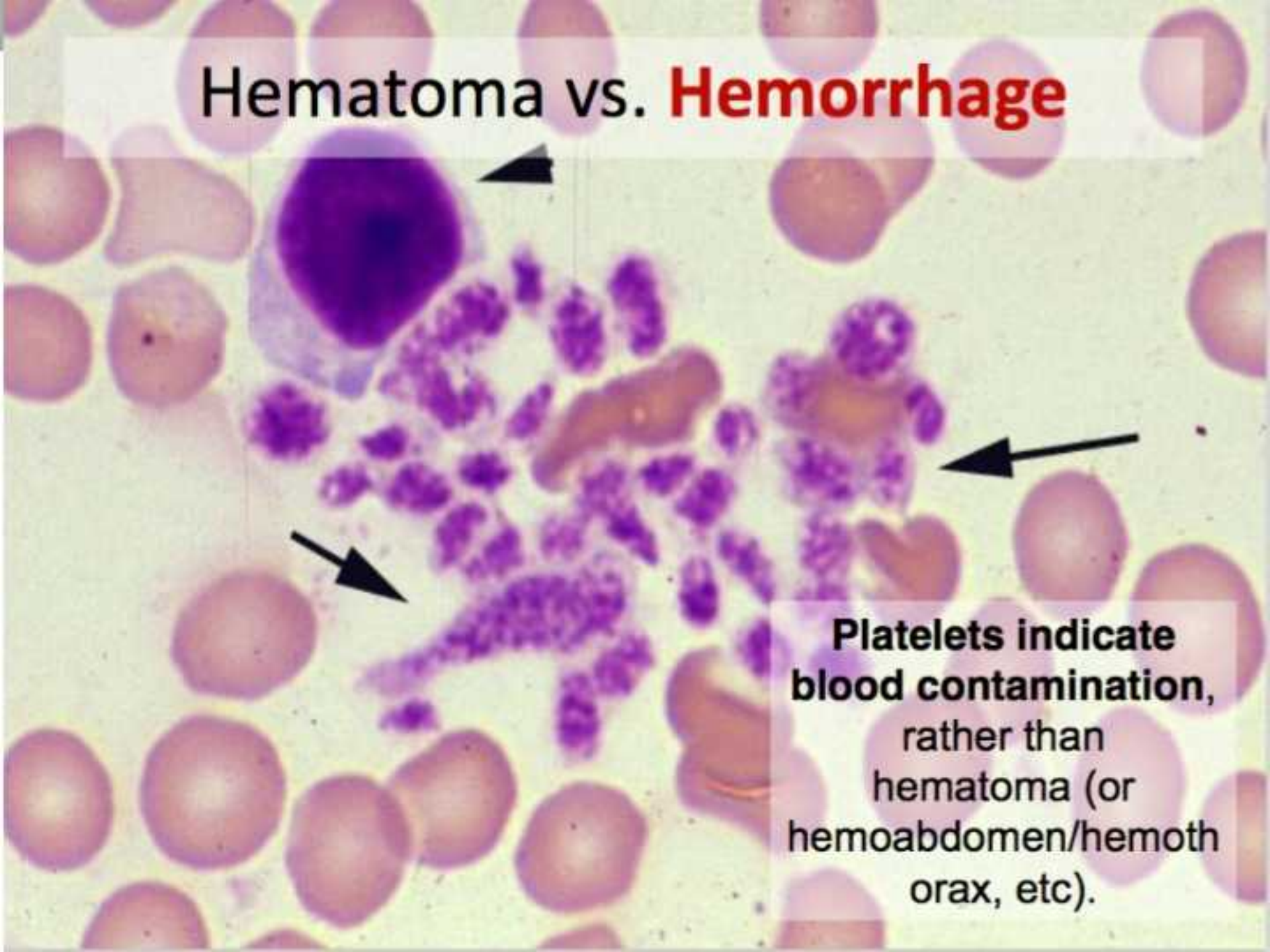

abundant amorphous keratinized debris
few cholesterol crystals
Cholesterol crystals
Keratin producing cyst or neoplasm

LIPOMA or SUBCUTANEOUS FAT



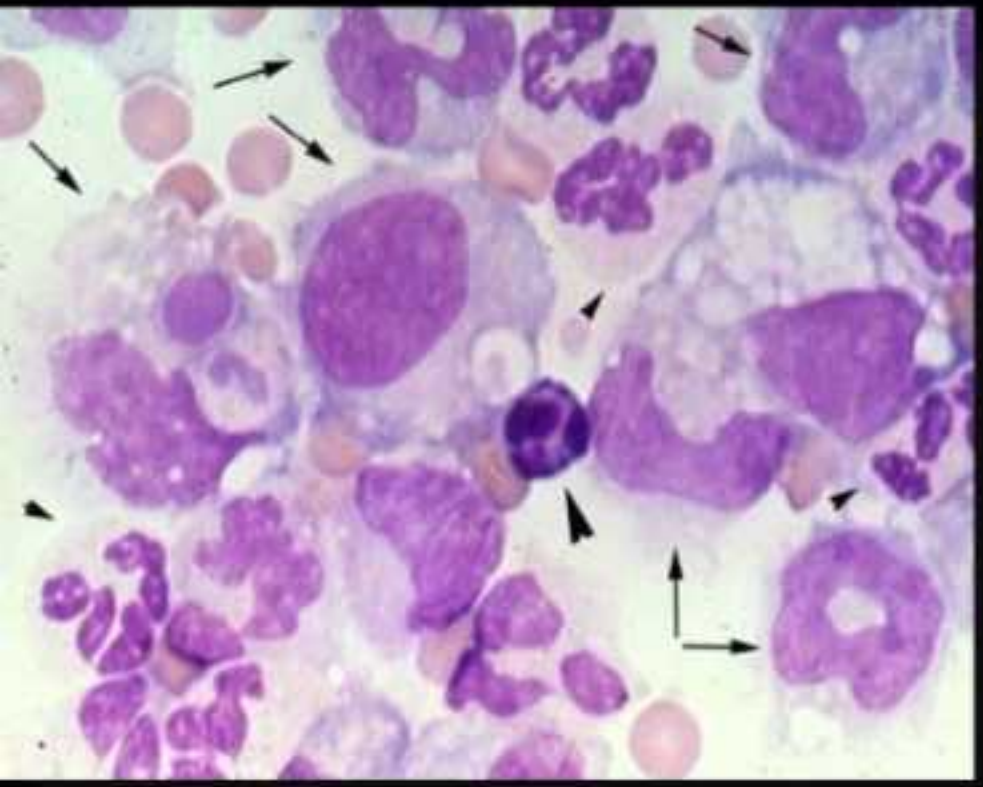

1.5 cm mass
aperio

Hematoma vs. Hemorrhage

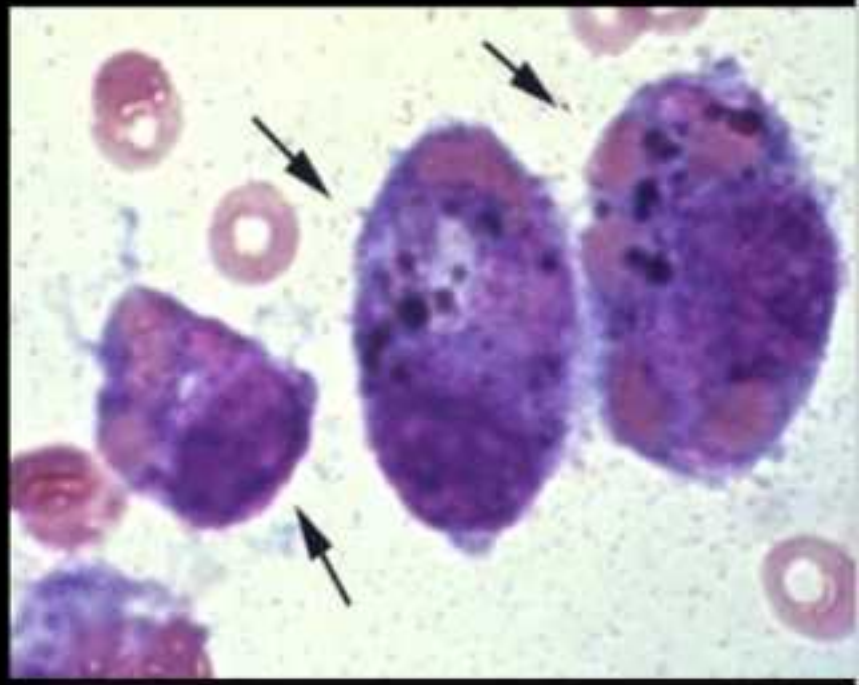


Platelets indicate blood contamination, rather than hematoma (or hemoabdomen/hemothorax, etc).

Hematoma vs. Hemorrhage

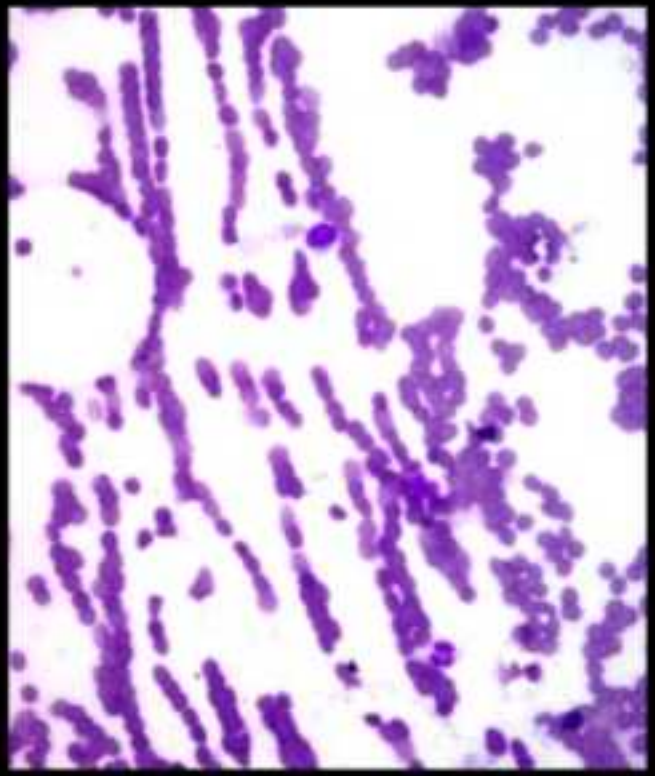


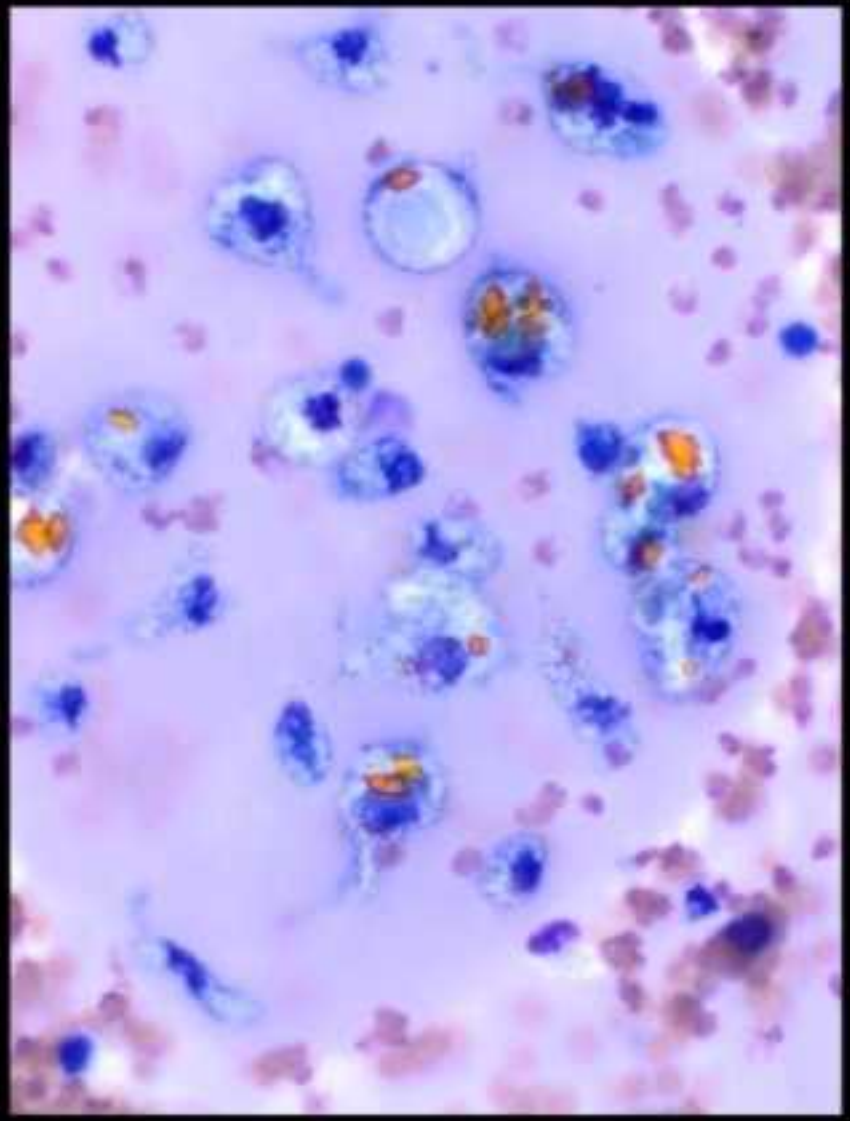
Erythrophagocytosis +
hemosiderin pigment



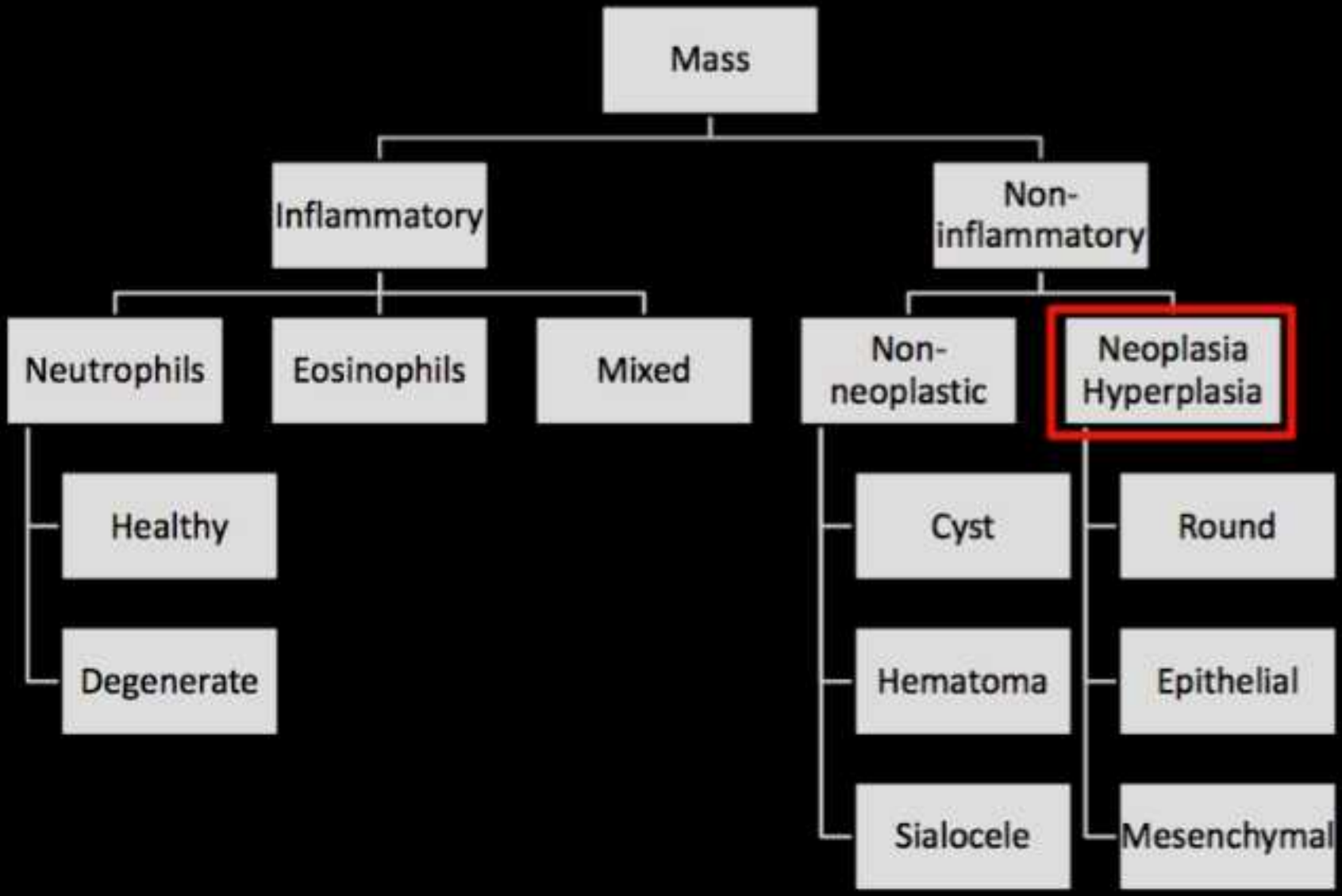
Sialocele

- Streams of blue mucinous material
- Rowing of erythrocytes
- Hematoidin crystals





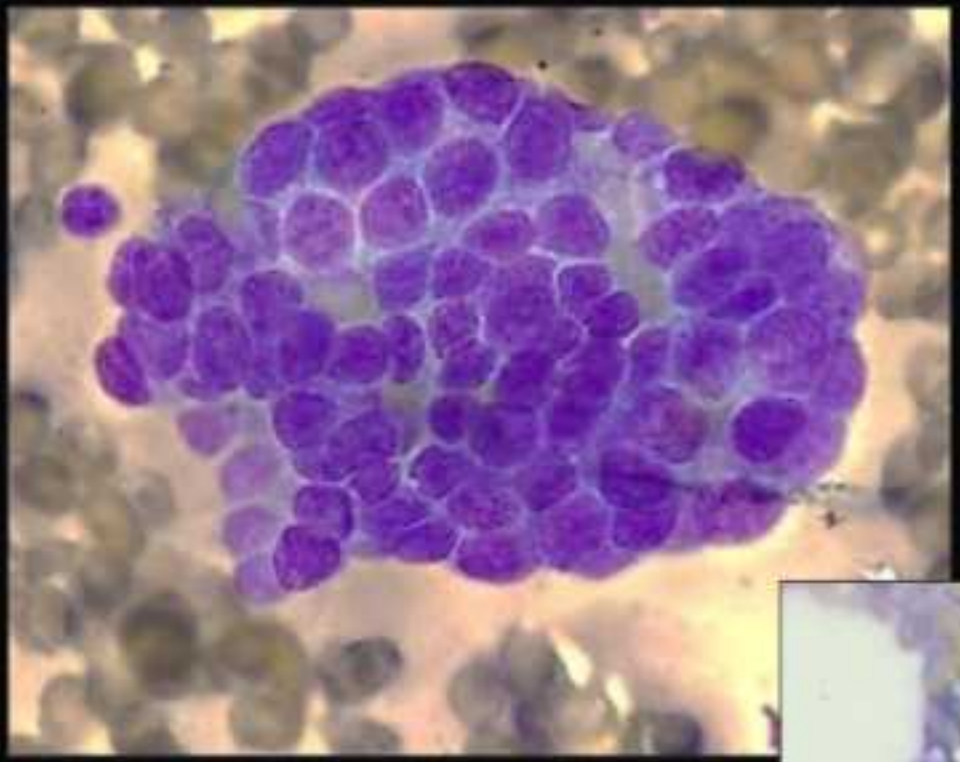
Diagnostic Tree



Neoplasms

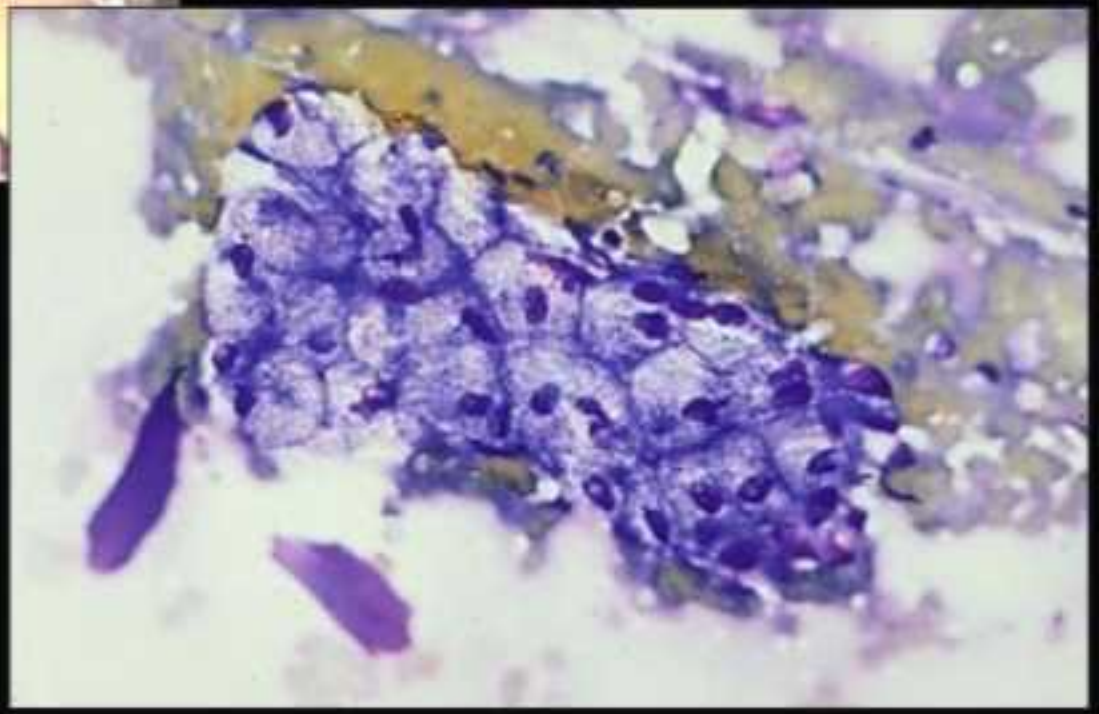
- Assess cellularity & slide quality
- Determine there are no (few) neutrophils
- Determine if cells are individual or in clusters
- Intra and/or extracellular?
- Cell shape
- Nuclear shape
- Nuclear shape?
- Feature of malignancy

Epithelial



Tight clusters

Highly cellular

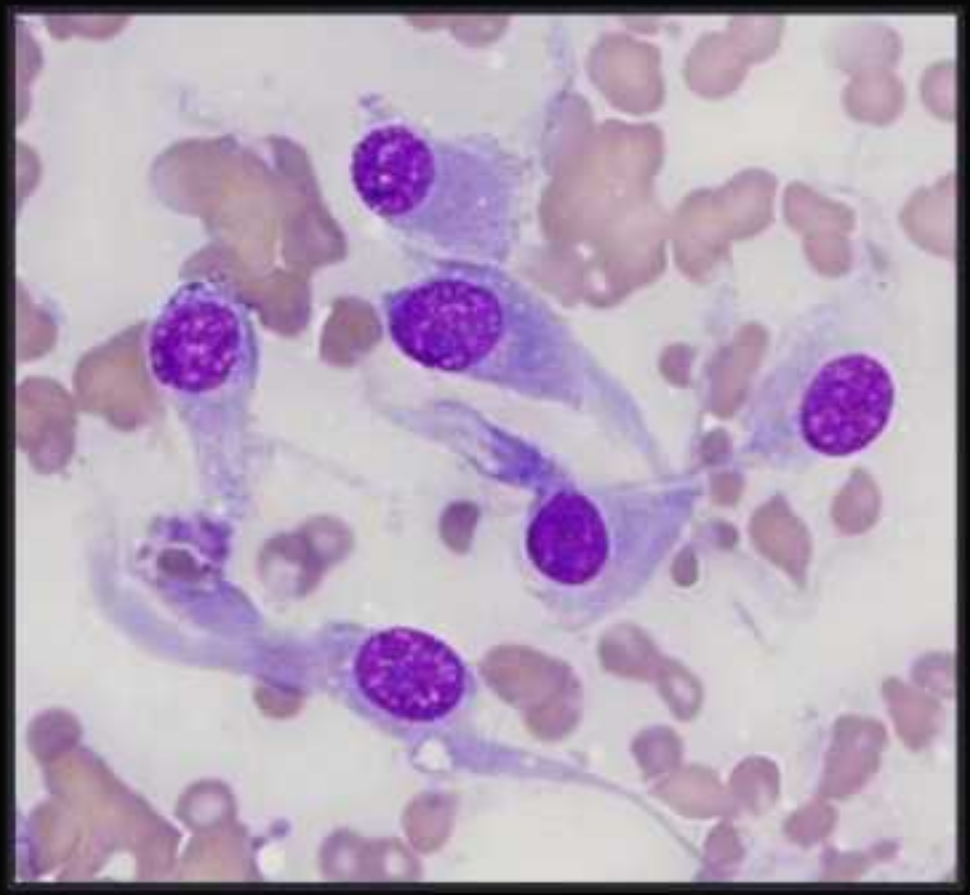


Polygonal

Central nucleus

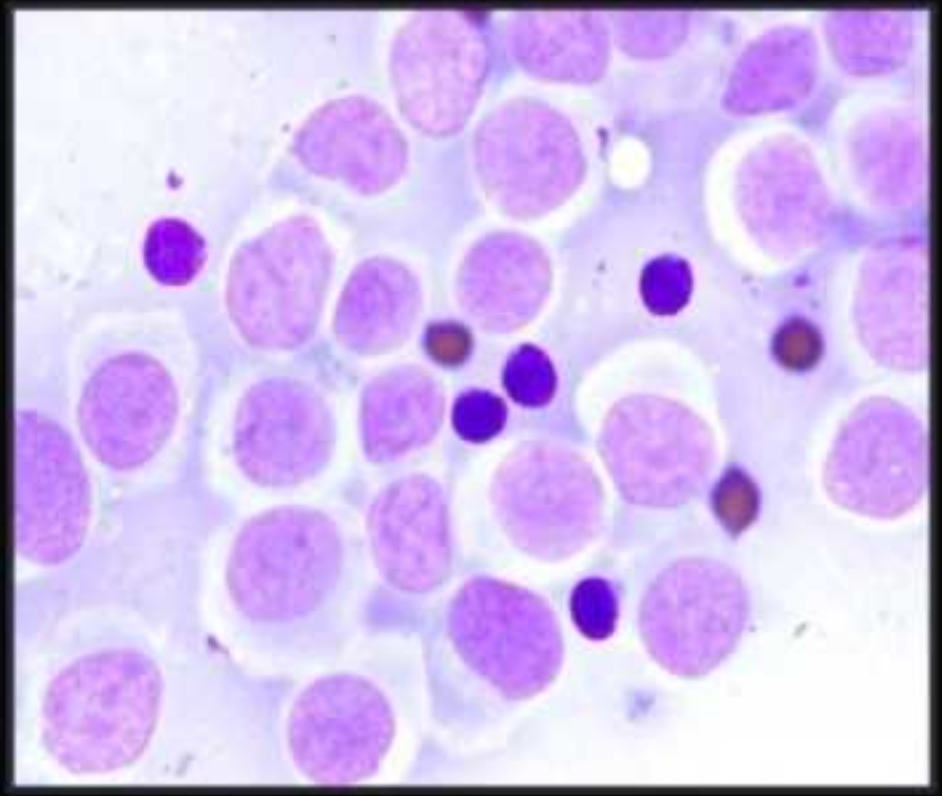
Mesenchymal

- Spindle-shaped
- Eccentric nucleus
- Individual to aggregated
- Low cellularity

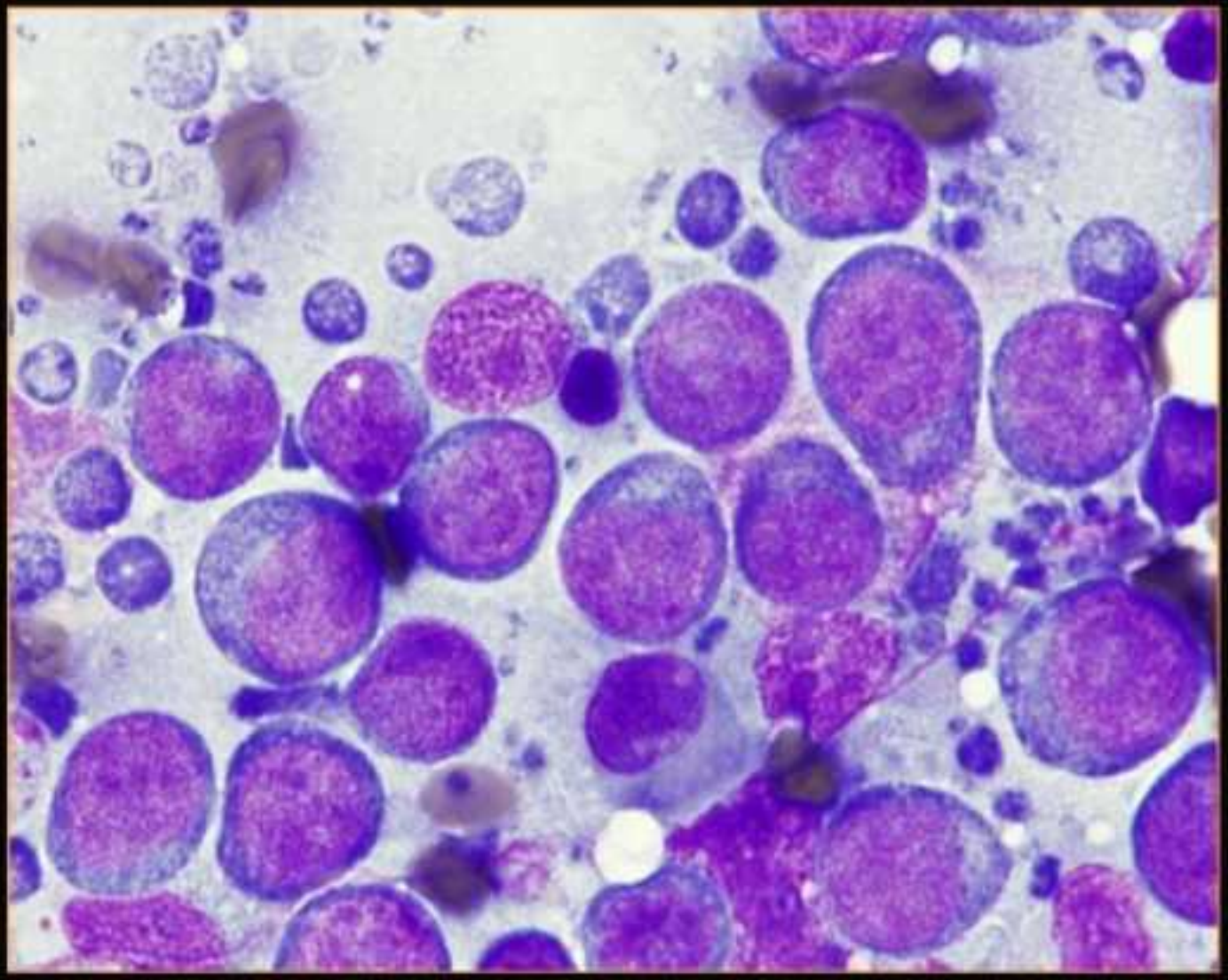


Round Cells

- Round
- Variable nuclear location
- Individual cells
- Highly cellular



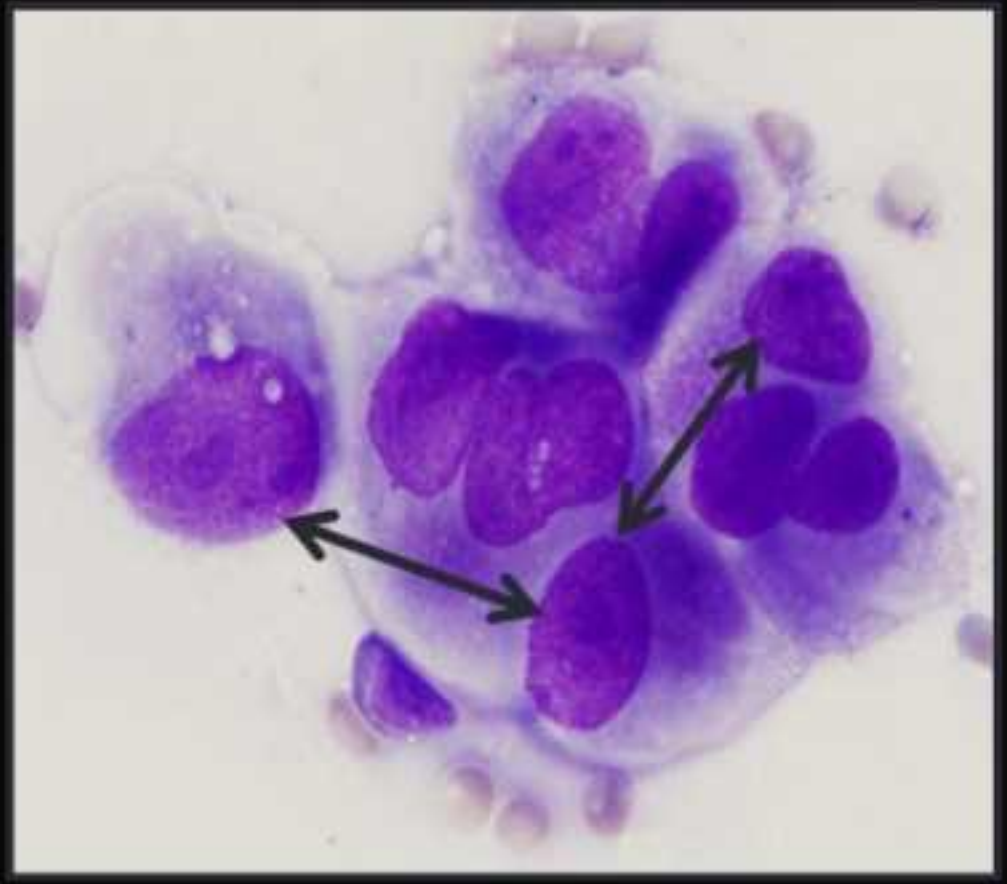
Cell type? Round Cell



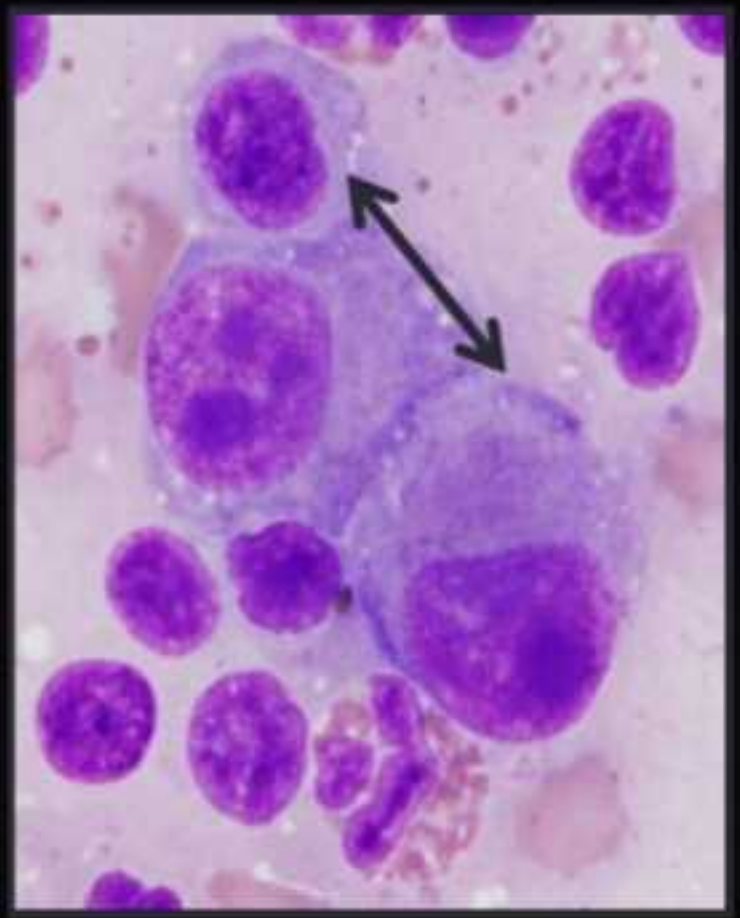
Cytologic Features of Malignancy

- Hypercellularity (decreased cohesiveness)
- Pleomorphism (anisocytosis, anisokaryosis)
- High/variable N:C ratio
- Multinucleation
- Karyomegaly
- Mitoses (+/- bizzarre)
- Nuclear molding (rapid cell growth)
- Coarse nuclear chromatin pattern
- Large, angular, or variable nucleoli (anisonucleolosis)

Criteria of Malignancy

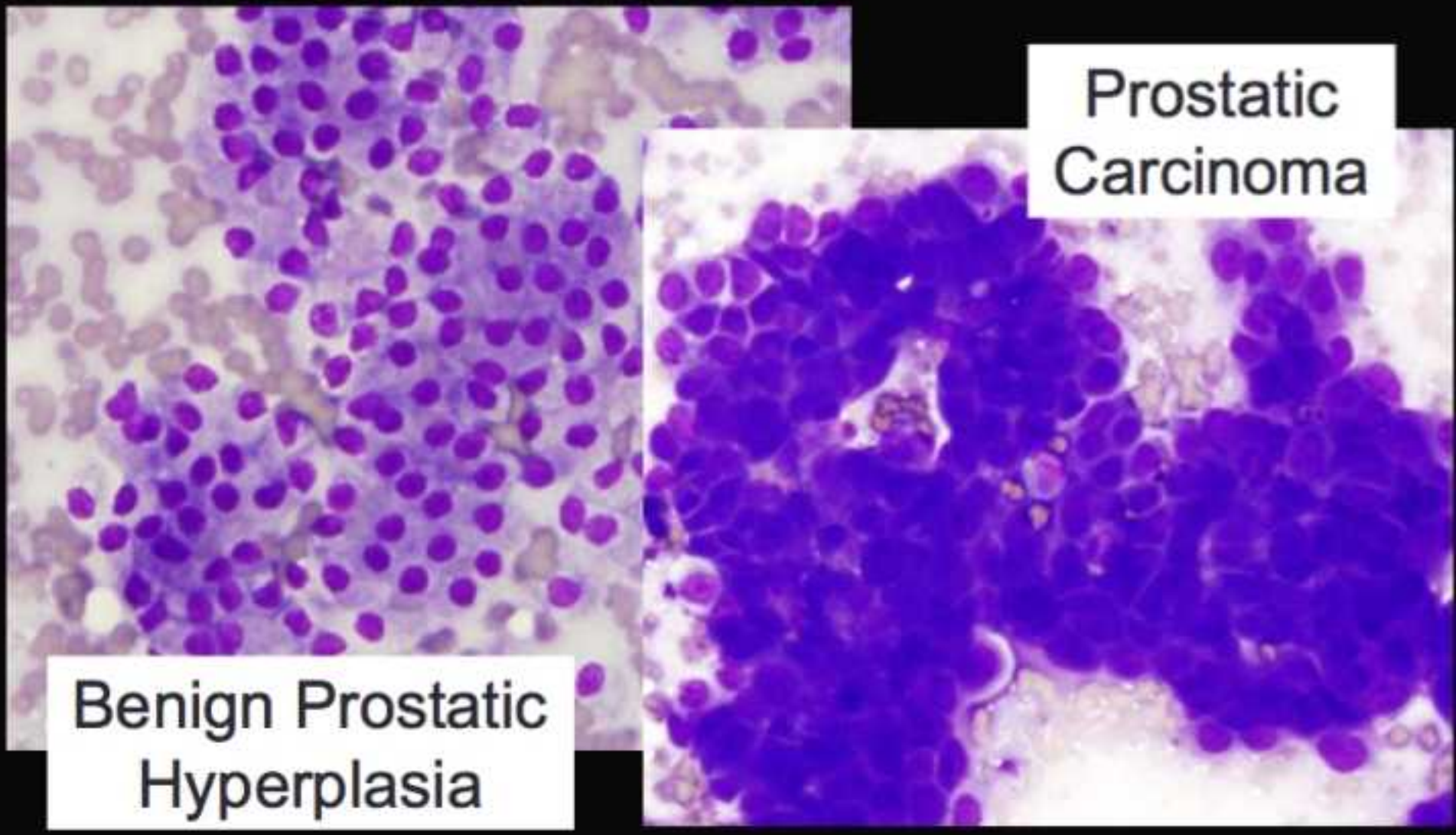


Anisokaryosis

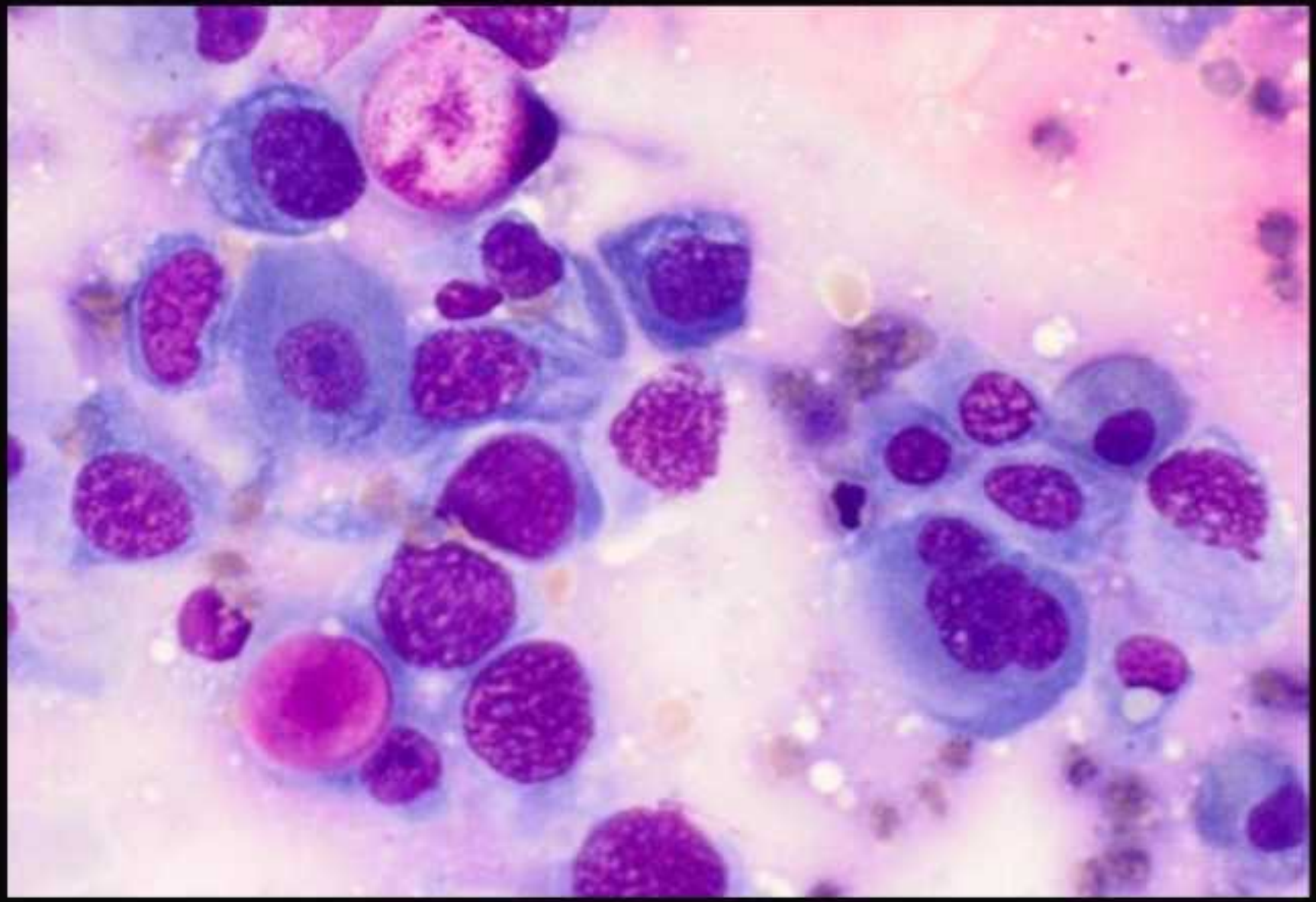


Anisocytosis

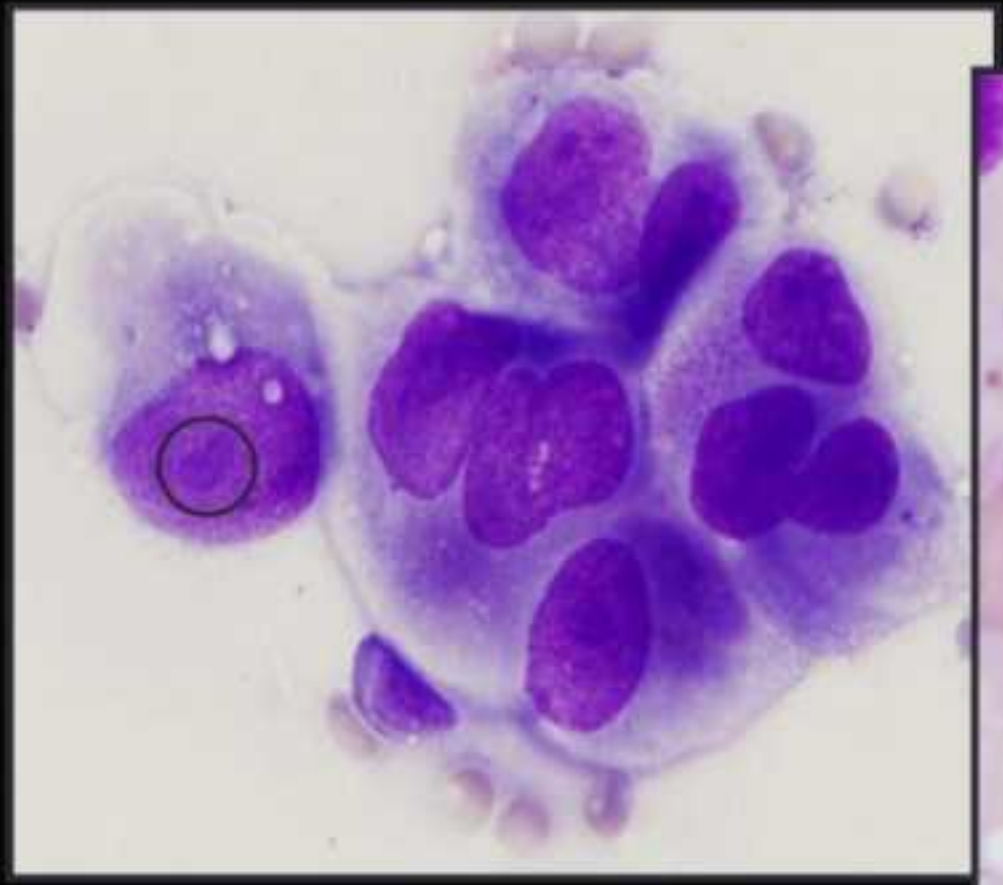
High N:C ratio

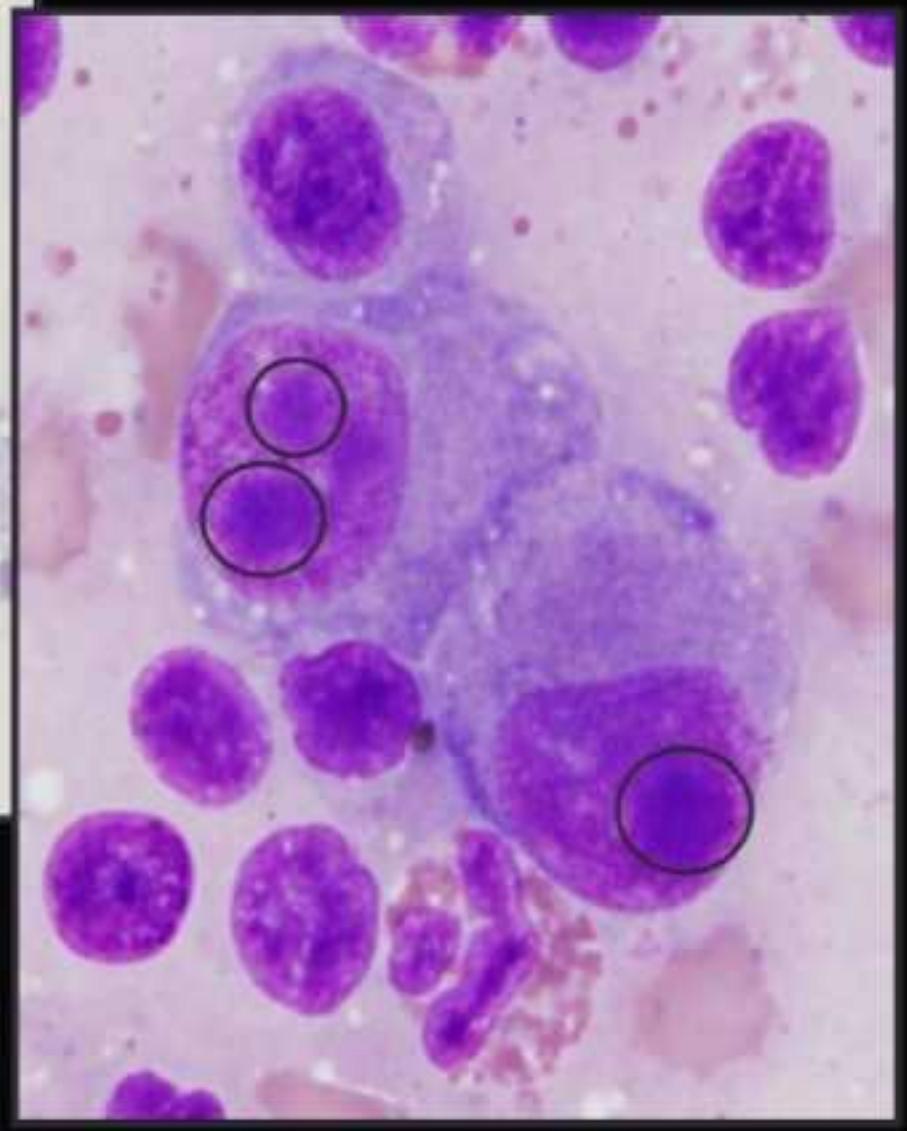


Variable N:C



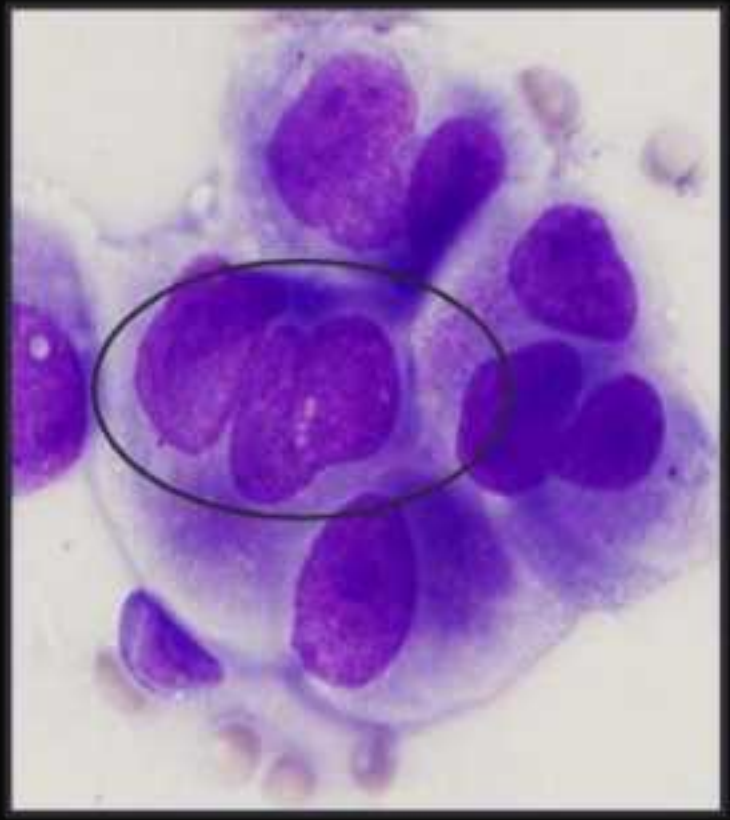
Anisonucleolosis



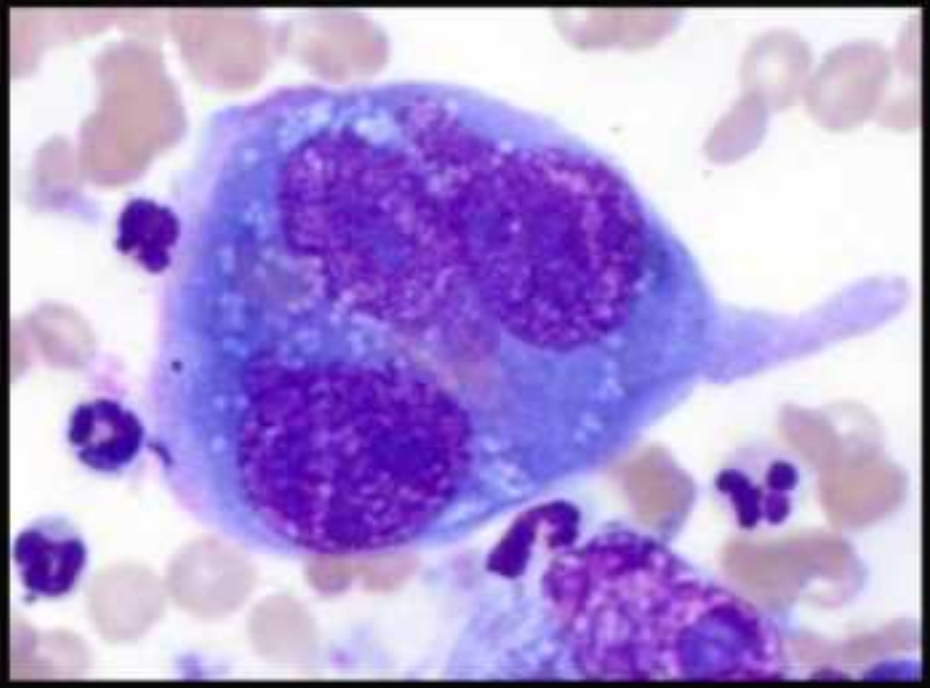


Prominent, multiple, & variably-sized nucleoli

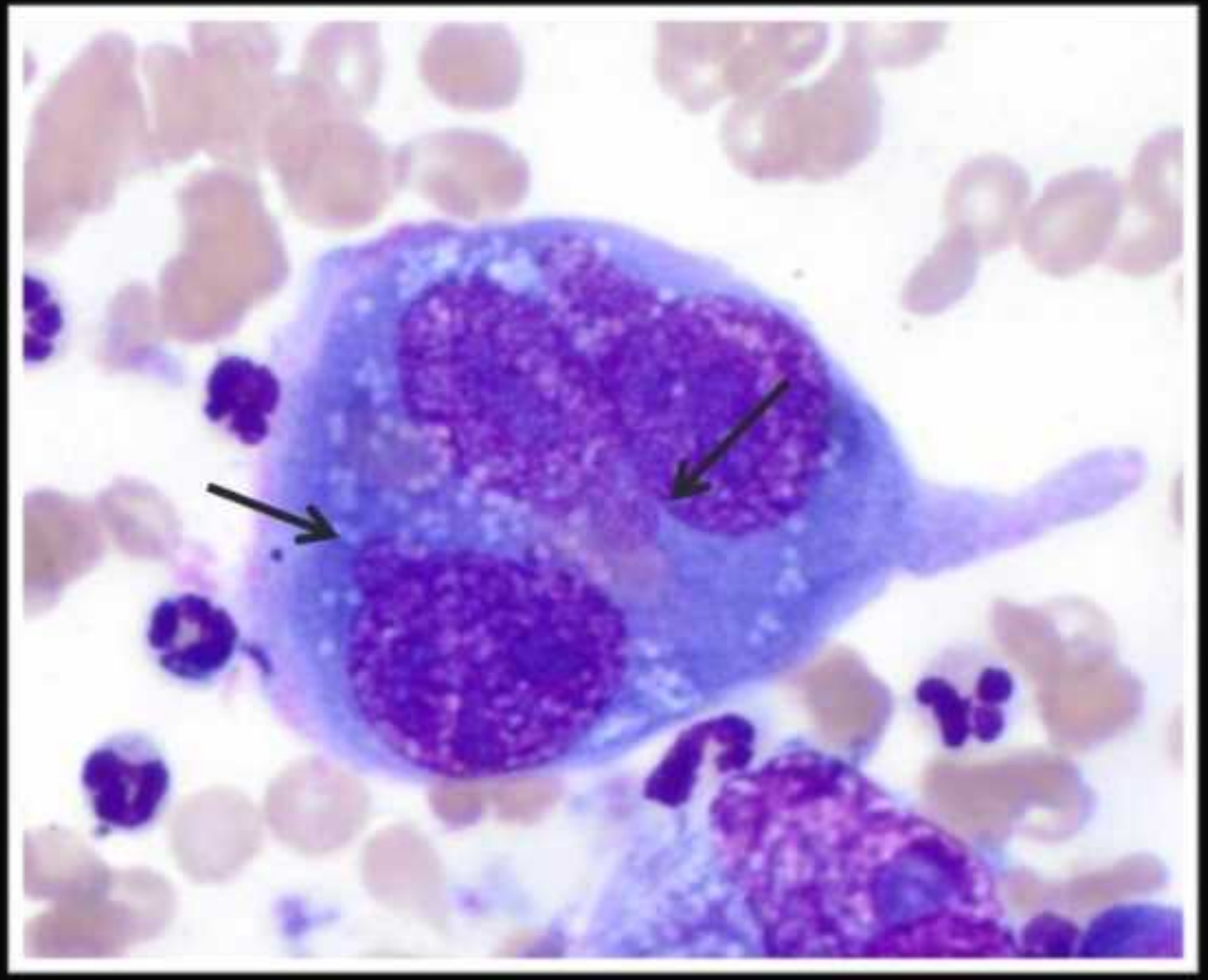
Criteria of Malignancy



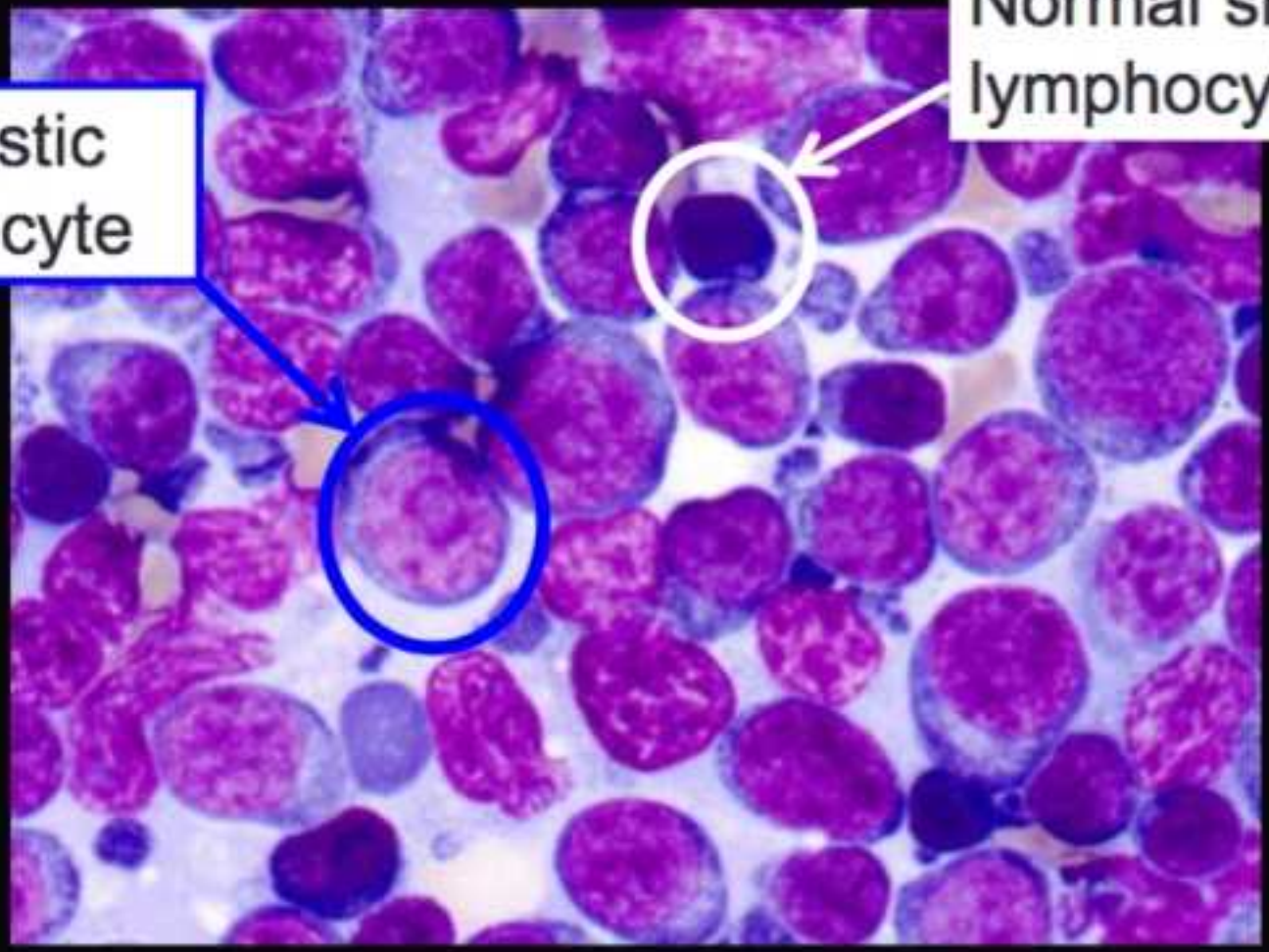
Nuclear molding



Multinucleation

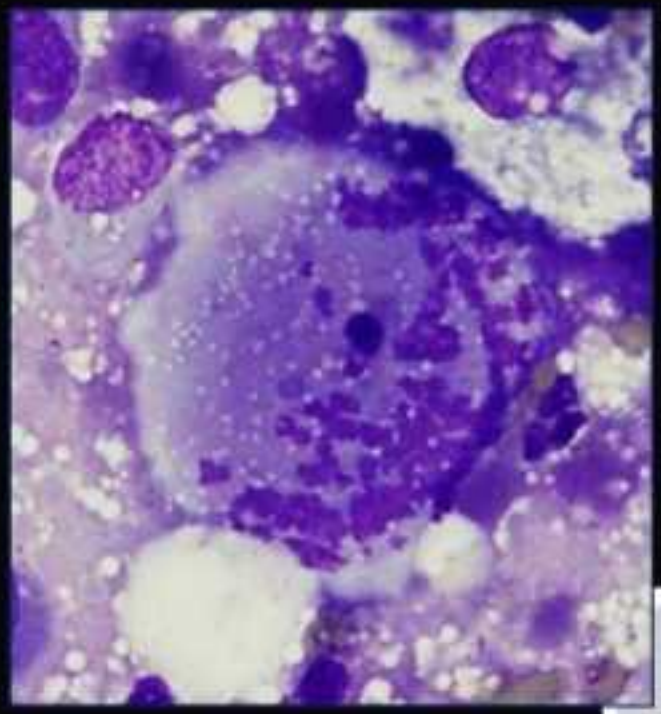


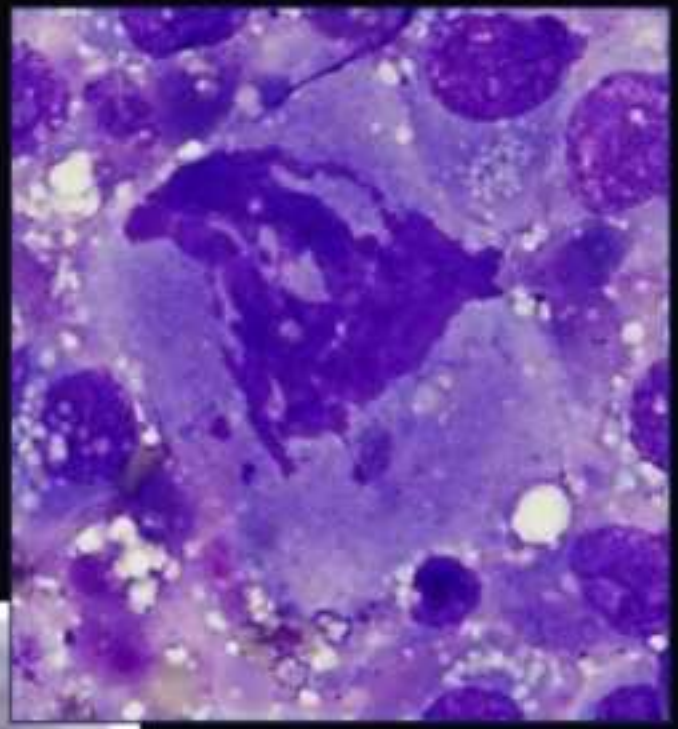
Nuclear blebs

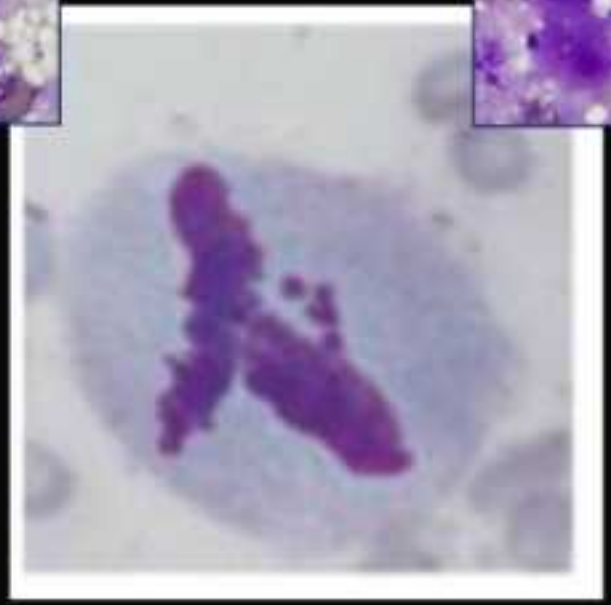


Altered chromatin pattern

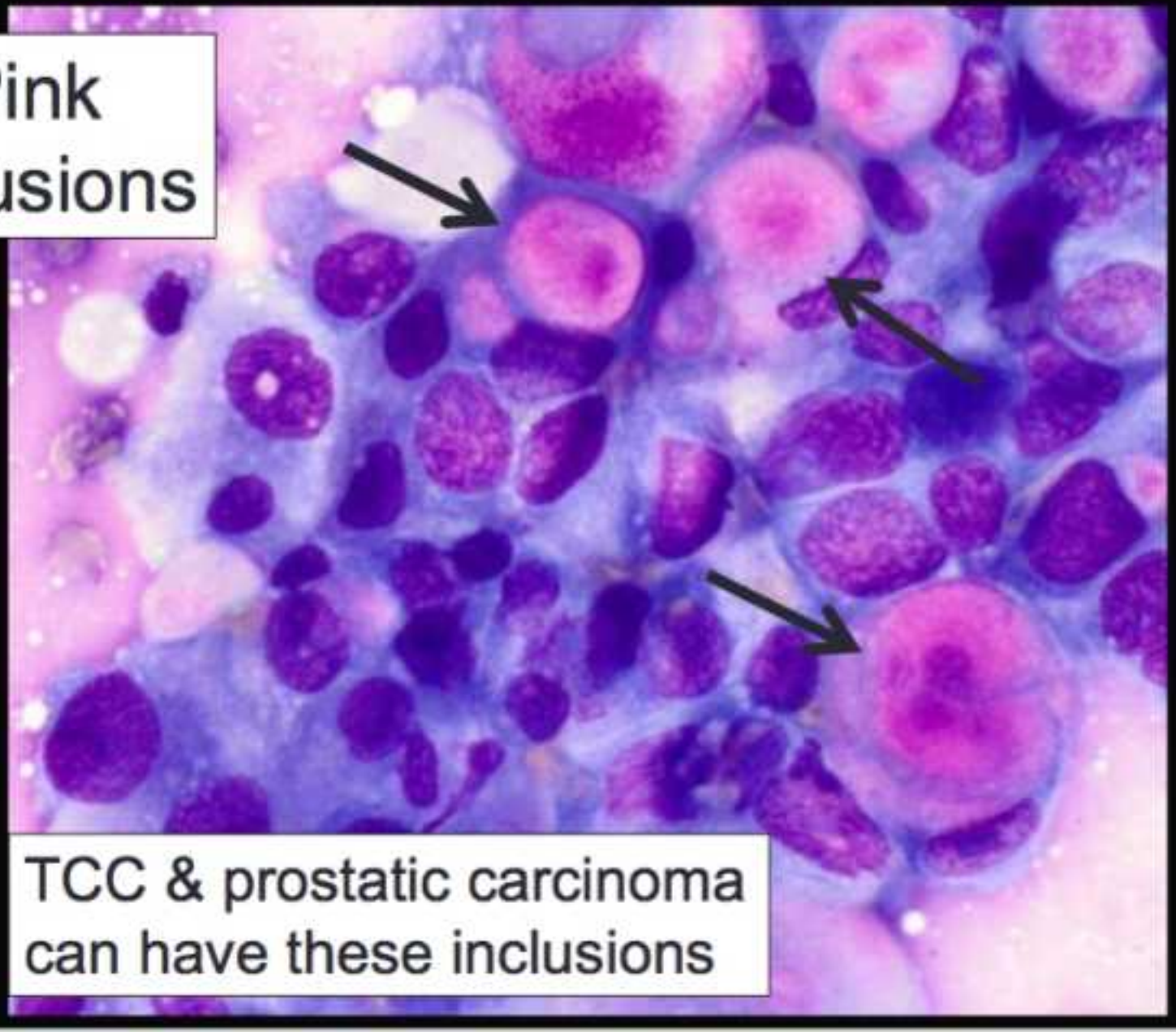
Aberrant mitotic figures



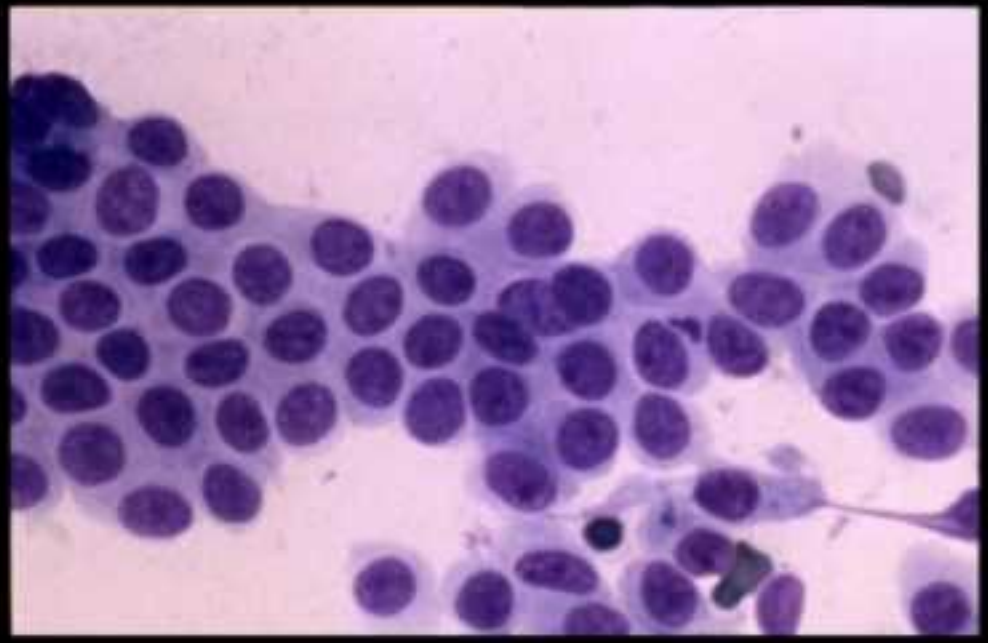




Atypical vacuolation or inclusions

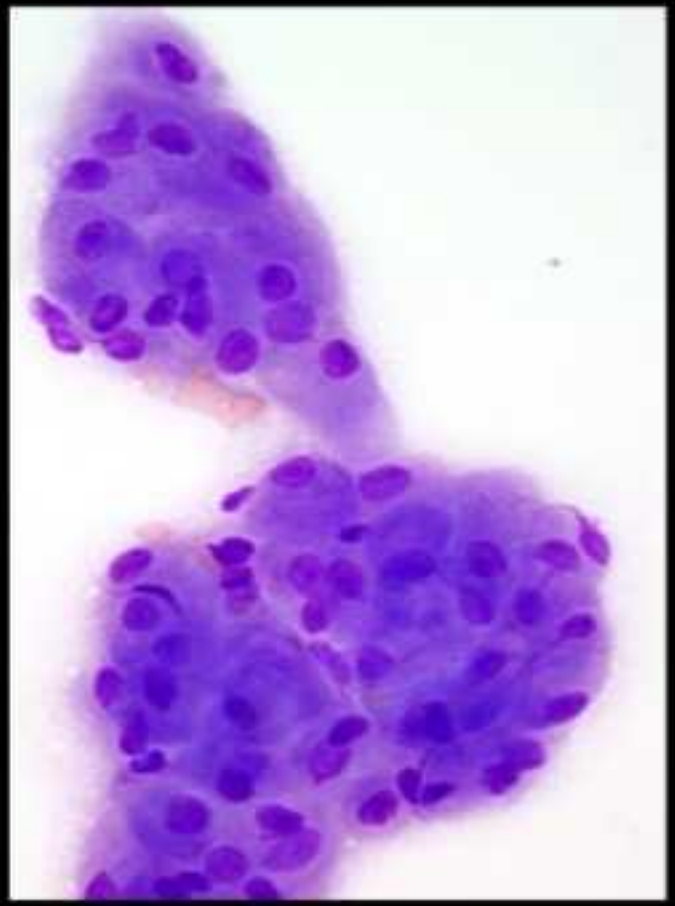


Benign Epithelial Tumors



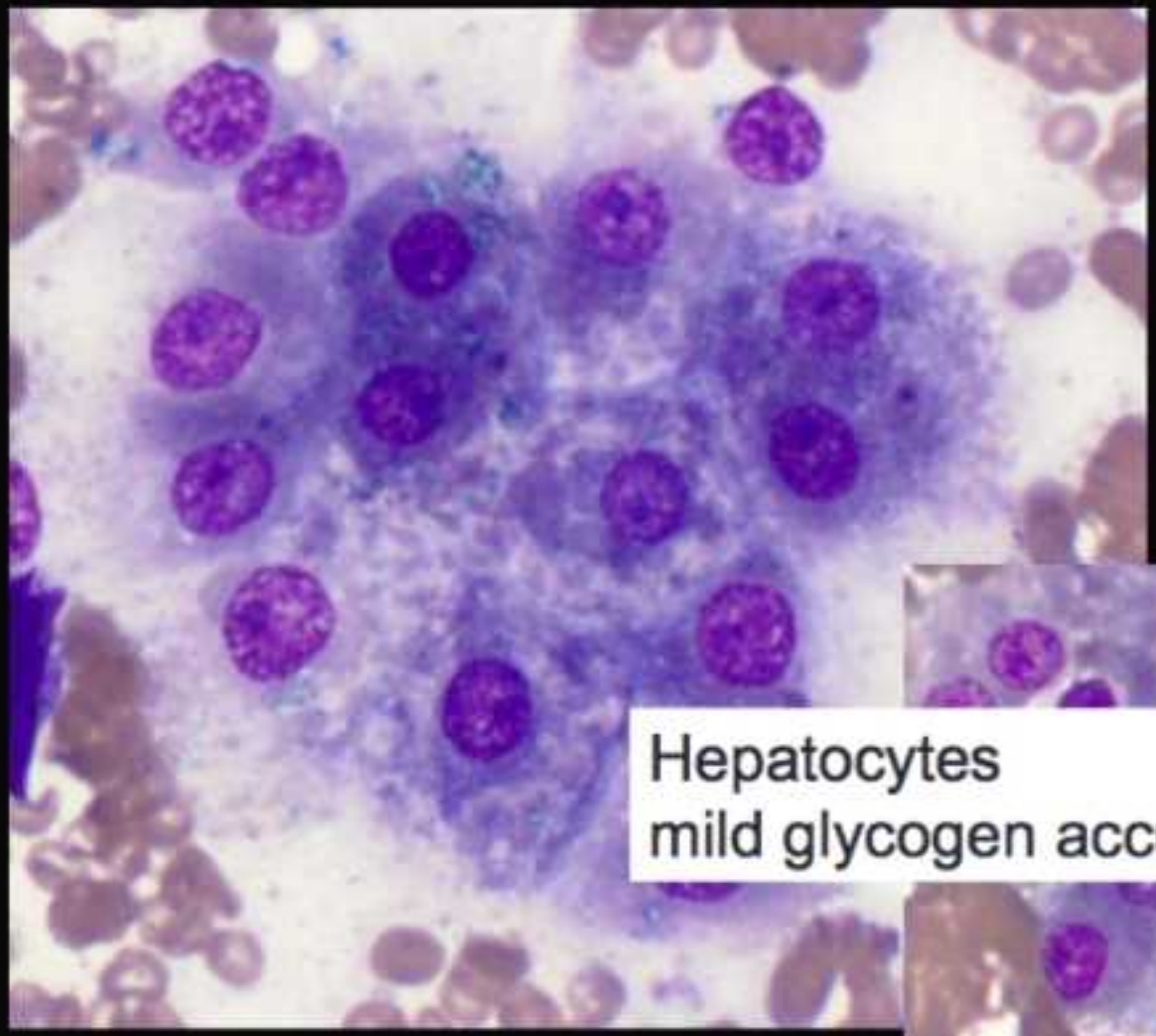
Mammary adenoma in a bitch

Uniform nuclear size & consistent N:C ratio



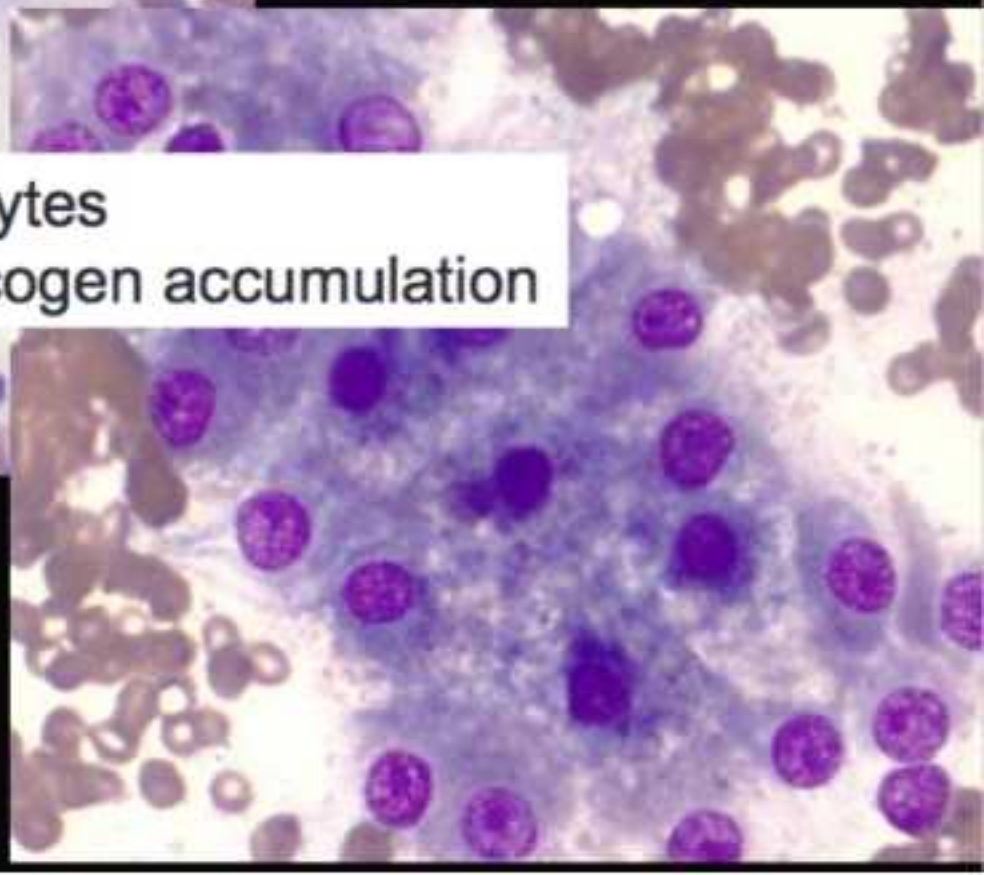
Perianal (hepatoid) gland adenoma in a dog

Both of these are "perfect" examples of cell-to-cell adhesion: sheets and uniformity of cells and nuclei with distinct cell borders!



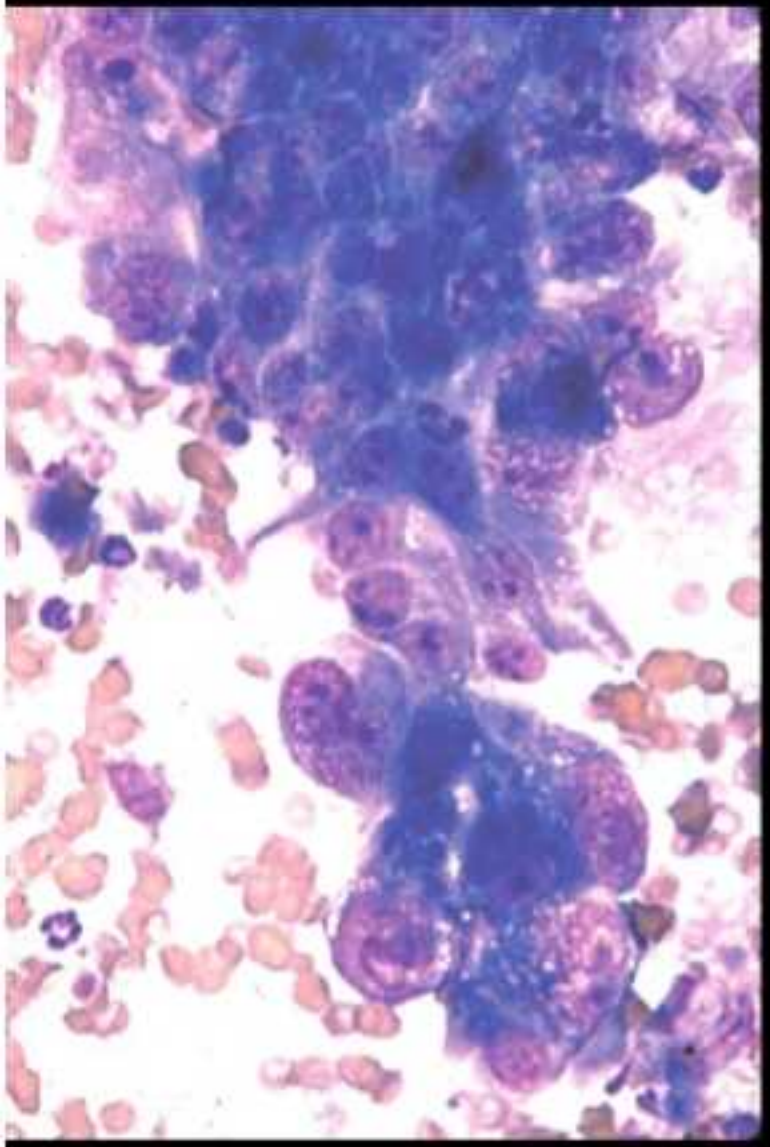
Epithelial

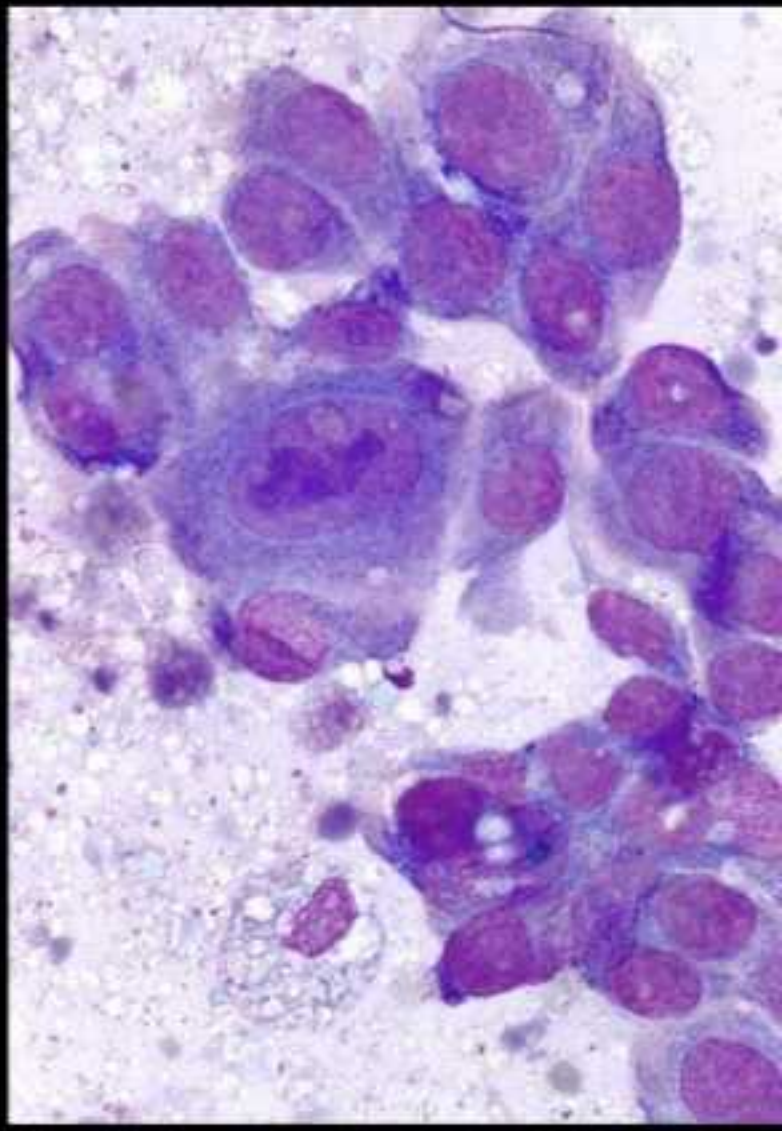
Hepatocytes
mild glycogen accumulation

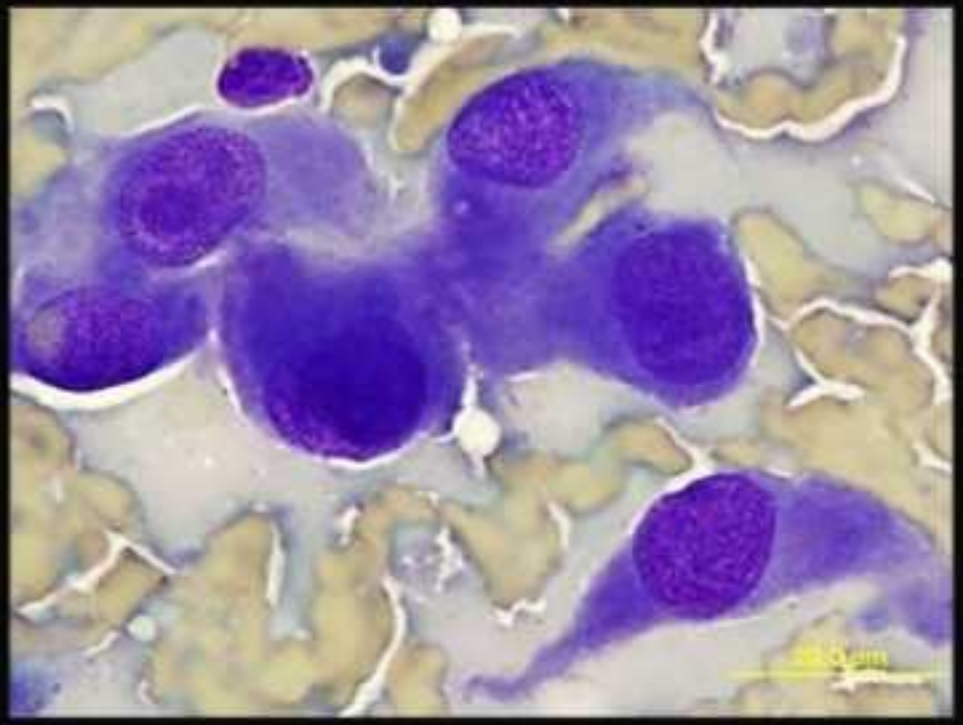


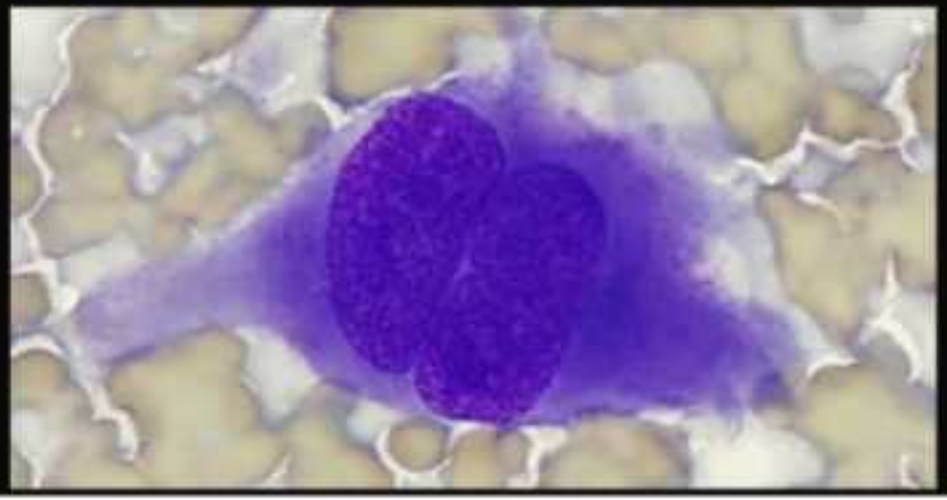
Benign

Malignant Epithelial Tumors









Mesenchymal malignant

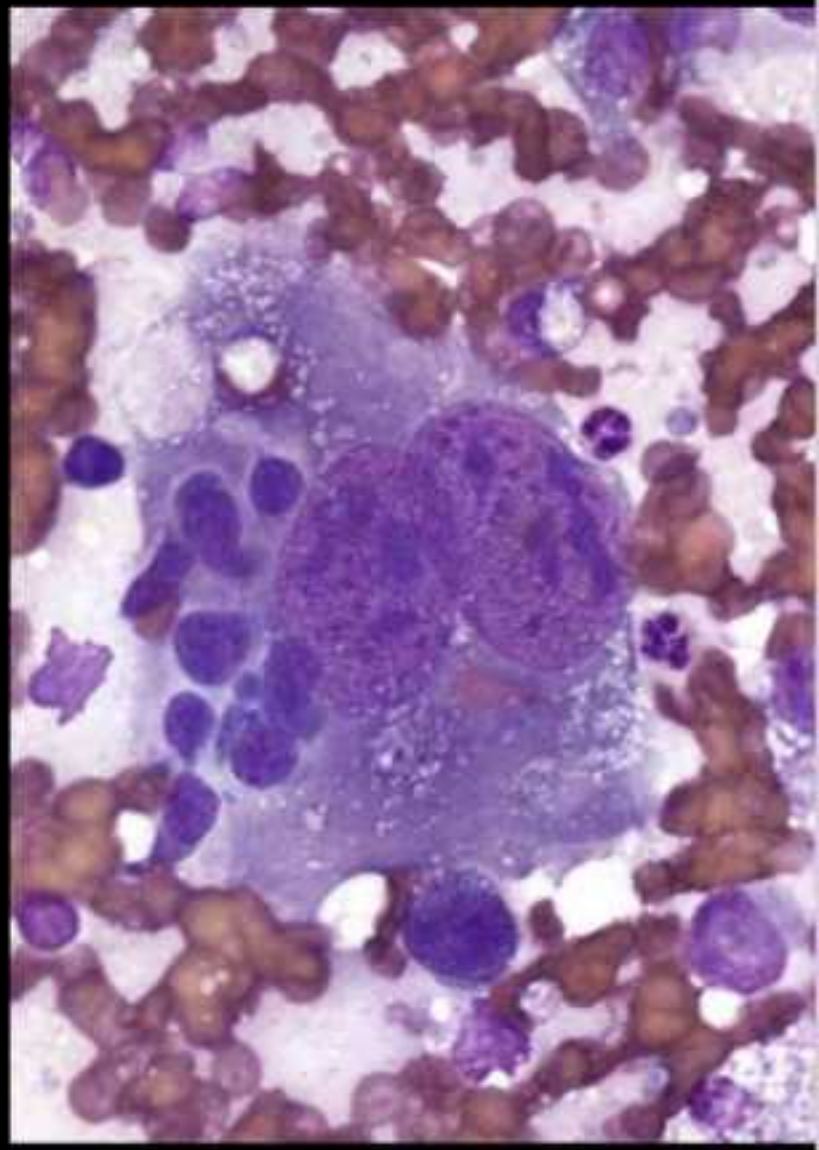
Malignant Mesenchymal Tumor

Fine needle aspirate from the lung of a dog with histiocytic sarcoma

Note the large binucleate cell with massive nuclei and multiple, irregular shaped nucleoli.

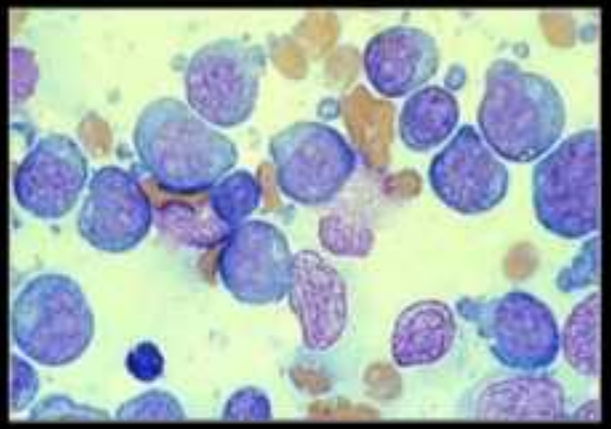
Note phagocytized erythrocytes (a feature of malignant histiocytic tumors)

The erythrocytes & neutrophils in the field provide an indication of the size of the tumor cell.



Diagnostic Tree

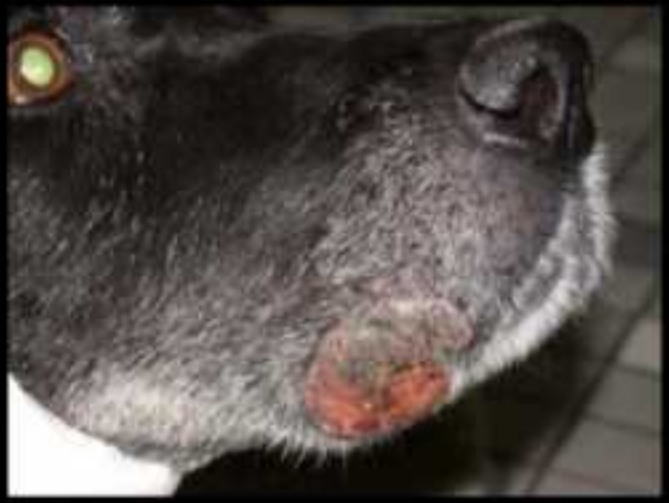
- Mass
  - Inflammatory
    - Neutrophils
      - Healthy
      - Degenerate
    - Eosinophils
    - Mixed
  - Non-inflammatory
    - Non-neoplastic
      - Cyst
      - Hematoma
      - Sialocele
    - Neoplasia Hyperplasia
      - Round
      - Epithelial
      - Mesenchymal

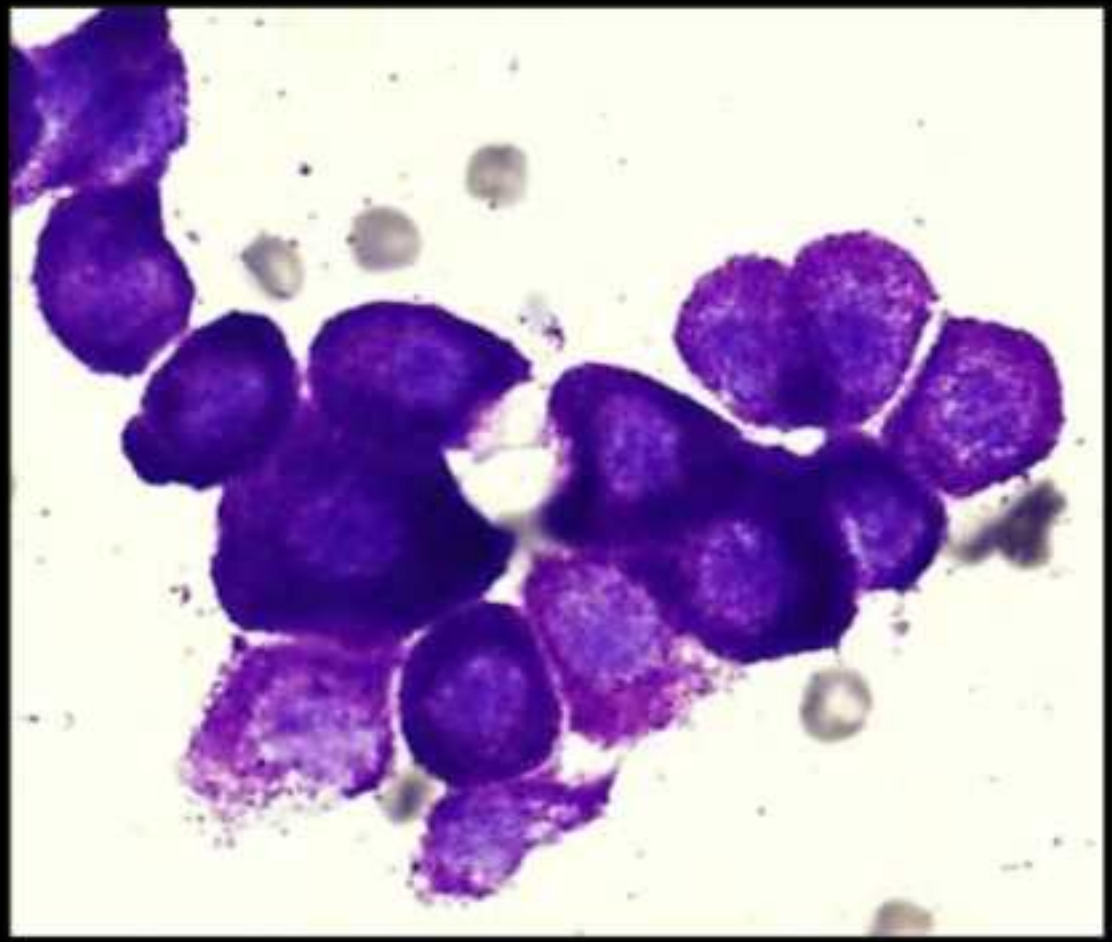


Round Cell Tumors

Lymphoma	Monomorphic Lymph population	
Mast Cell Tumor	Metachromatic granules	Eosinophils; Diff-Quik
Histiocytoma	Plain; 'fried egg'	Young dogs; lymphocytes
Plasmacytoma	Eccentric nucleus	Golgi
TVT	Ropey chromatin; vacuoles	location

11-year-old Labrador Retriever

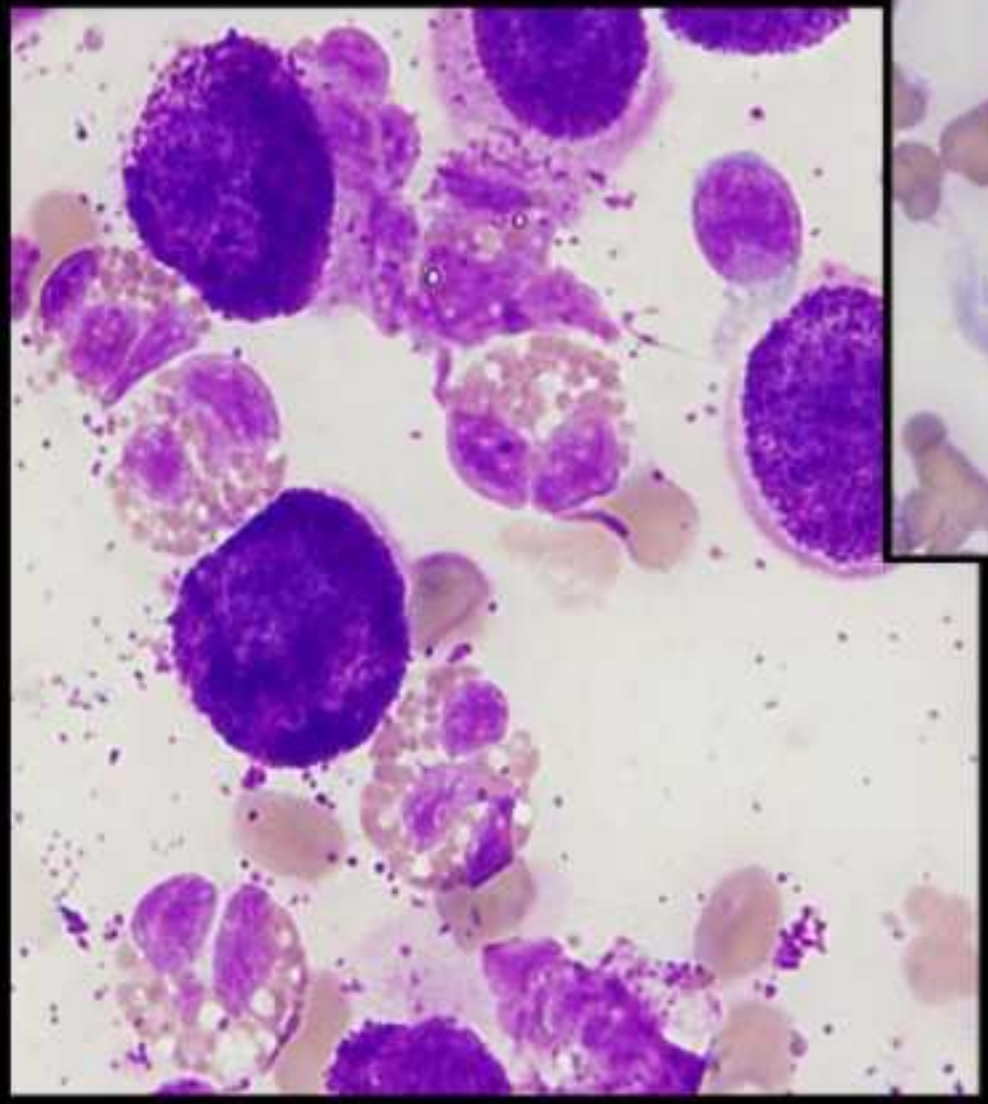


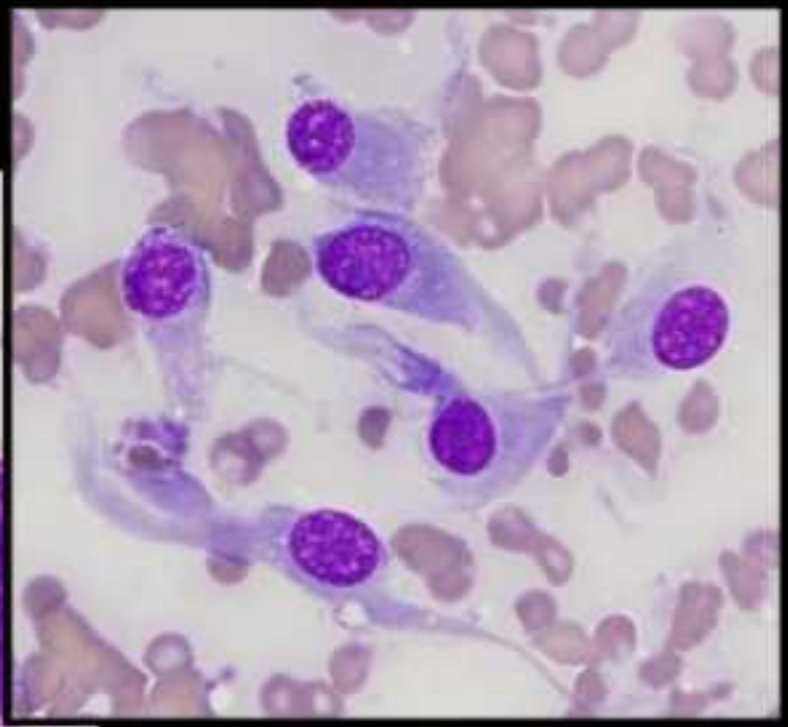


Granules do not always stain with *Diff Quik*.

Some tumors just don't have cells with many granules.

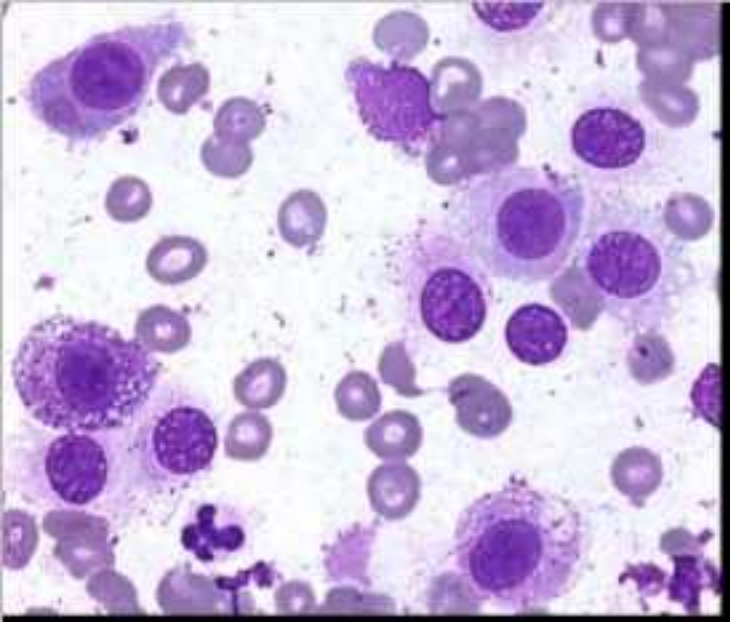
Mast cell tumors

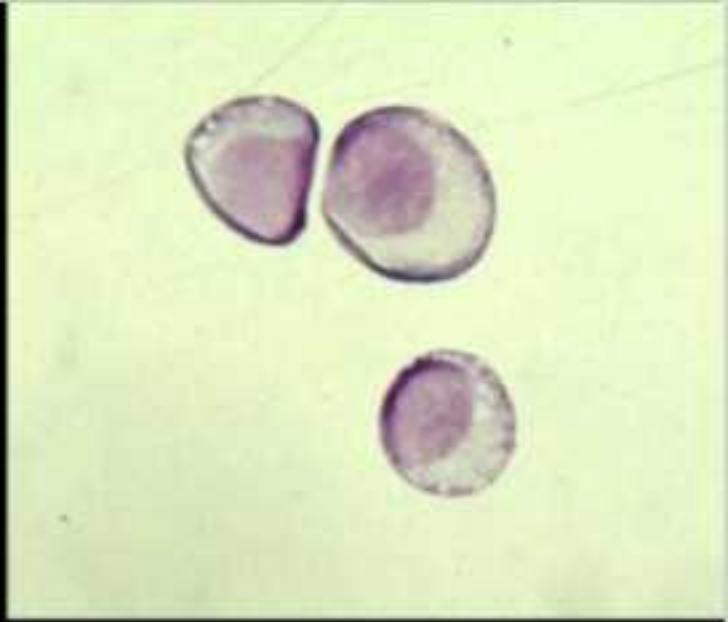




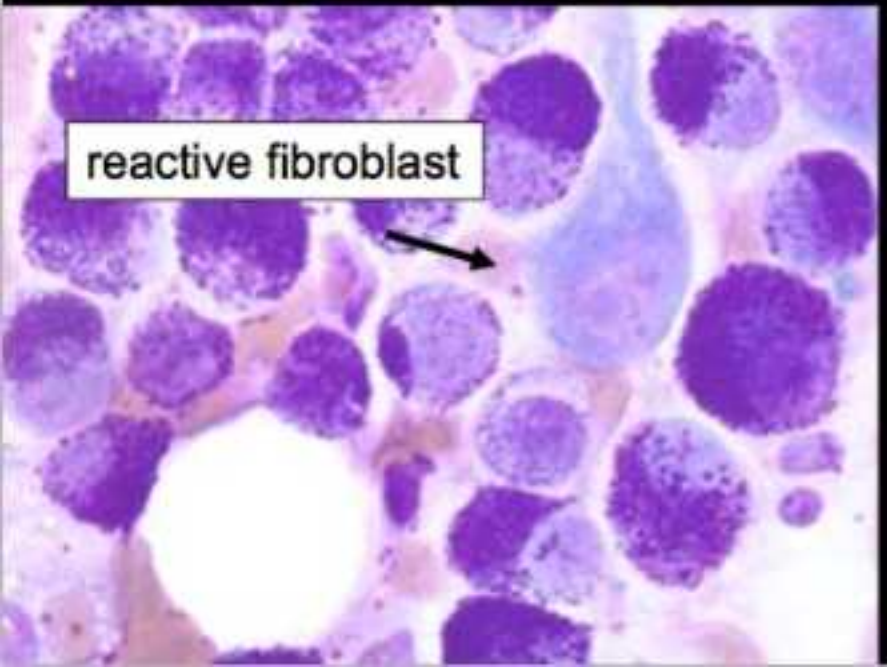
Canine mast cell tumors:

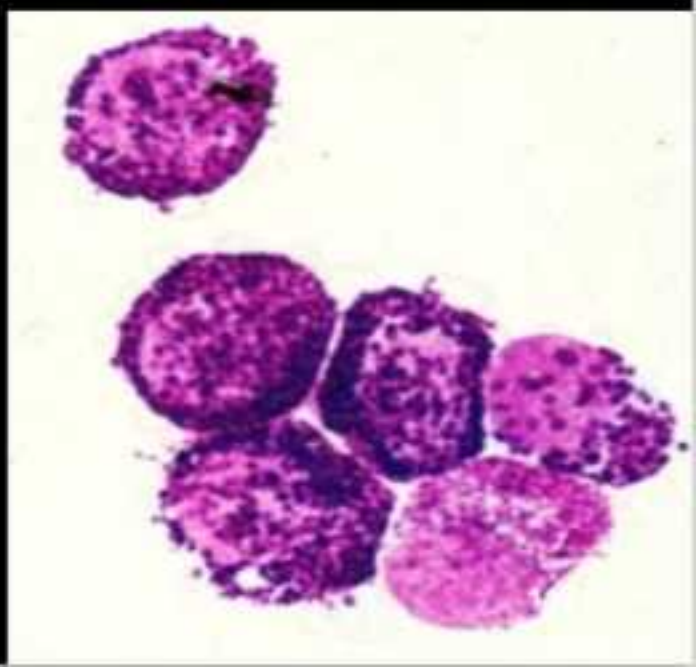
- mast cells
- eosinophils
- reactive fibroblasts





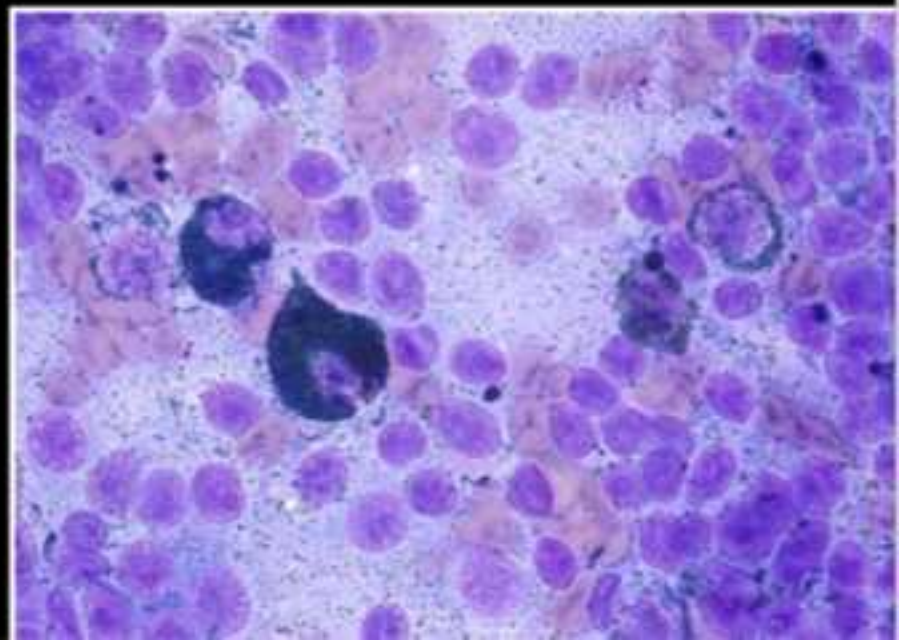
Variable granule staining





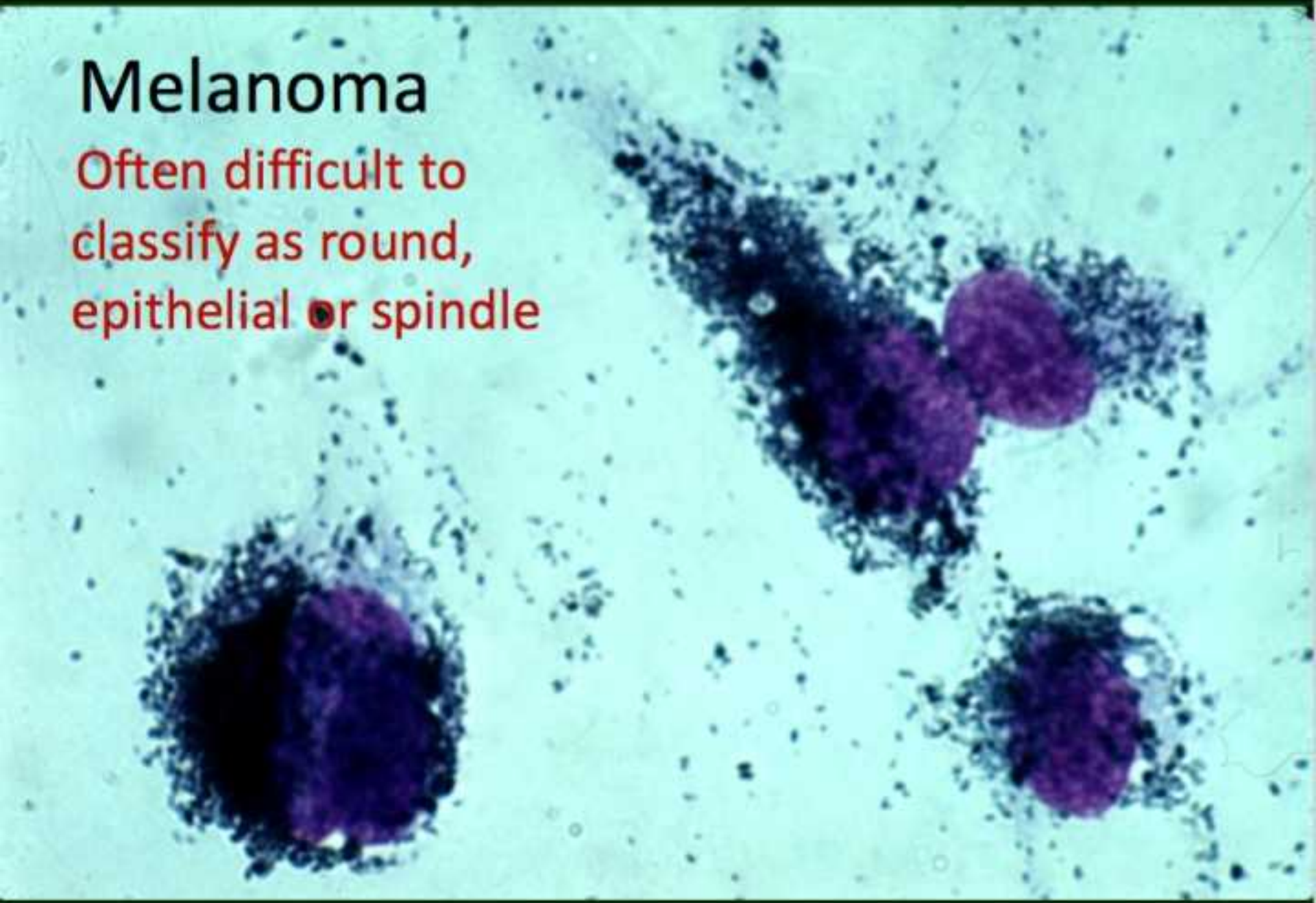
Neoplasms With Cytoplasmic Granules

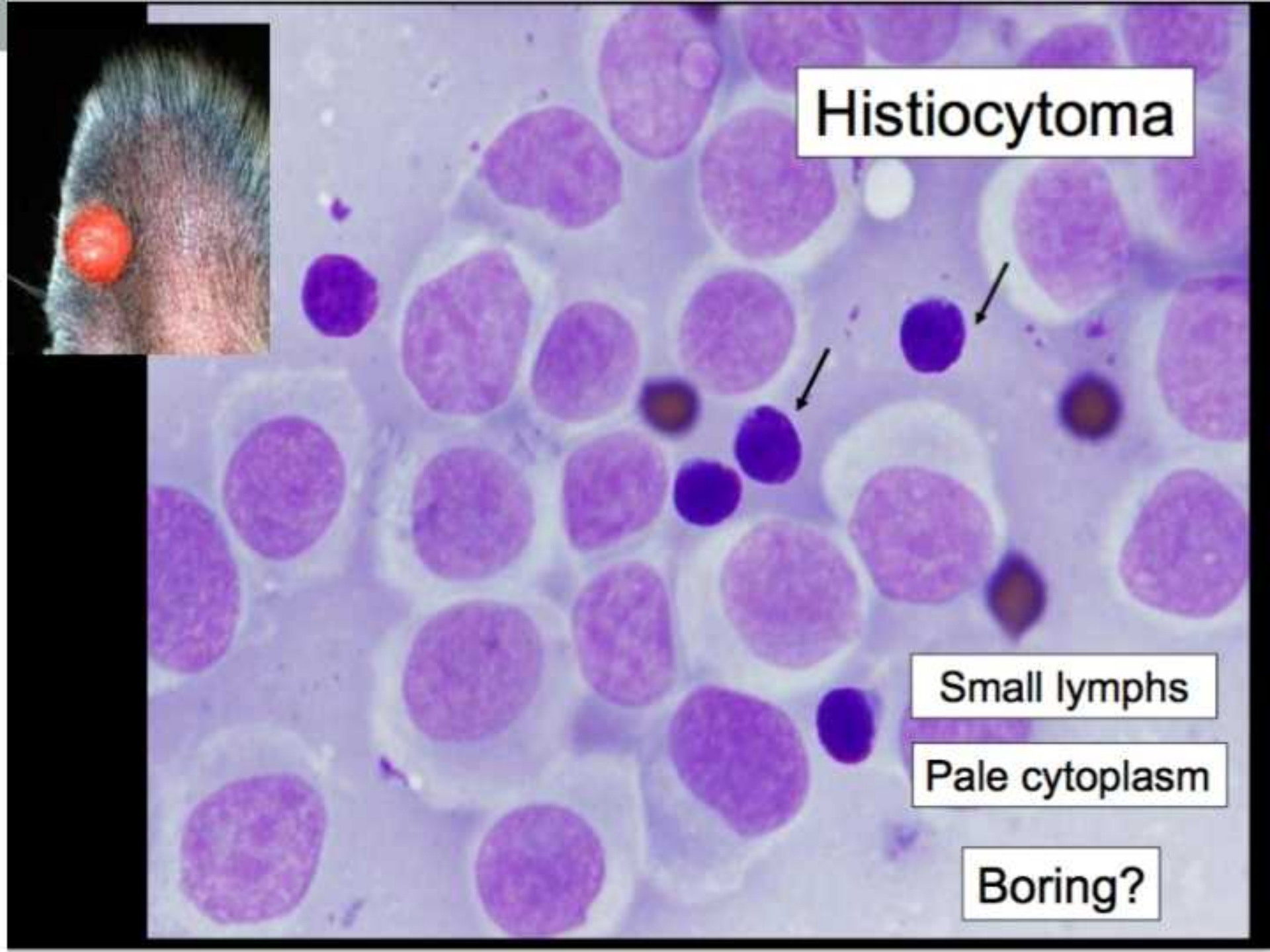
- Mast cell tumor
- Melanoma
  - Black, green, blackish-green granules
  - Variable numbers of granules
  - Pleomorphic cells
    - Round
    - Polygonal
    - Spindle
    - Mixture of shapes

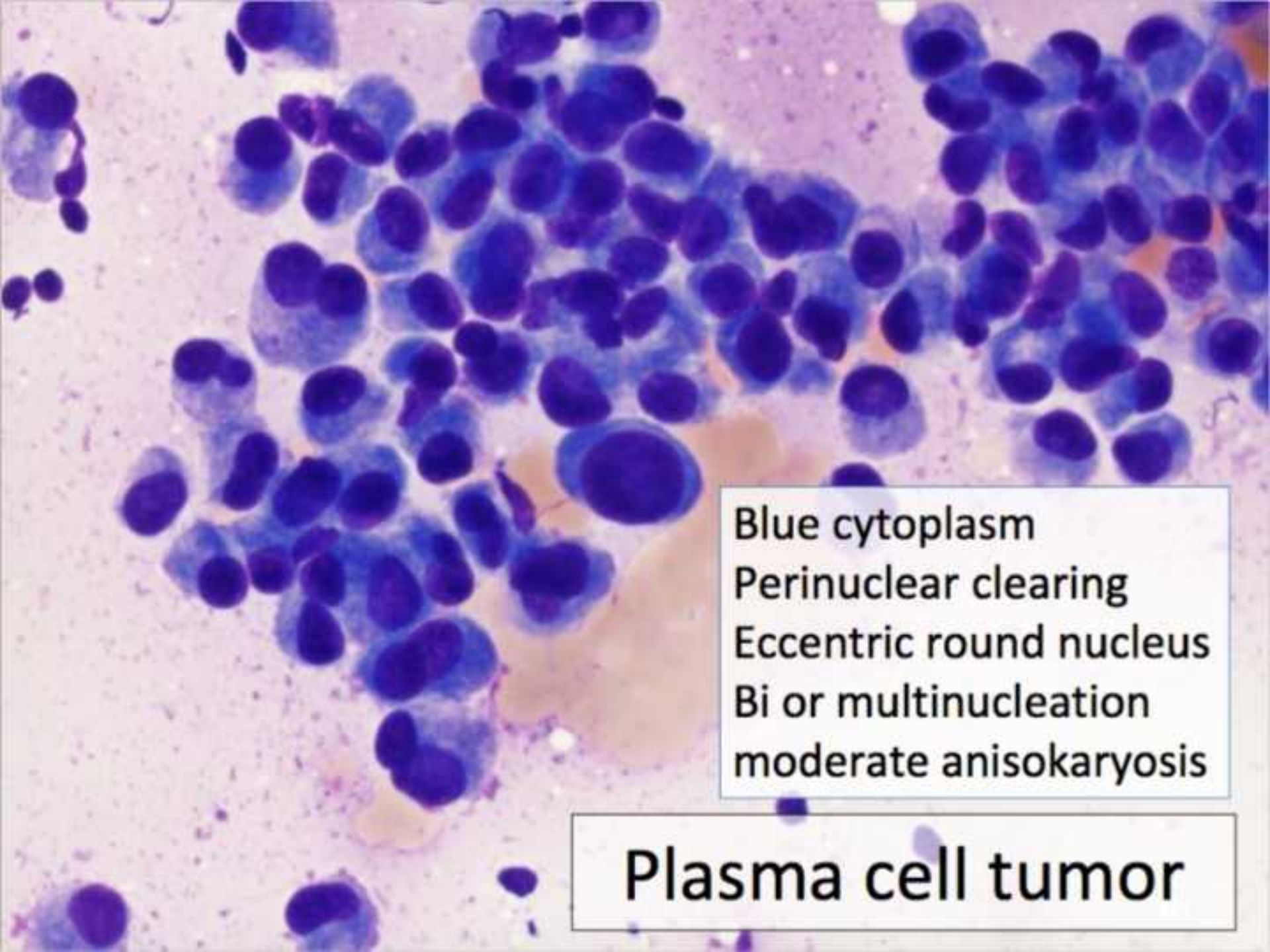


Melanoma

Often difficult to classify as round, epithelial or spindle

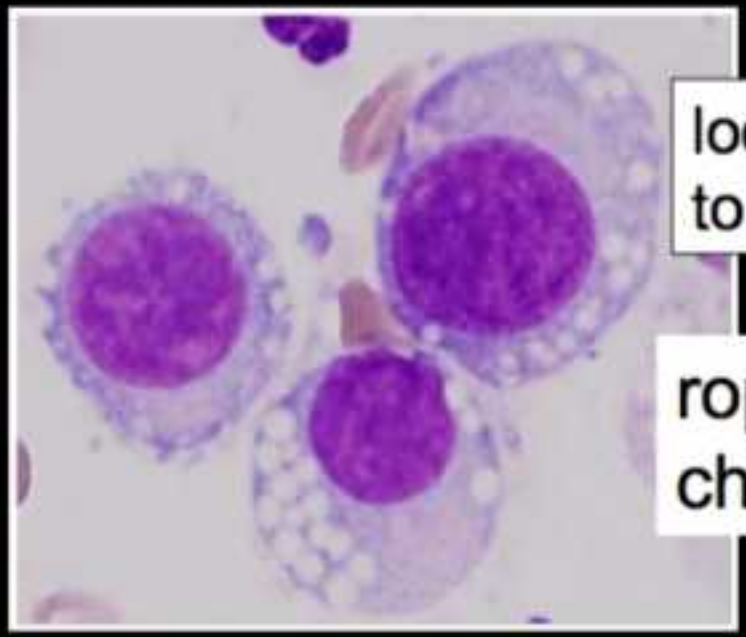



Histiocytoma
Small lymphs
Pale cytoplasm
Boring?

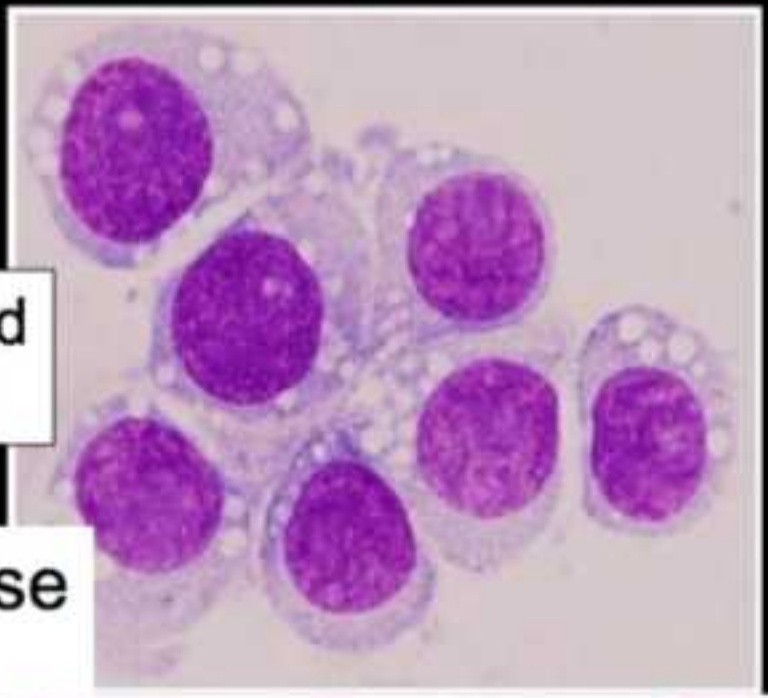

Blue cytoplasm
Perinuclear clearing
Eccentric round nucleus
Bi or multinucleation
moderate anisokaryosis
Plasma cell tumor

TVT

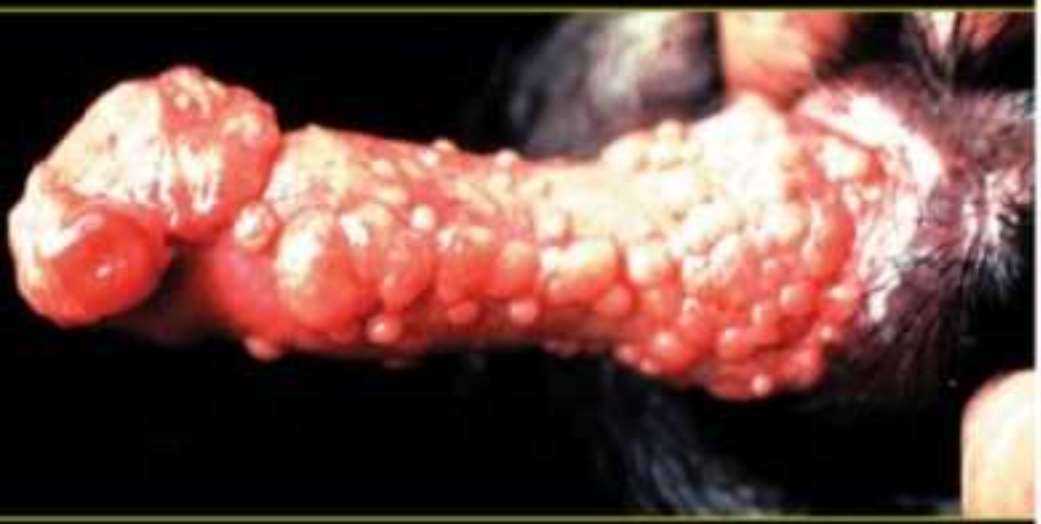
punctate vacuoles

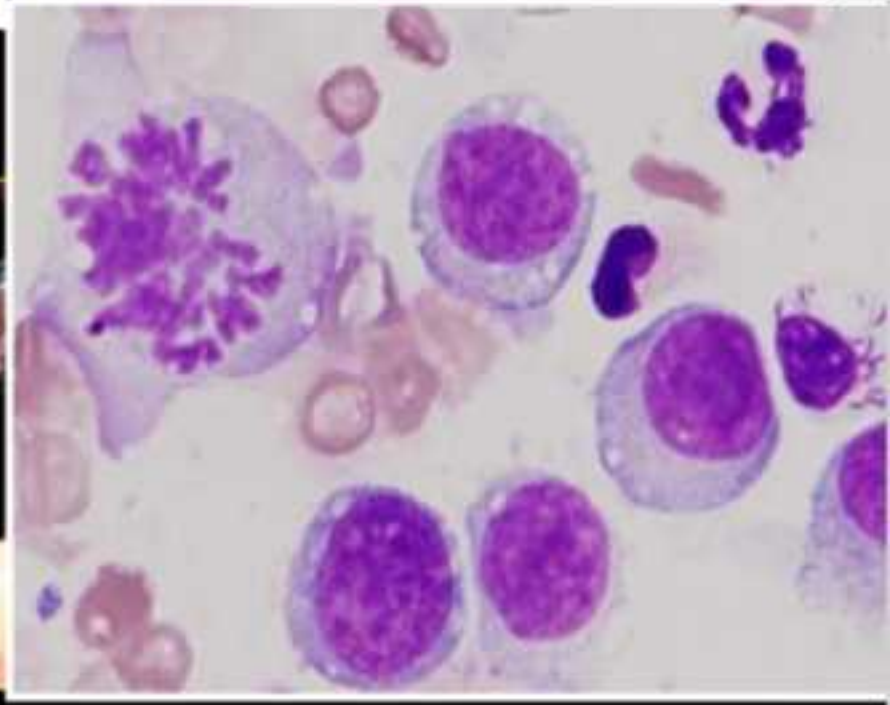


look lymphoid to histiocytic



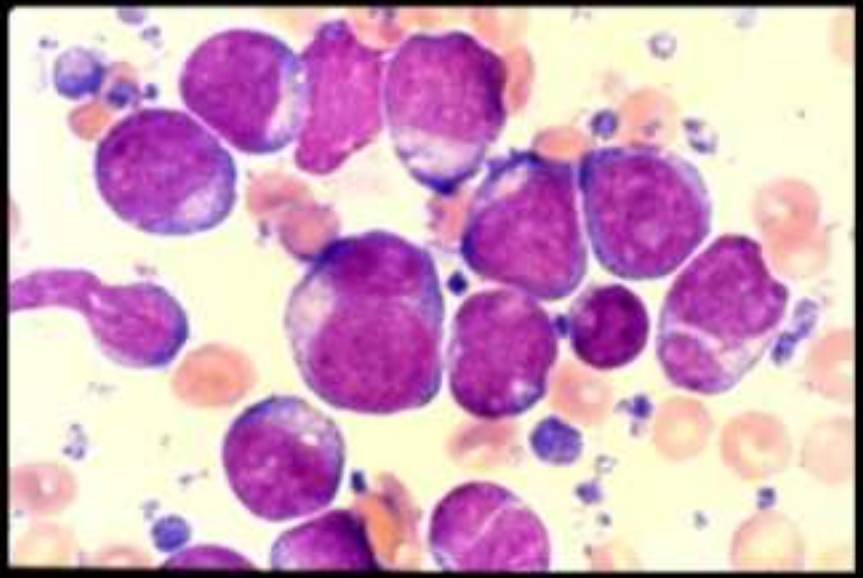
ropey, coarse chromatin

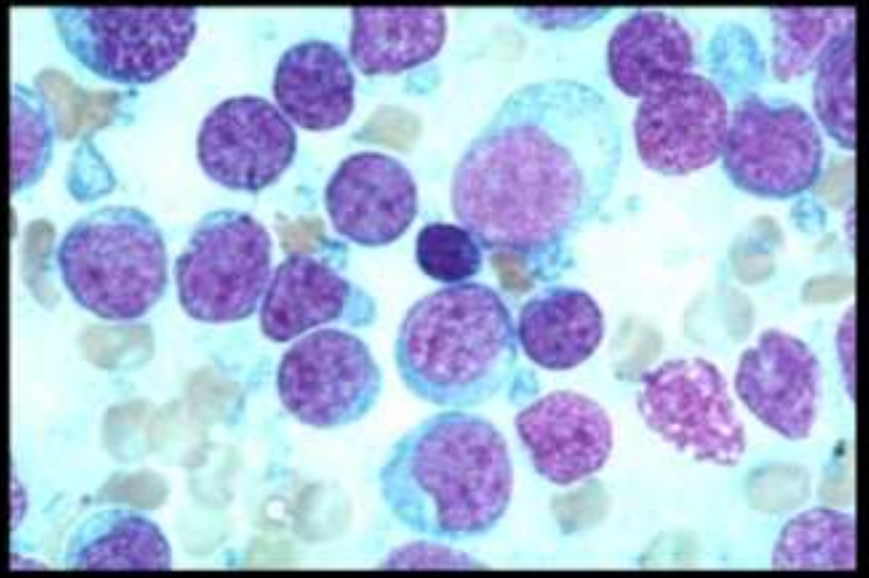




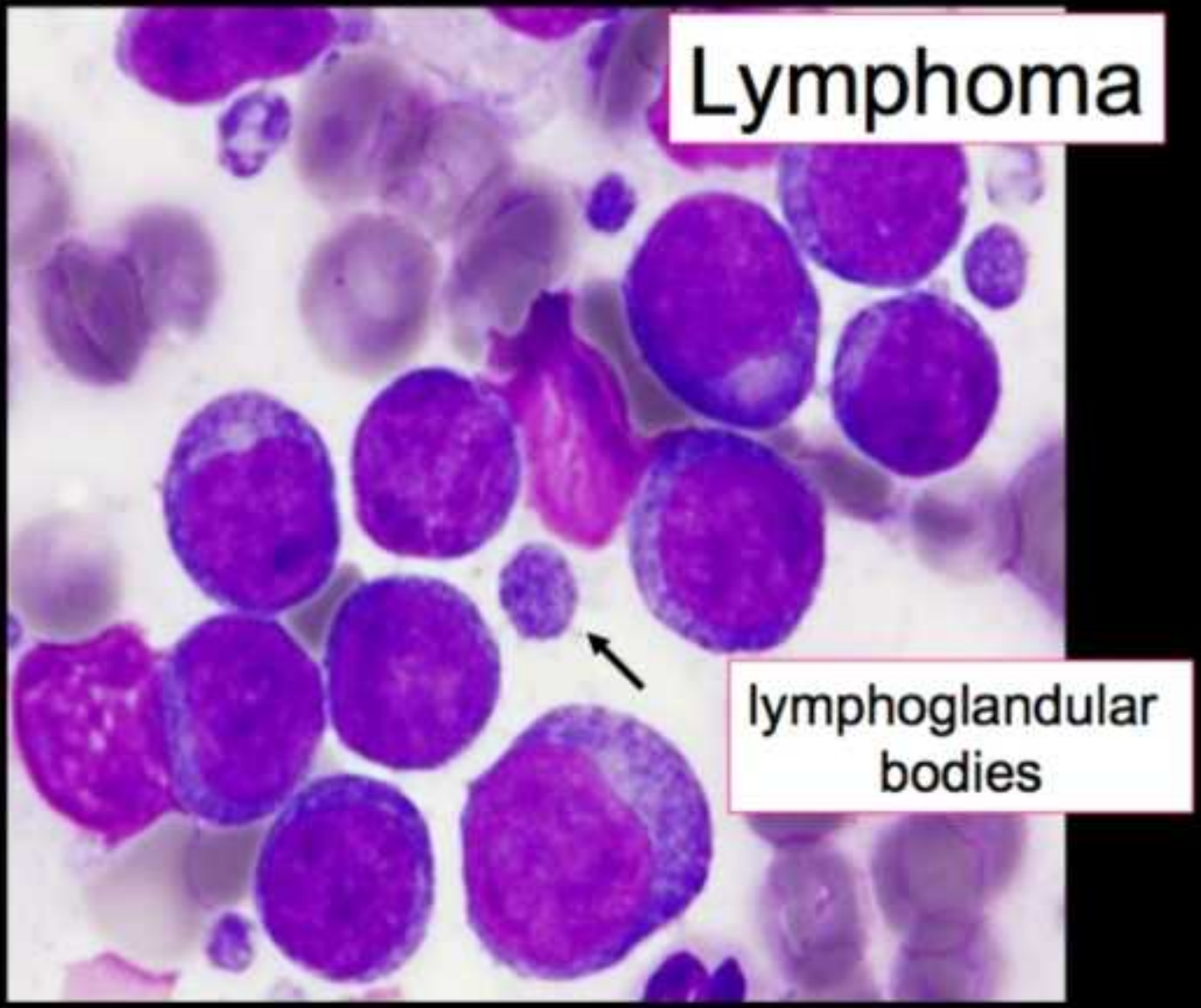
Lymphoma

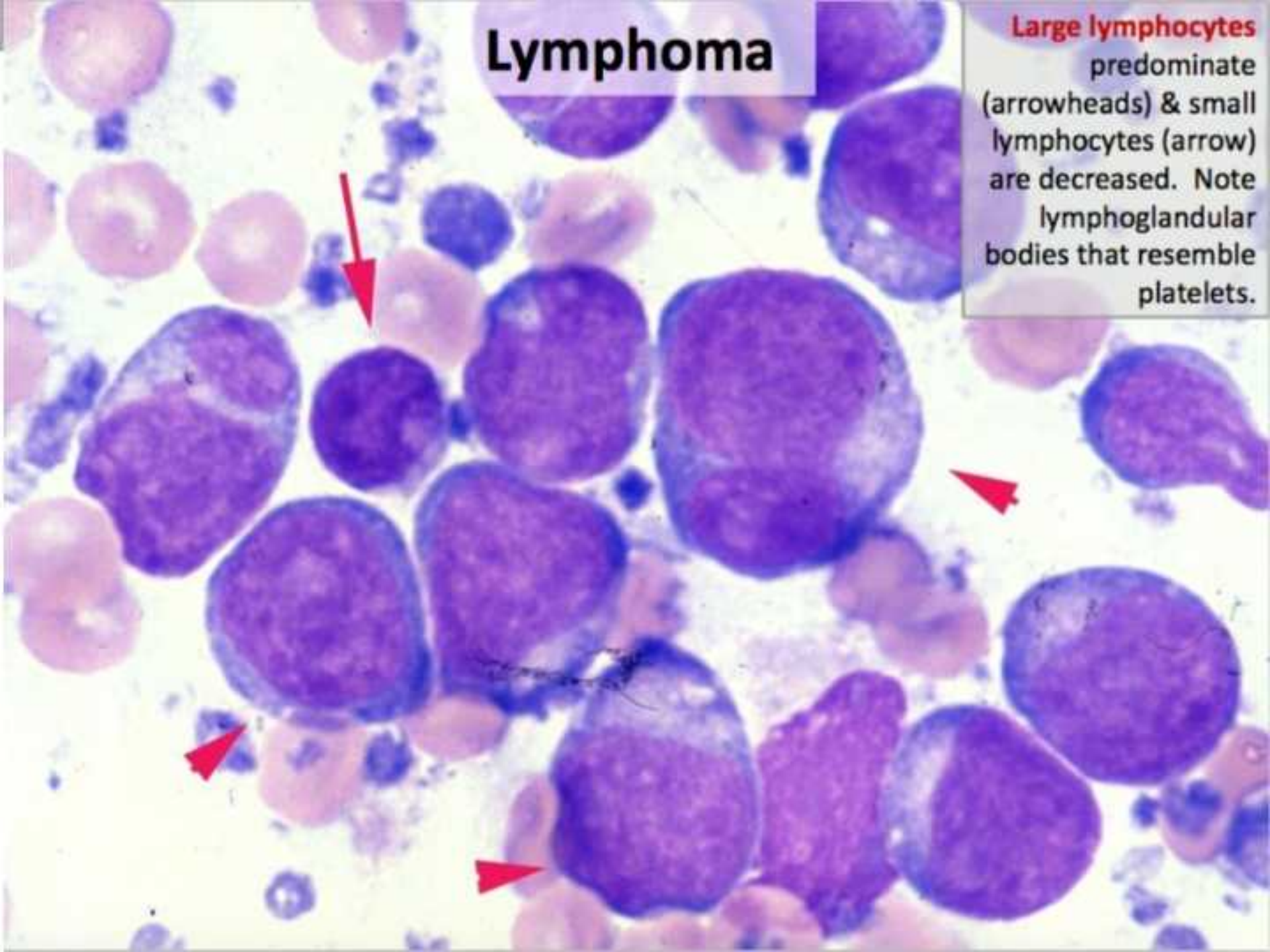
- Monomorophic population of medium to large lymphocytes (>50% of cells); decreased small lymphocytes; rare plasma cells
- May have numerous lymphoglandular bodies (cytoplasmic remnants)
- Presence of mitotic figures variable





Large 'inguinal'
mass in 12 year old, grey, horse.

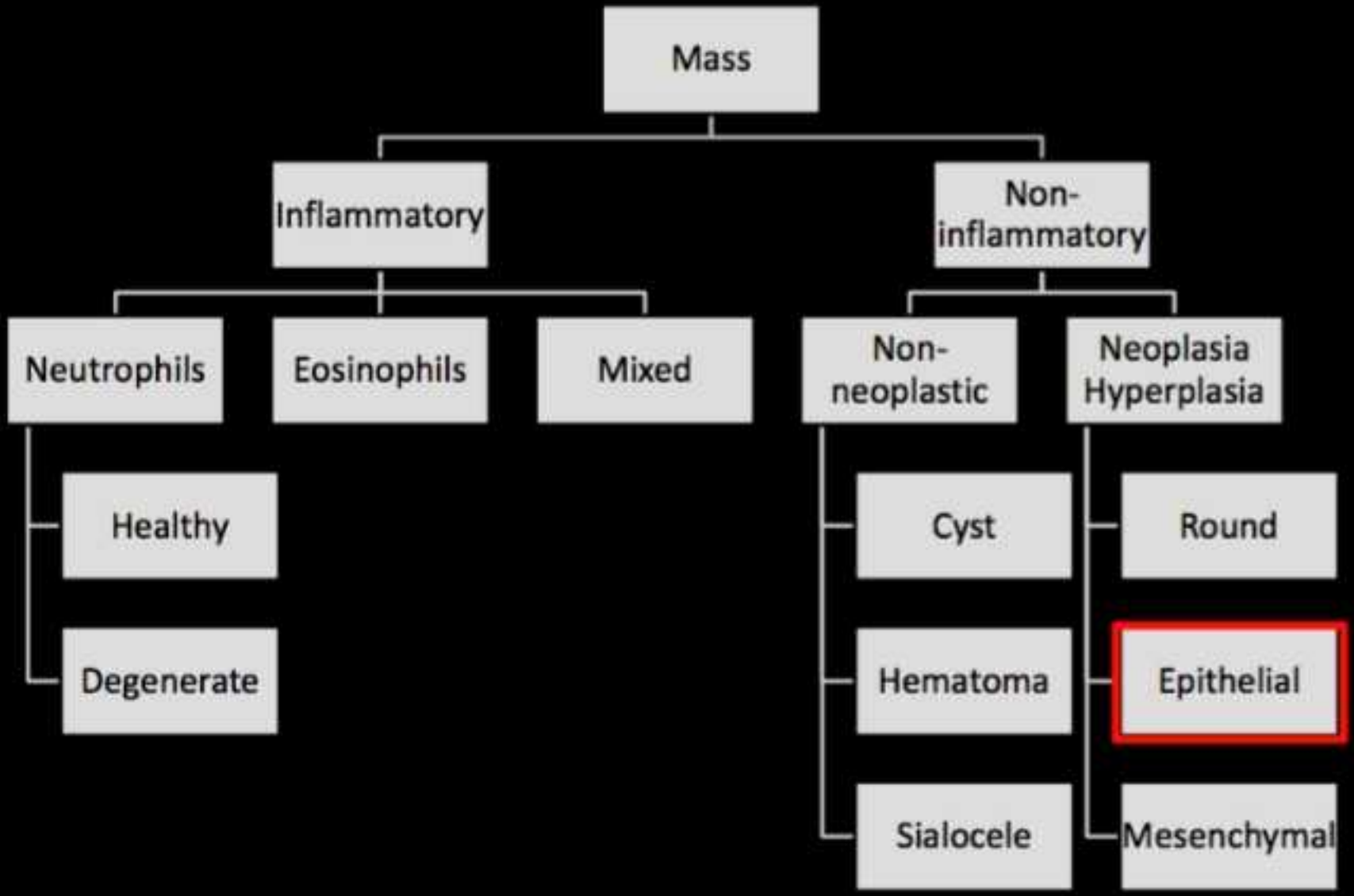



Lymphoma
Large lymphocytes predominate (arrowheads) & small lymphocytes (arrow) are decreased. Note lymphoglandular bodies that resemble platelets.

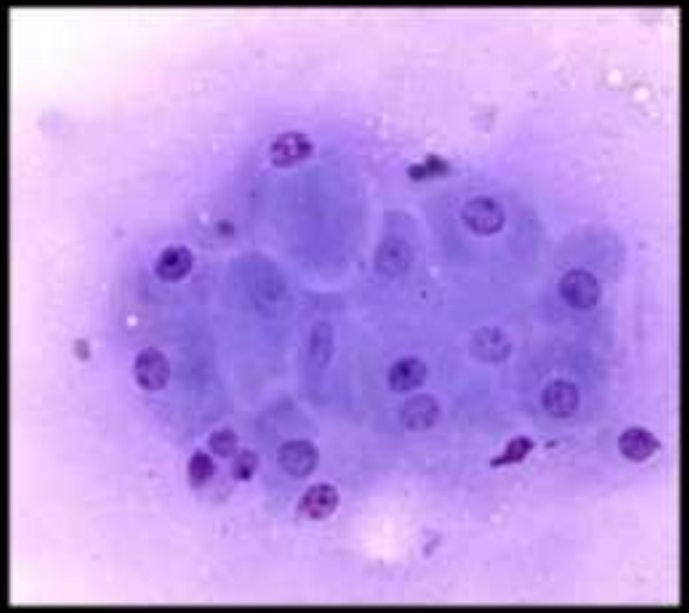
Summary: Round Cell Tumors

- *Mast cell tumor* – granules; eosinophils
- *Histiocytoma* – young dog, abundant pale blue cytoplasm; kidney-shaped nuclei
- *TVT* – location; look like histiocytoma, cytoplasmic vacuolation
- *Lymphoma* – scant cytoplasm; blast cells
- *Plasma cell* – eccentric nuclei, Golgi zone, abundant cytoplasm

Diagnostic Tree



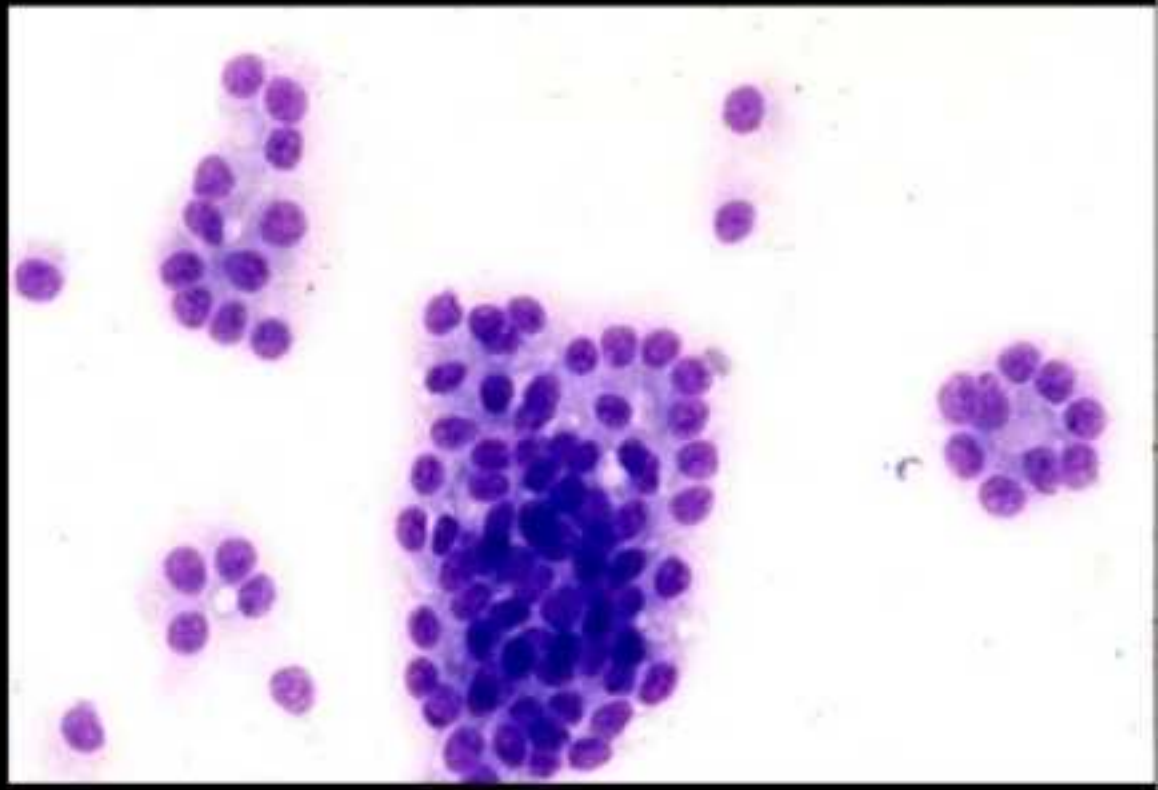
Epithelial Cell Tumors



- Adenoma vs. carcinoma
- Sebaceous gland tumors
- Mammary neoplasms
- Prostatic neoplasms
- Nasal tumors
- Transitional (urothelial) cell tumors
- Perianal neoplasms
- Basaloid epithelial tumors

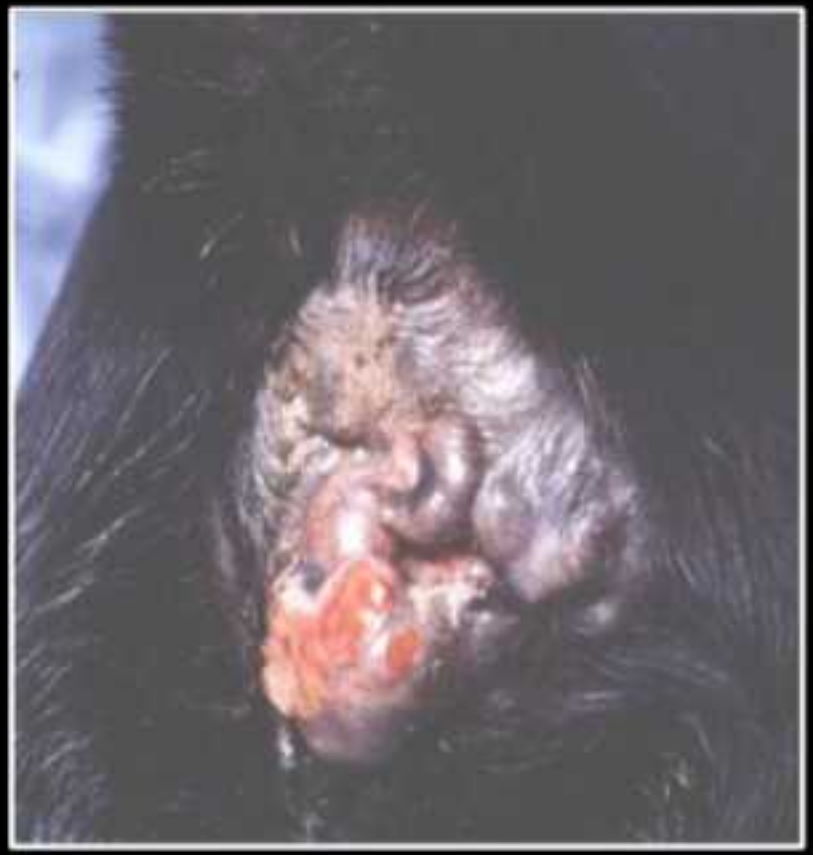
Epithelial Tumors

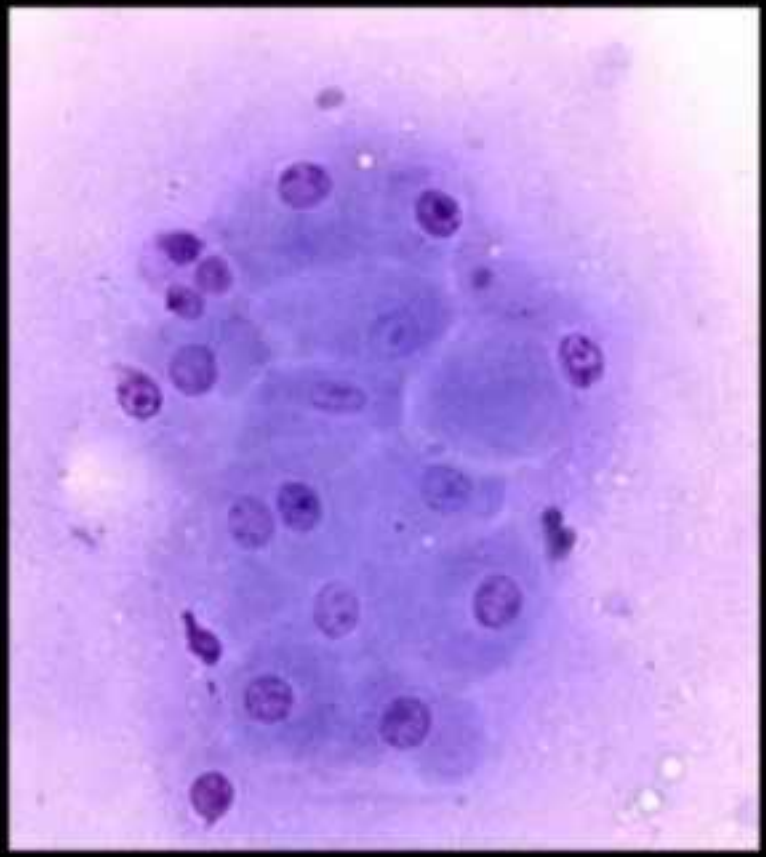
- Cells in sheets or clusters
- Usually many cells present
- Cytoplasmic borders usually distinct
- Often large cells w/ abundant cytoplasm
- May show signs of differentiation



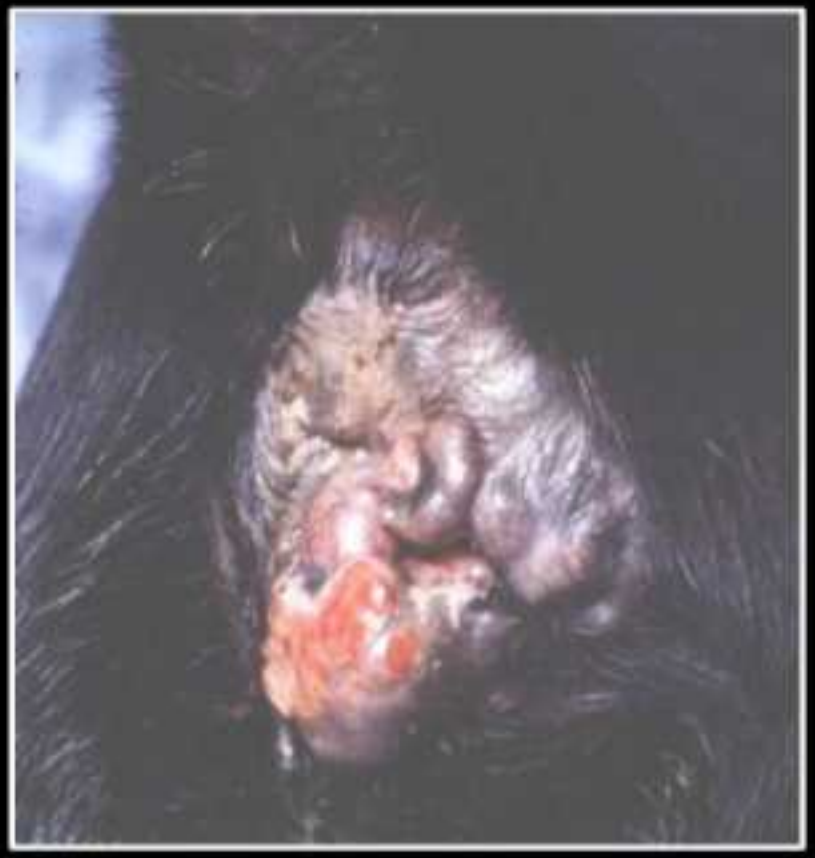
Adenoma: Uniform cells.
Acinar formation on far right.

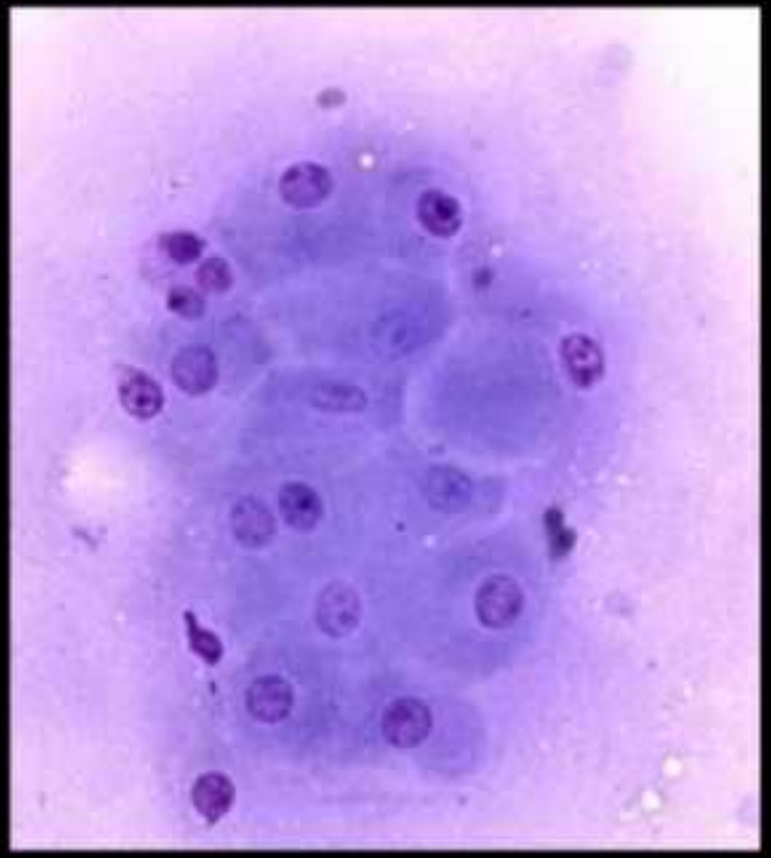
Diagnosis?





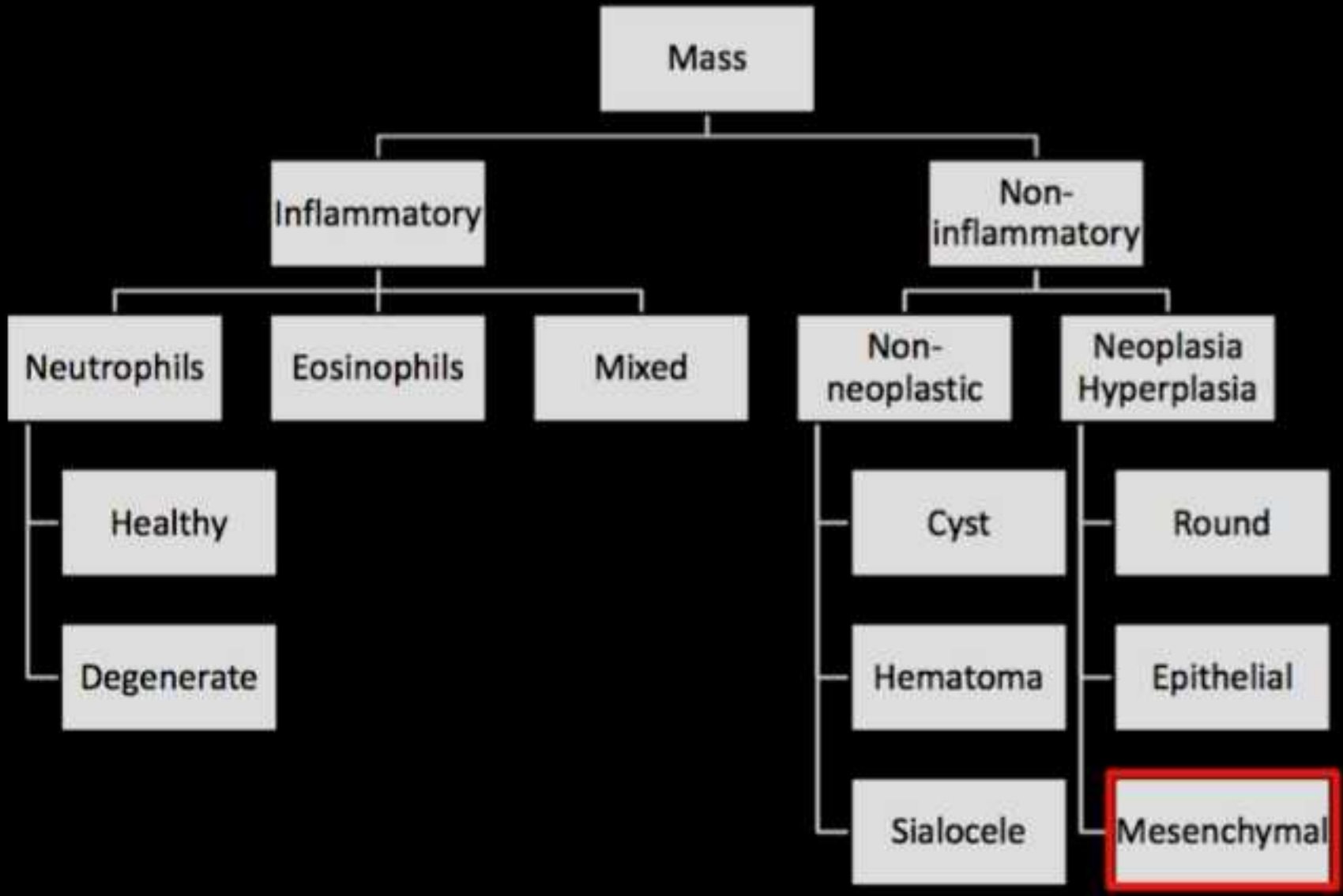
Perianal/hepatoid adenoma adenoma (not anal sac adenocarcinoma)





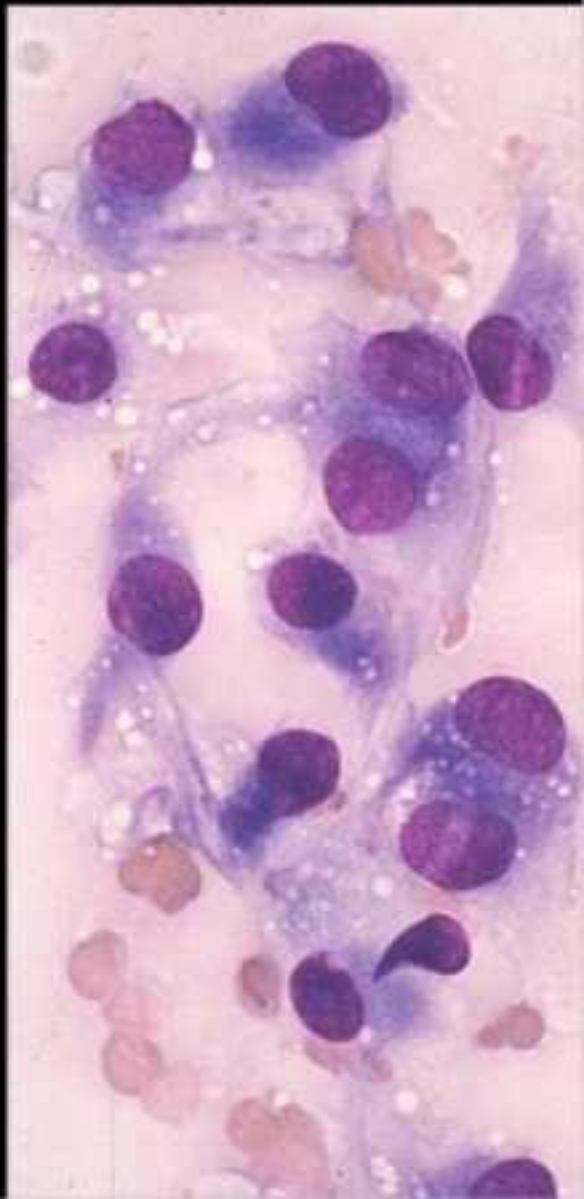
- Hormonally dependent
- May regress after castration; castration may prevent recurrence

Diagnostic Tree



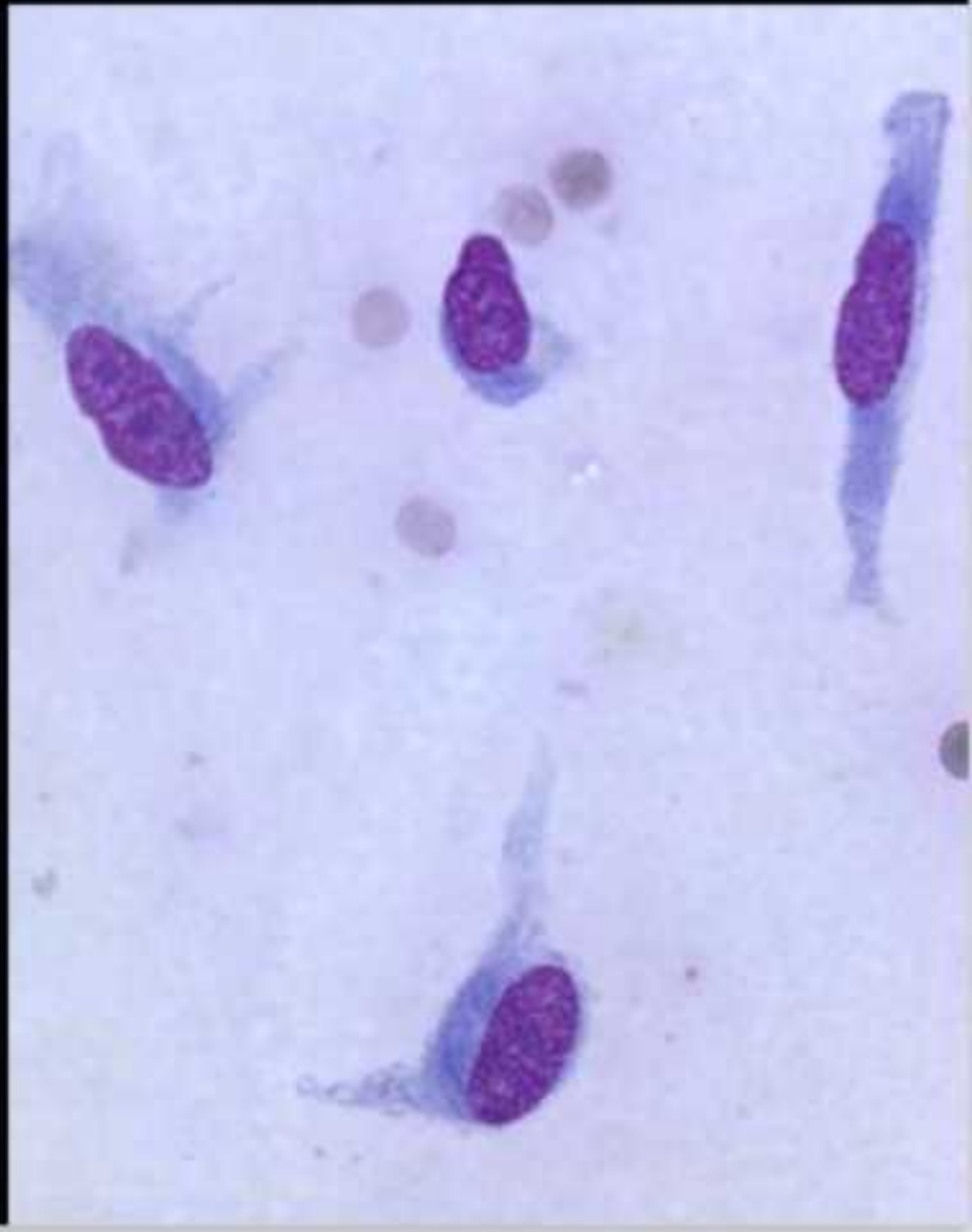
Mesenchymal (Spindle) Cell Tumors

- ‘oma vs. sarcoma
- Fibroma / Fibrosarcoma
- Osteoma / Osteosarcoma
- Hemangioma / Hemangiosarcoma
- Peripheral nerve sheath tumor (hemangiopericytoma) vs. perivascular wall tumor difficult to distinguish histologically
  - PWT: veiled cells, crown cells

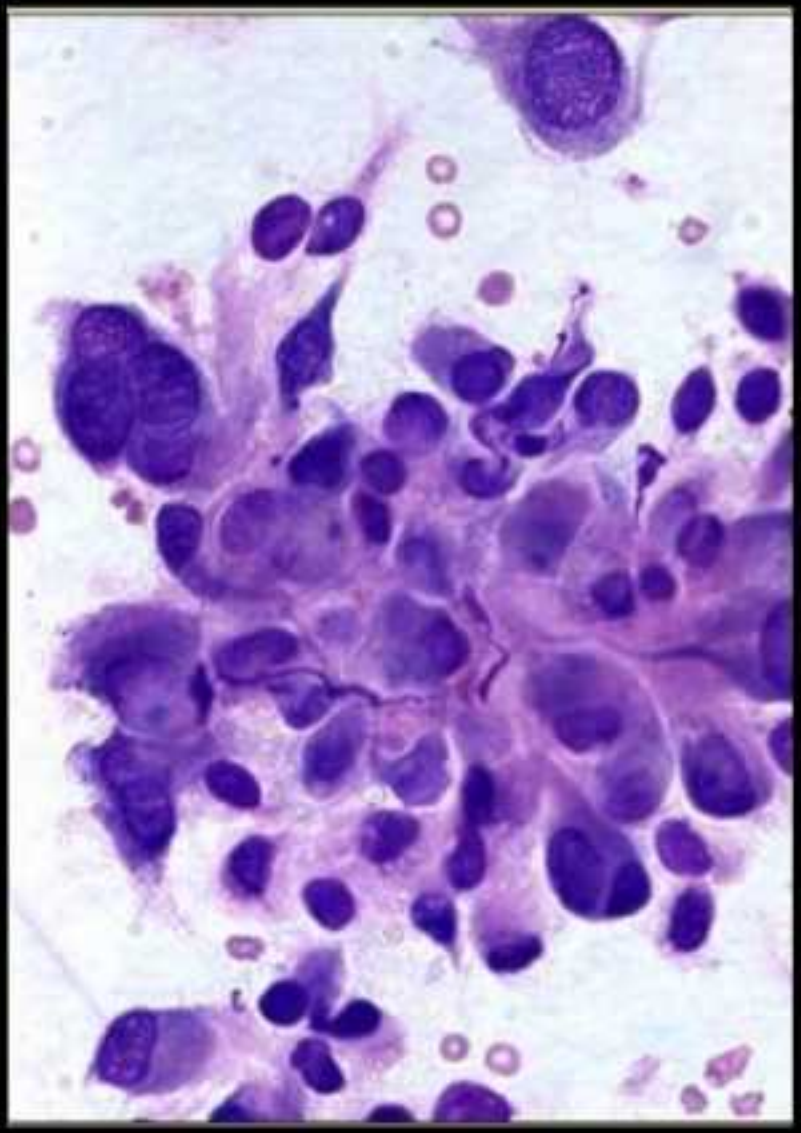


Mesenchymal Tumors

- Exfoliate poorly in FNAs and imprints; few cells present
  - Exceptions: PNST, osteosarcoma , feline vaccine site sarcomas
- Usually elongated nuclei
- Cytoplasmic tails (spindle cells)
- Usually individual cells but sometimes clusters with intercellular matrix
- **Active fibroblasts resemble malignant mesenchymal cells**



Ten cm diameter, non-ulcerated mass; hind leg of dog



History of a mass is key

Mesenchymal tumor is more likely here than granulation tissue

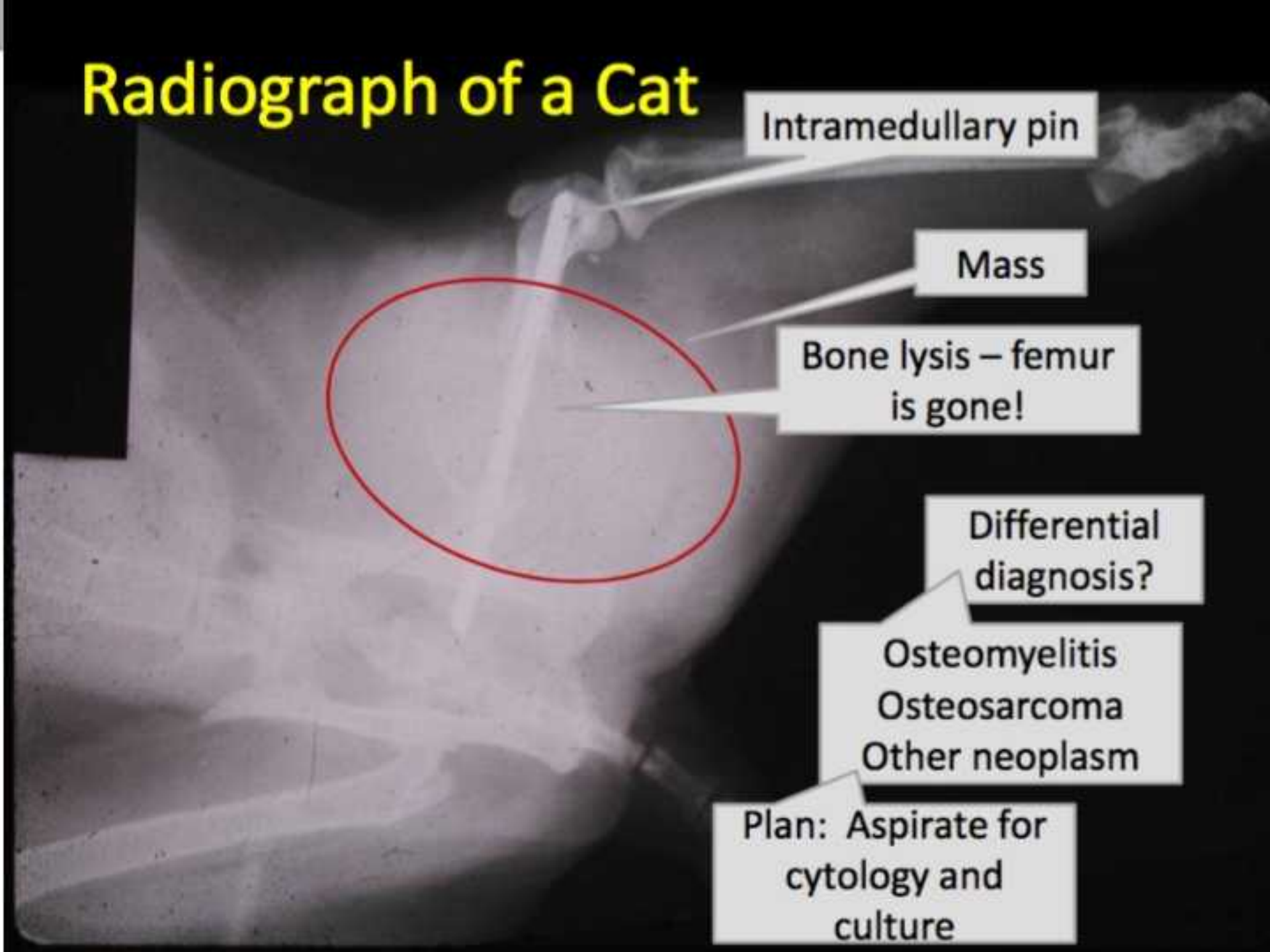
Cell variability indicates malignancy

Recommend histopathology (incisional vs. excisional biopsy)

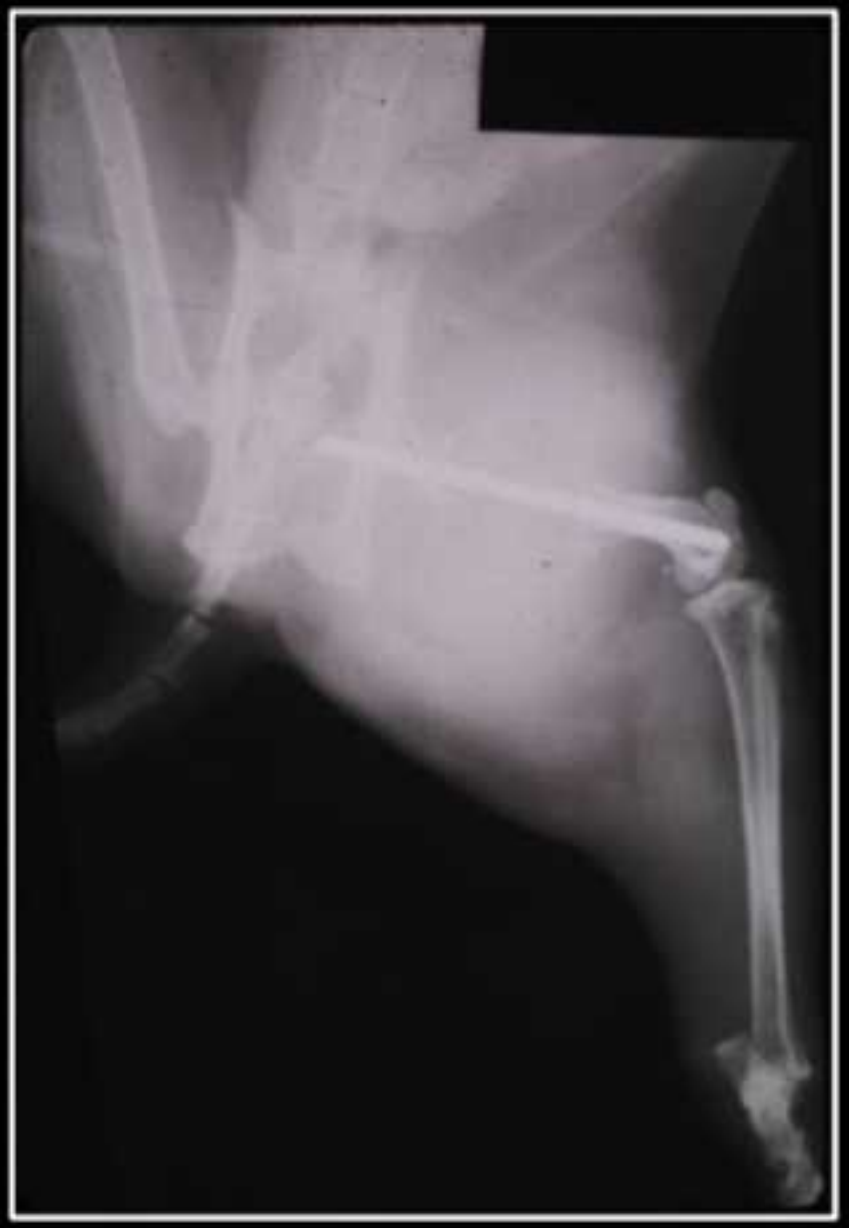
Mesenchymal Tumors

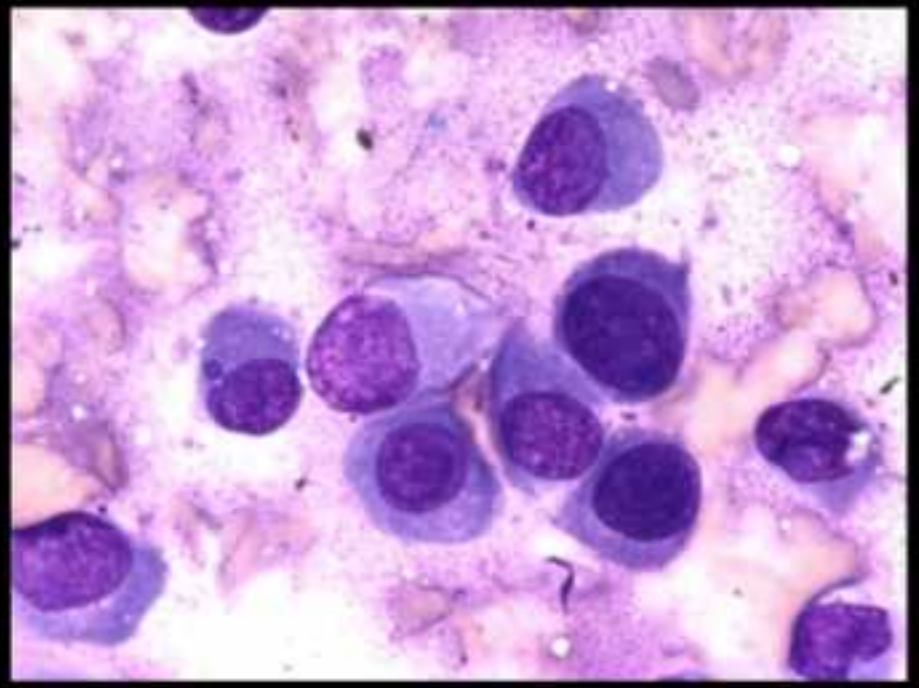
- Don't worry about the exact name
- Grading schemes DO NOT differentiate based on the name:
  - fibrosarcoma, hemangiopericytoma, neurofibrosarcoma, peripheral nerve sheath tumor, perivascular wall tumor, poorly differentiated sarcoma etc.
- Grading schemes are based on histopathology: degree of differentiation, necrosis, and mitotic index (some use other criteria)

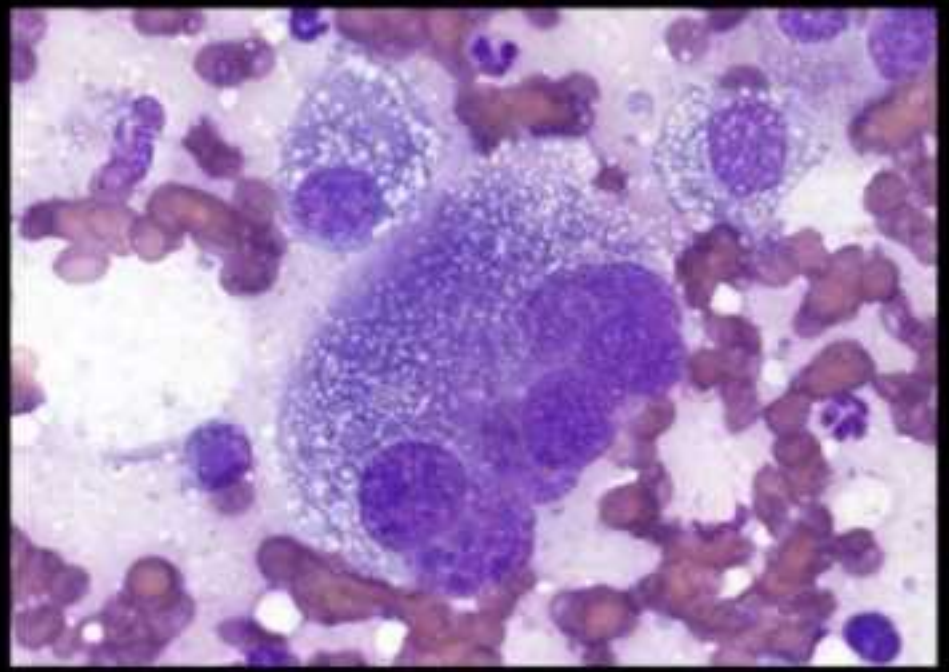
Radiograph of a Cat

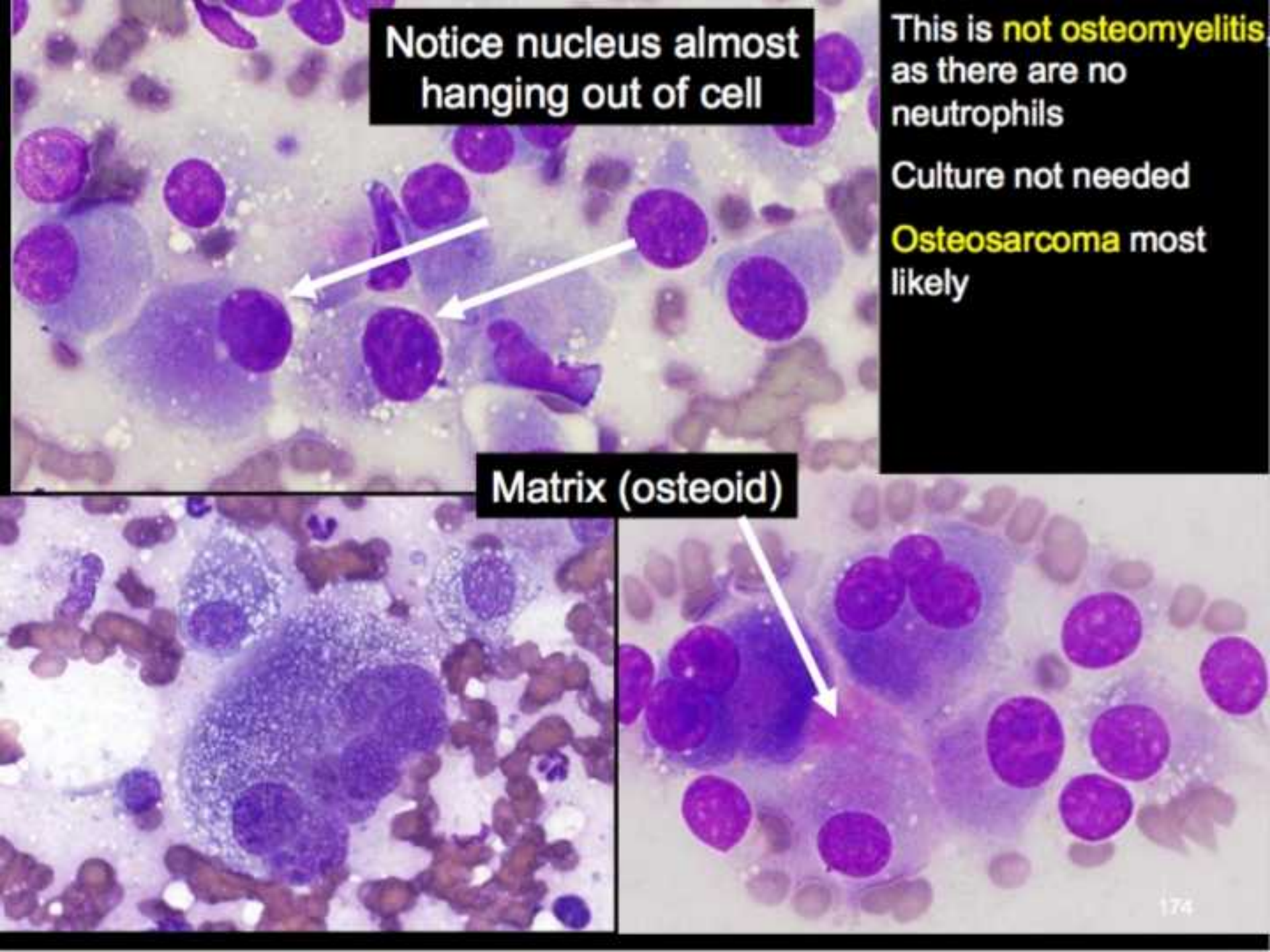


Cat

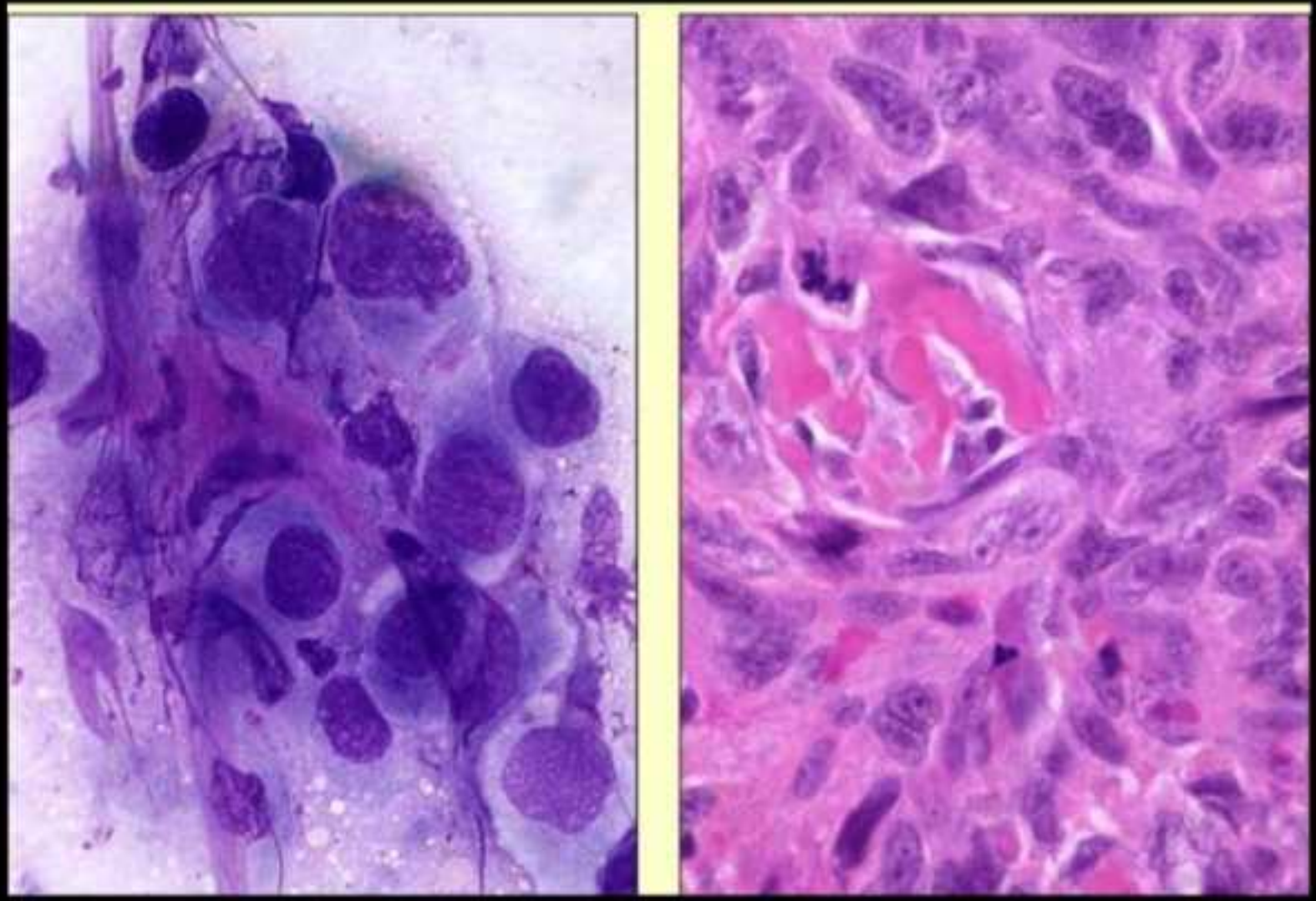







Notice nucleus almost hanging out of cell
This is not osteomyelitis as there are no neutrophils
Culture not needed
Osteosarcoma most likely
Matrix (osteoid)
174

Lytic Bone Lesion From a Dog

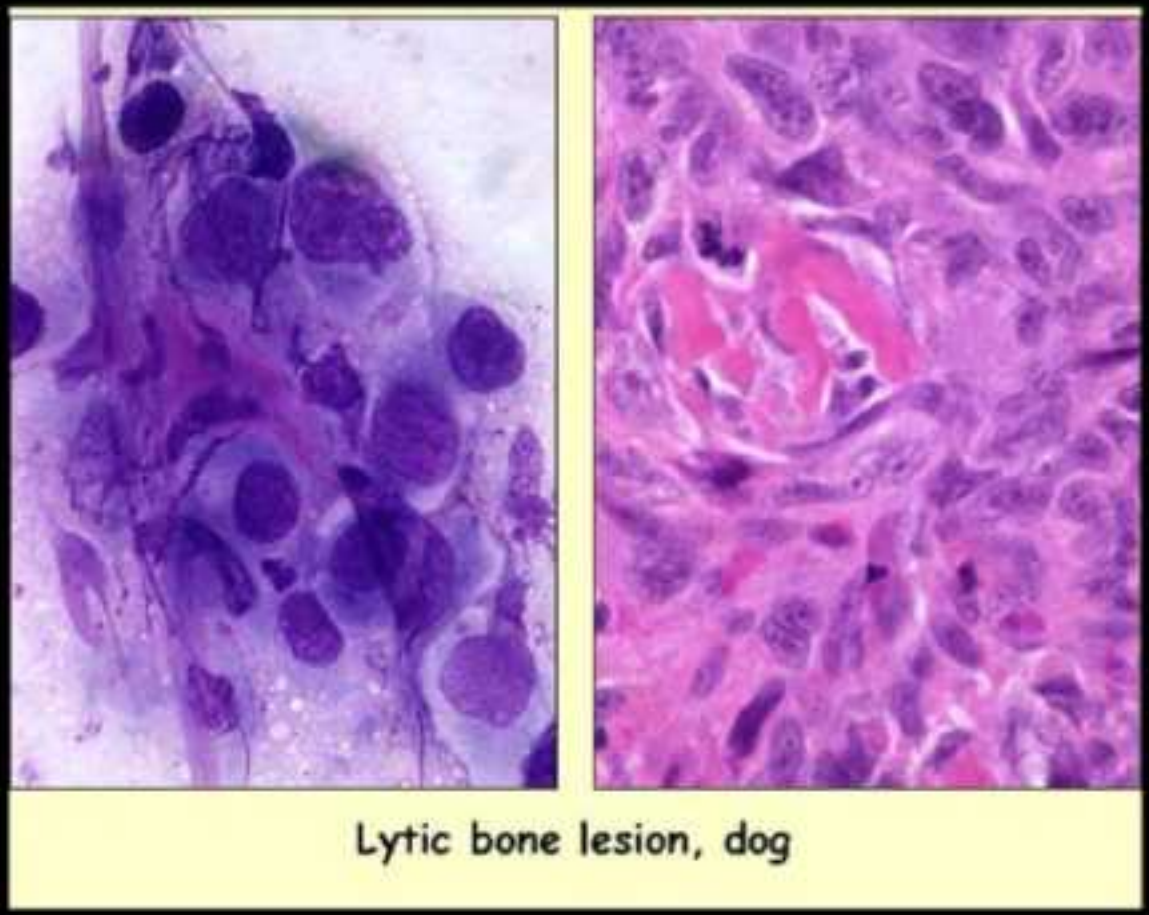


Neutrophils? Are cells individualized or organized? Shape of cells? Shape of nuclei? Variability? Any other diagnostic clues?

Diagnosis: Osteosarcoma

Keys to diagnosis:

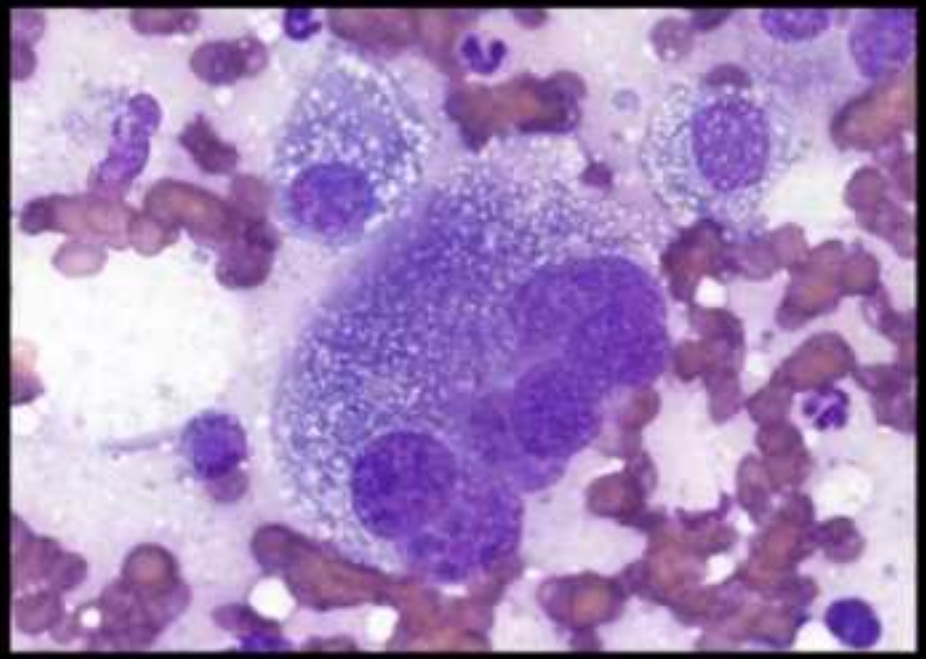
1. **LOCATION:** bone with lysis
2. **Morphology:** spindle cells
3. **Product:** note the pink material in both specimens; this is osteoid (very useful diagnostically)

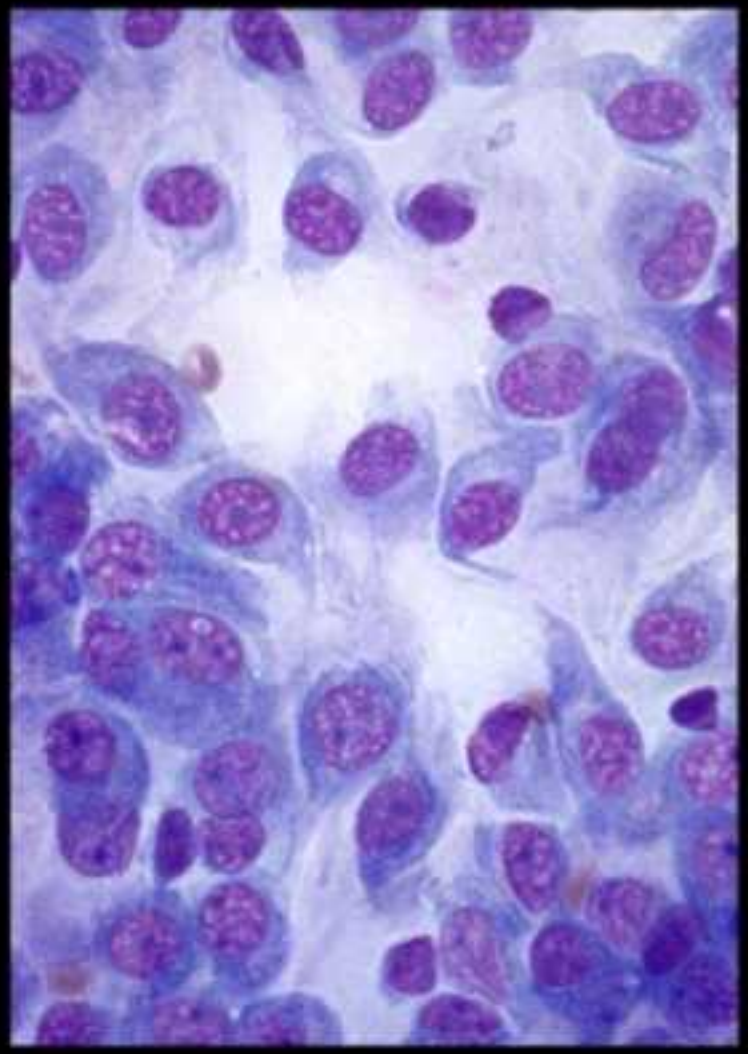


Lytic bone lesion, dog

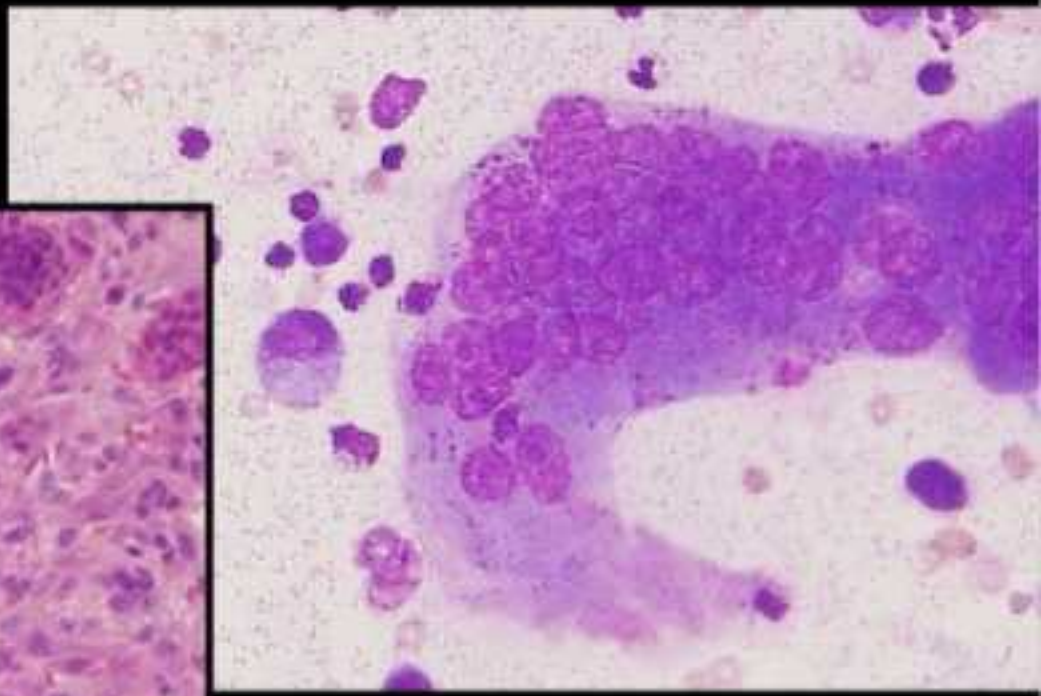
6-year-old cat with mass at the base of the neck.

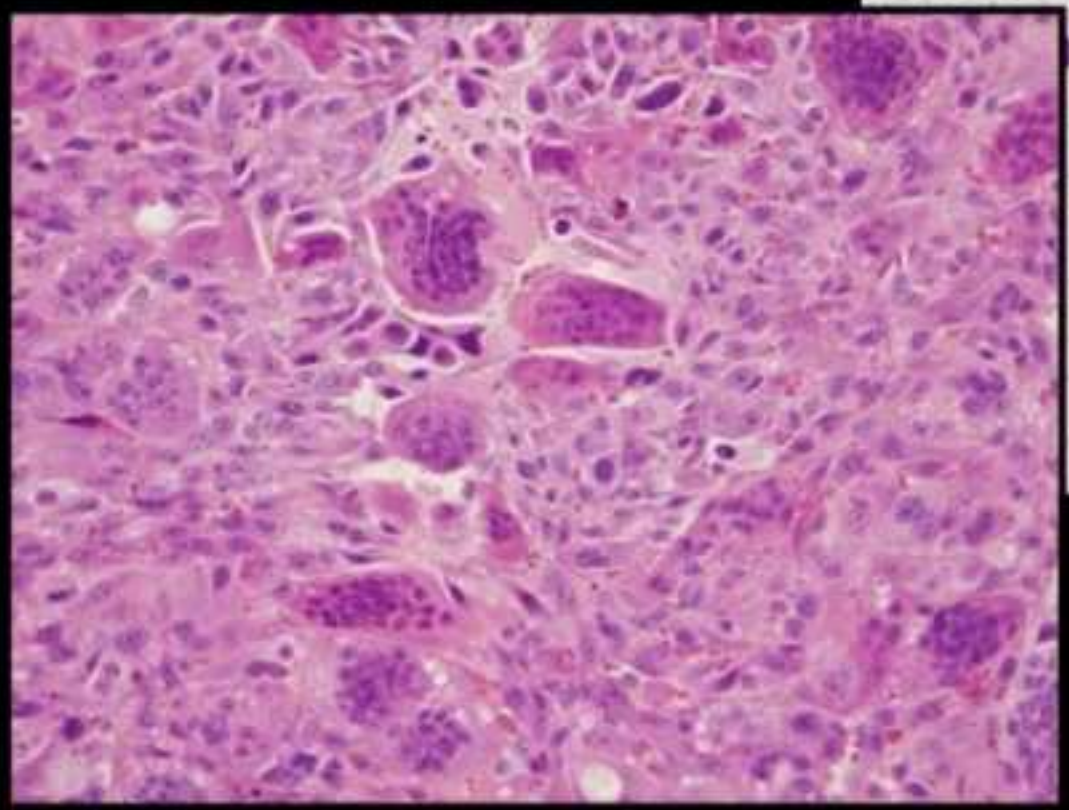
Fine needle aspirate





Diagnosis: Injection site sarcoma; fibrosarcoma with giant cells; giant cell tumor of soft parts

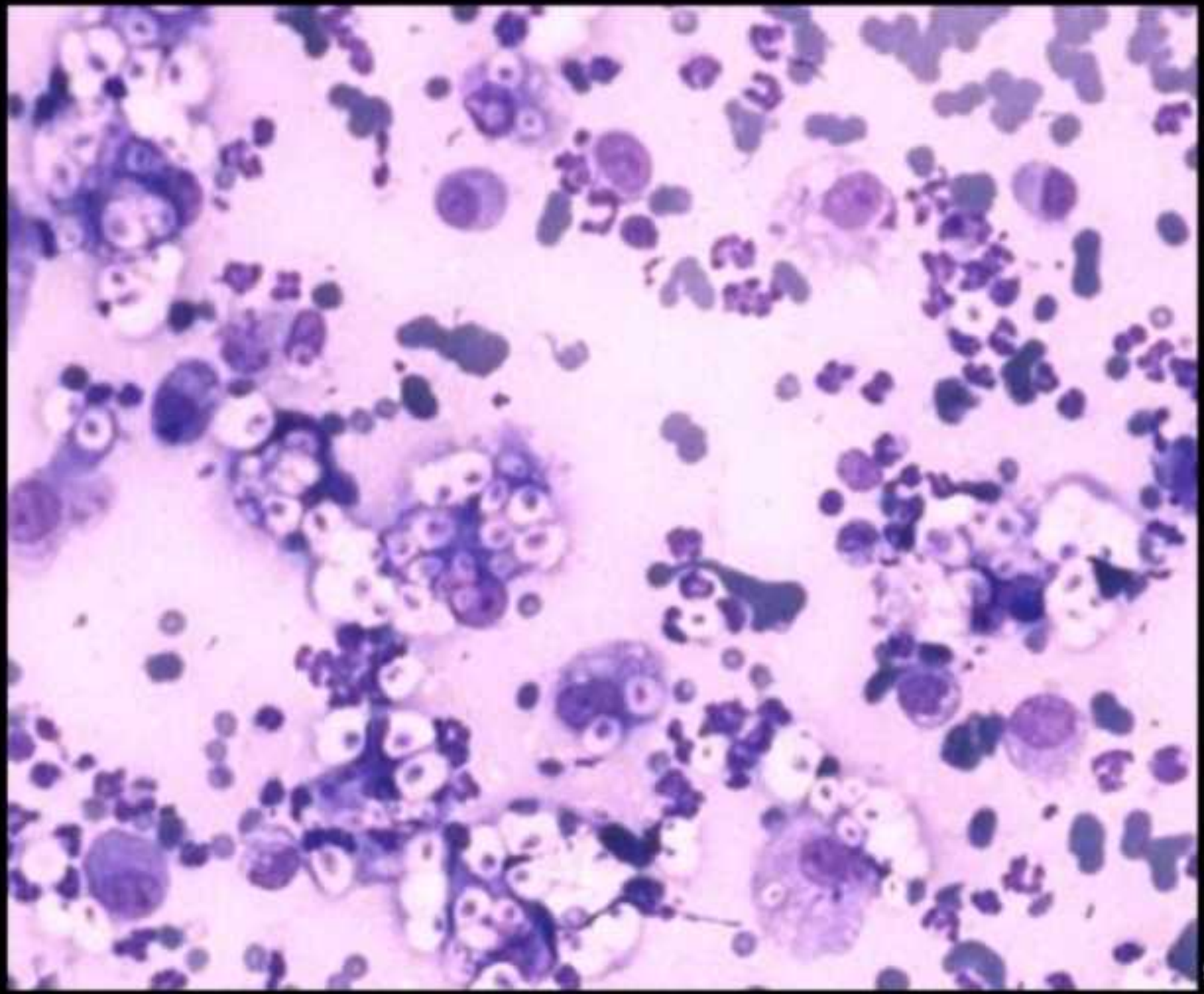




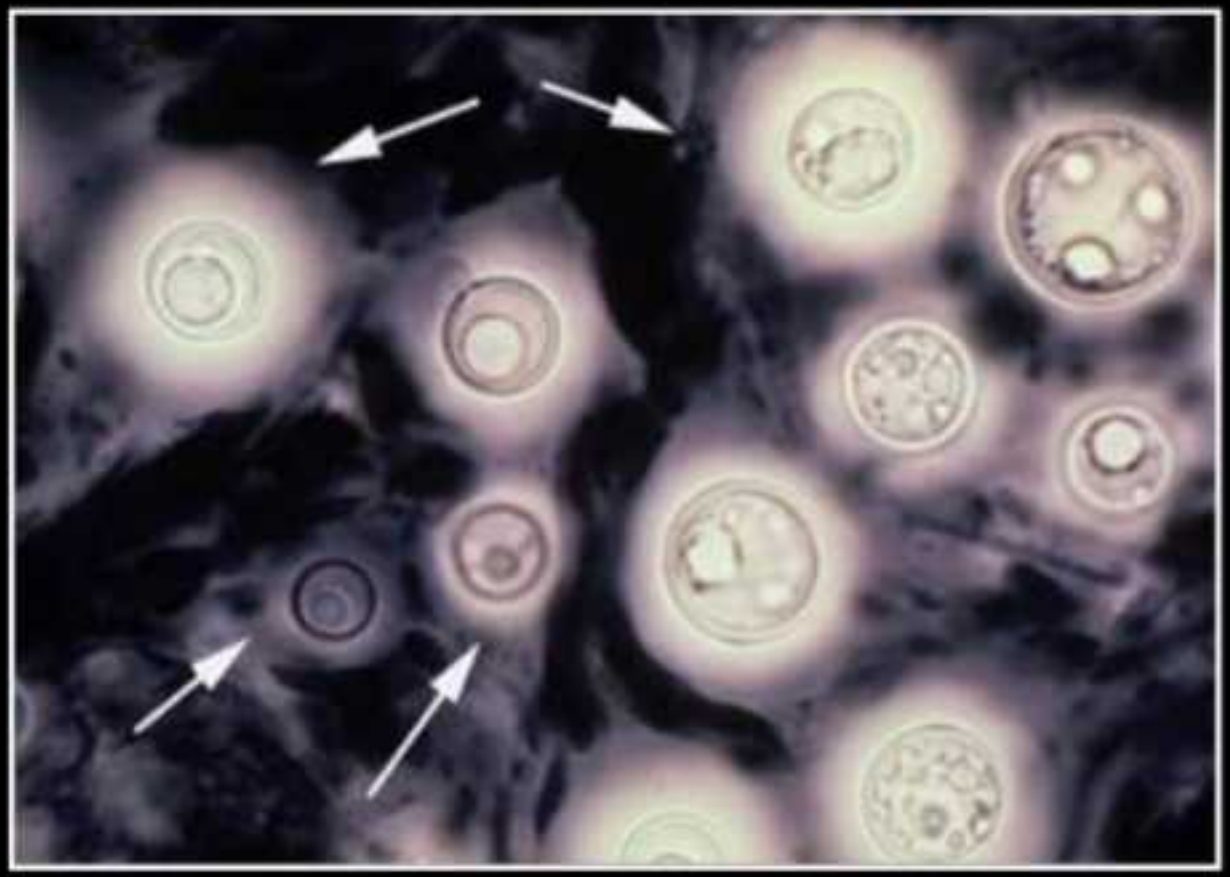
Keys to diagnosis: cat, anatomic location, cell appearance

CASE EXAMPLES

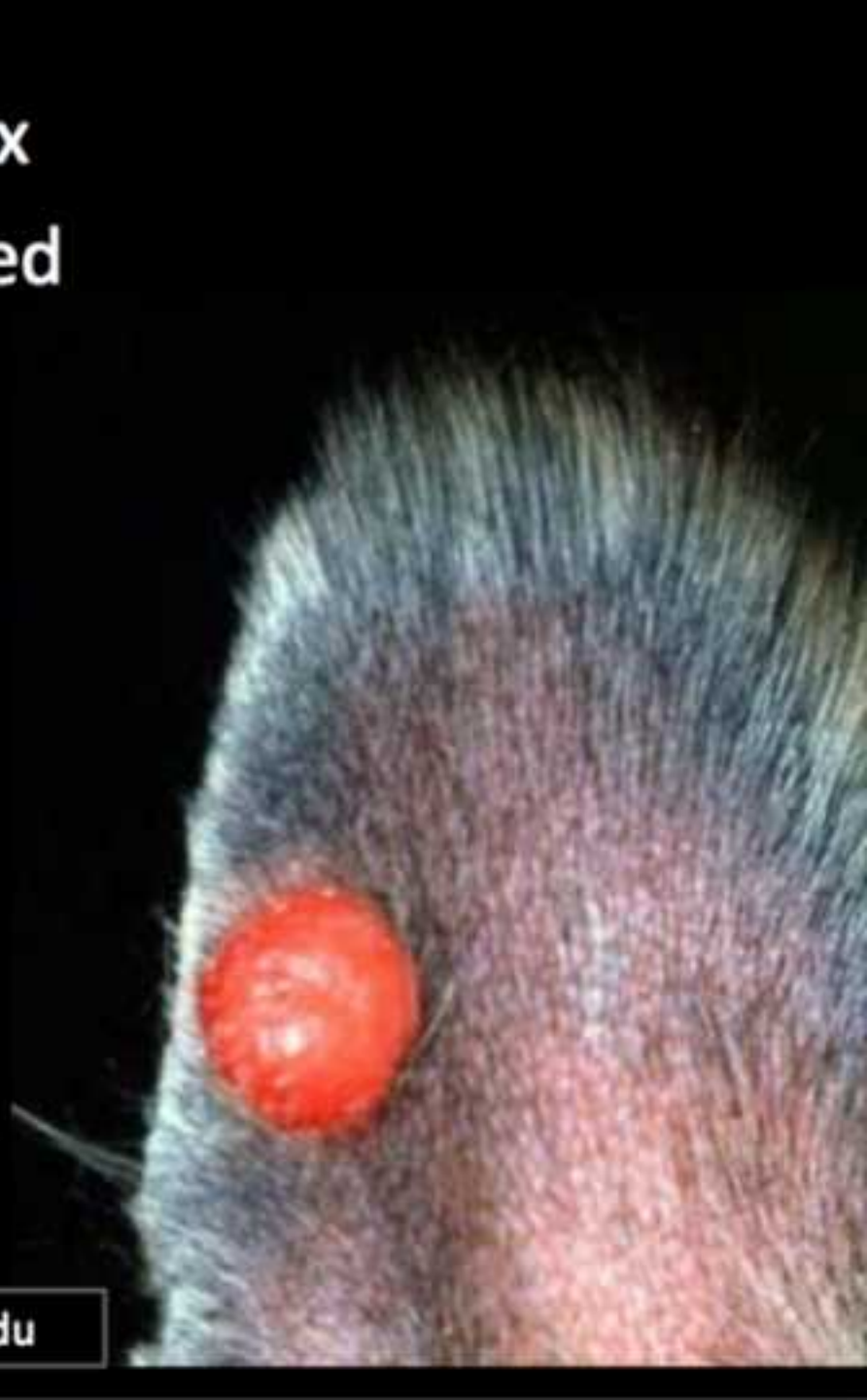
- Adult cat with swelling over bridge of the nose
- Fine needle aspirate
- What's the first question?

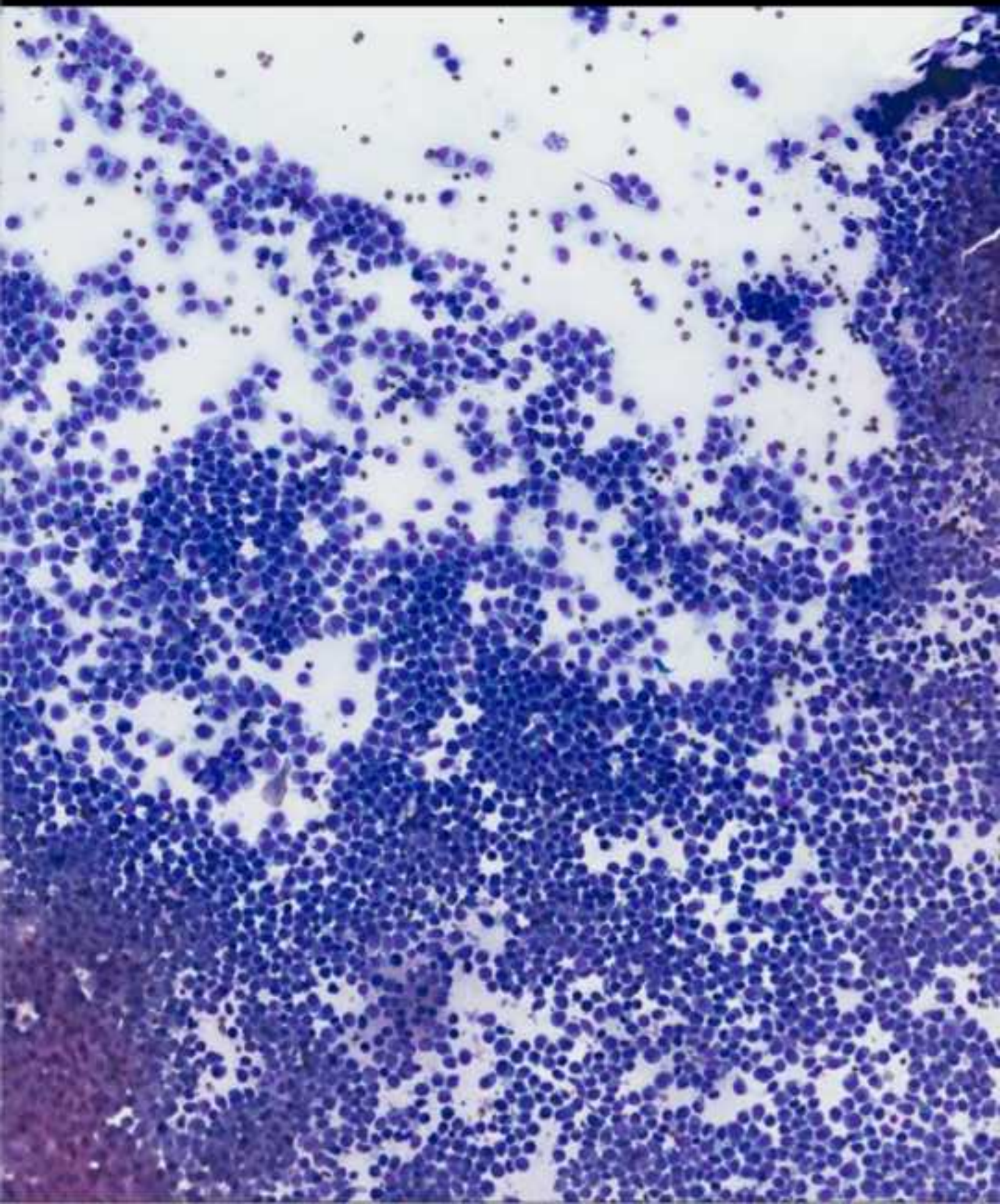


Cause: *Cryptococcus neoformans*

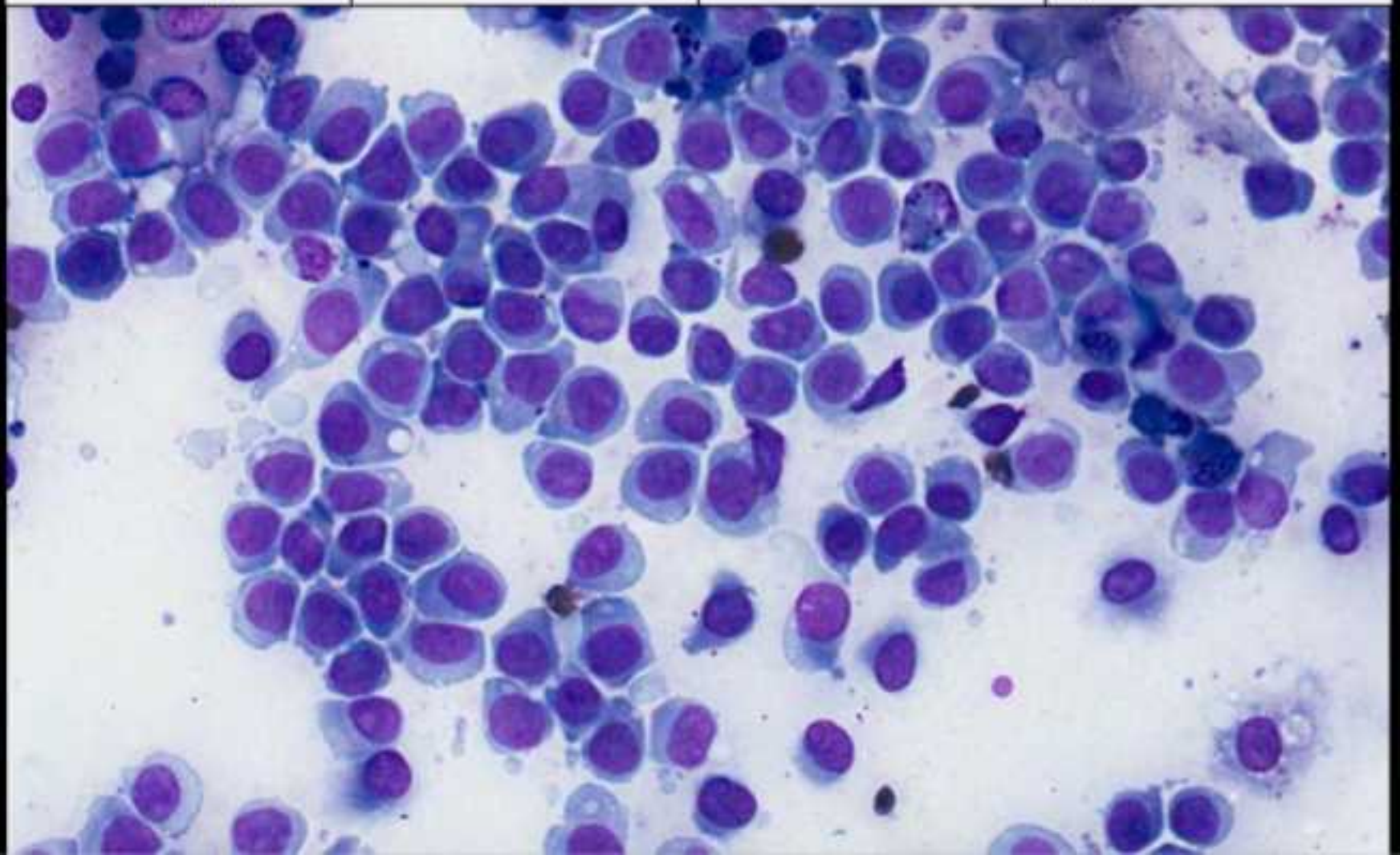


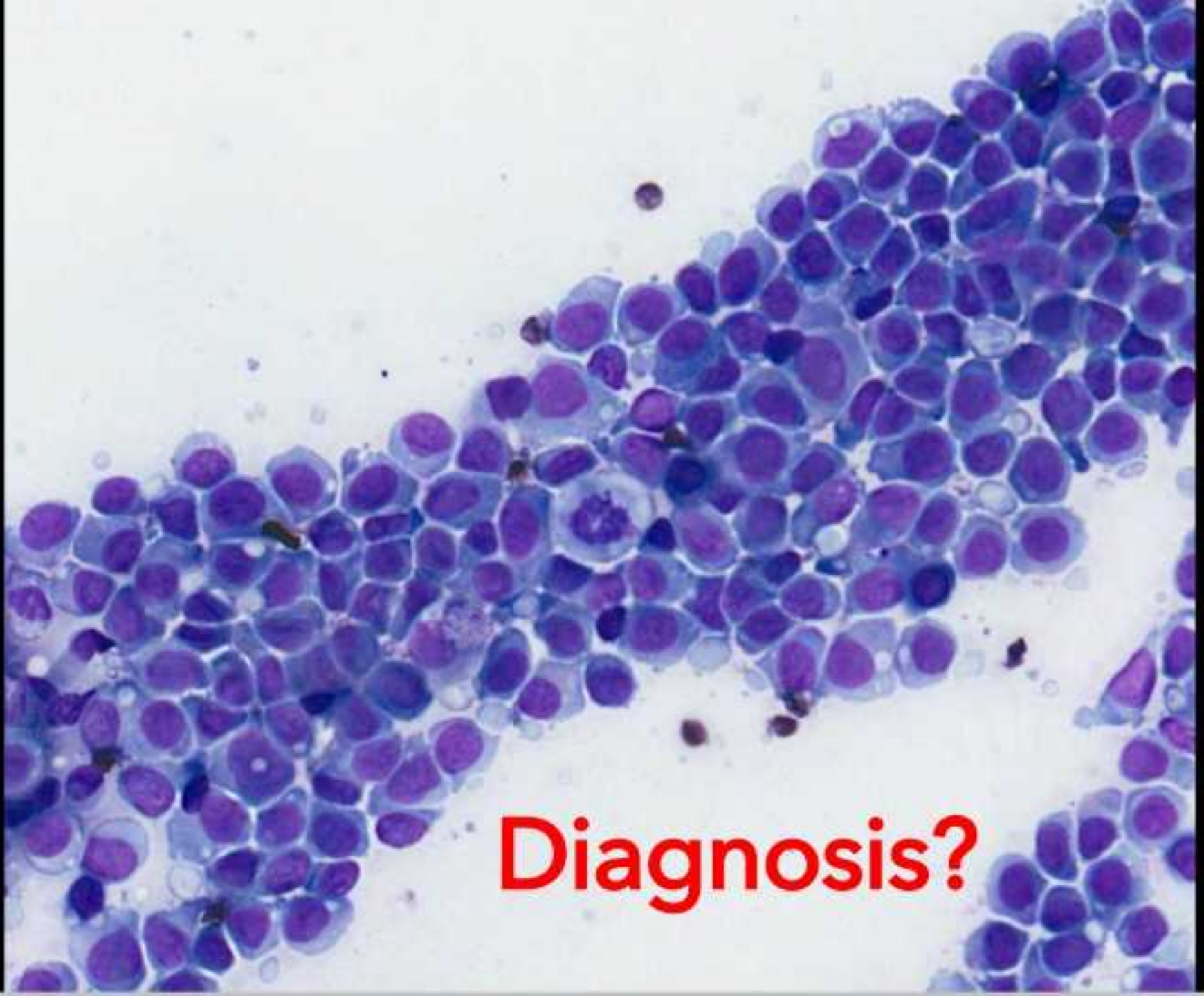
- 10-month-old shepherd mix
- Hairless, round, 1.5cm raised cutaneous mass on pinna
- Submitted FNAs





Type	Cellularity	Arrangement	Shape
Round cell	High	Individual	Round
Epithelial	High	Clusters	Polygonal*
Mesenchymal	Low**	Individual	Spindle





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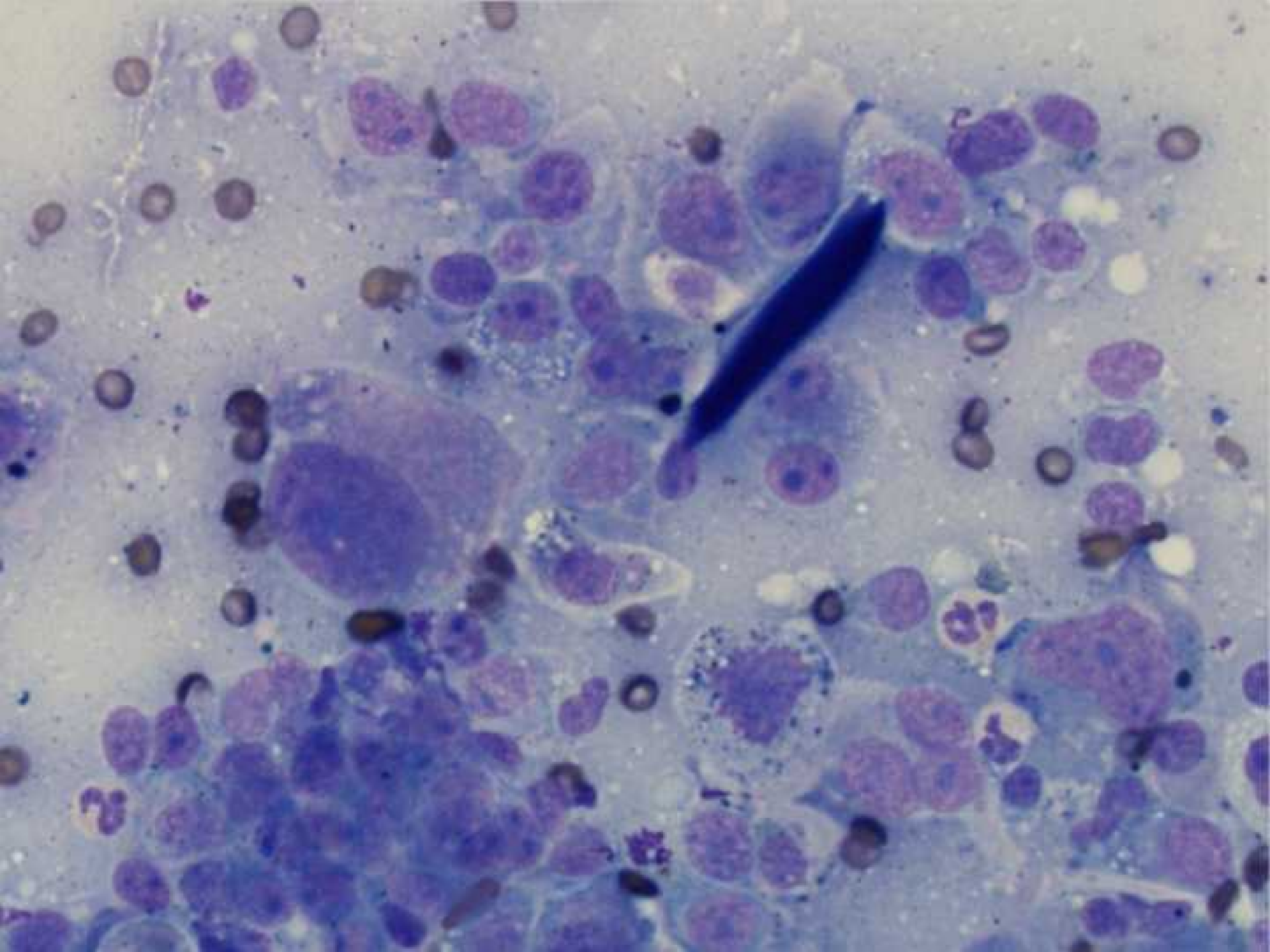
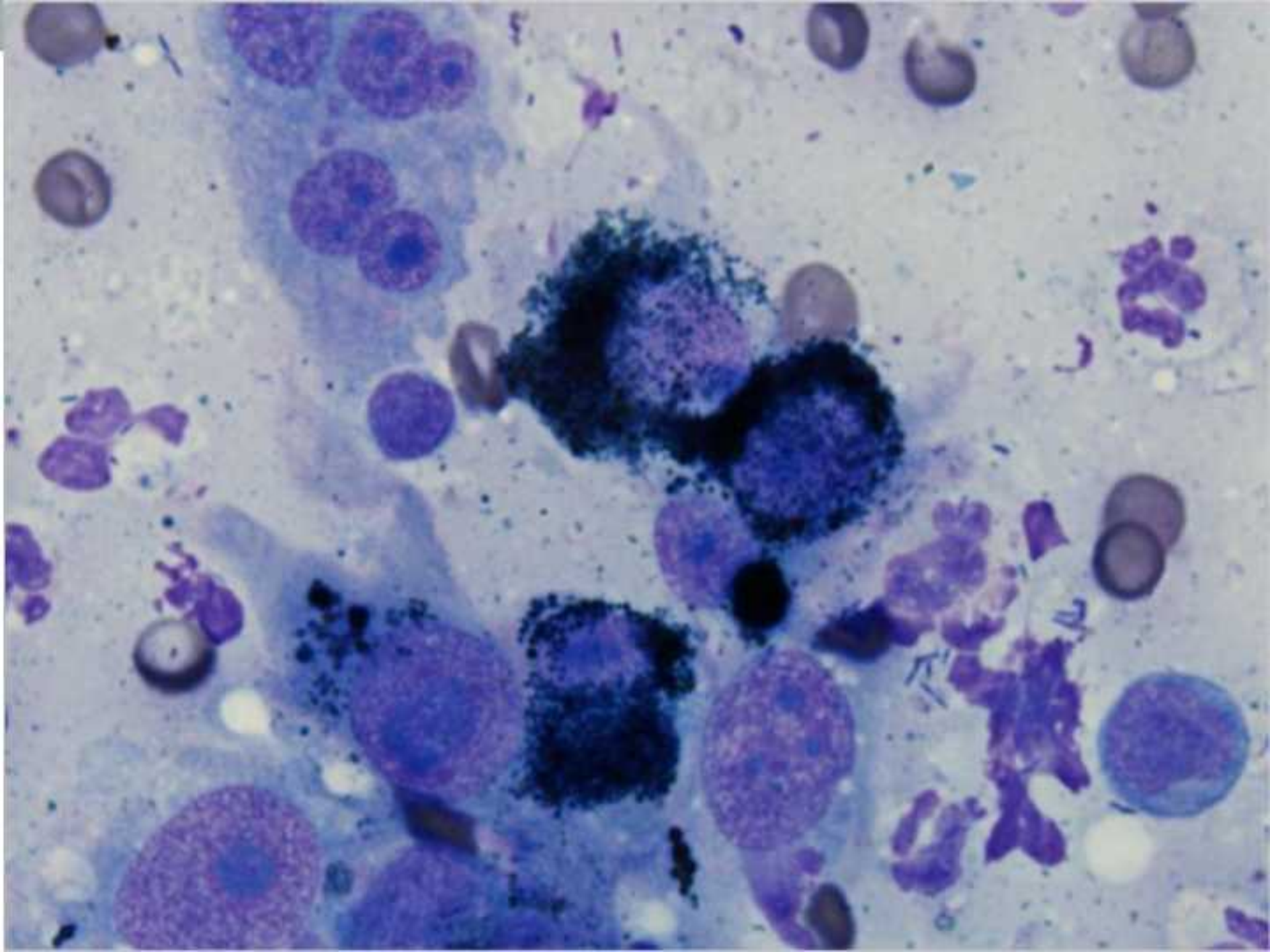
Ans: Canine Cutaneous Histiocytoma

- Benign dermal neoplasms
- Langerhans cell origin (histiocyte)
- Typically grow rapidly and spontaneously regress.

Cutaneous mass, dog

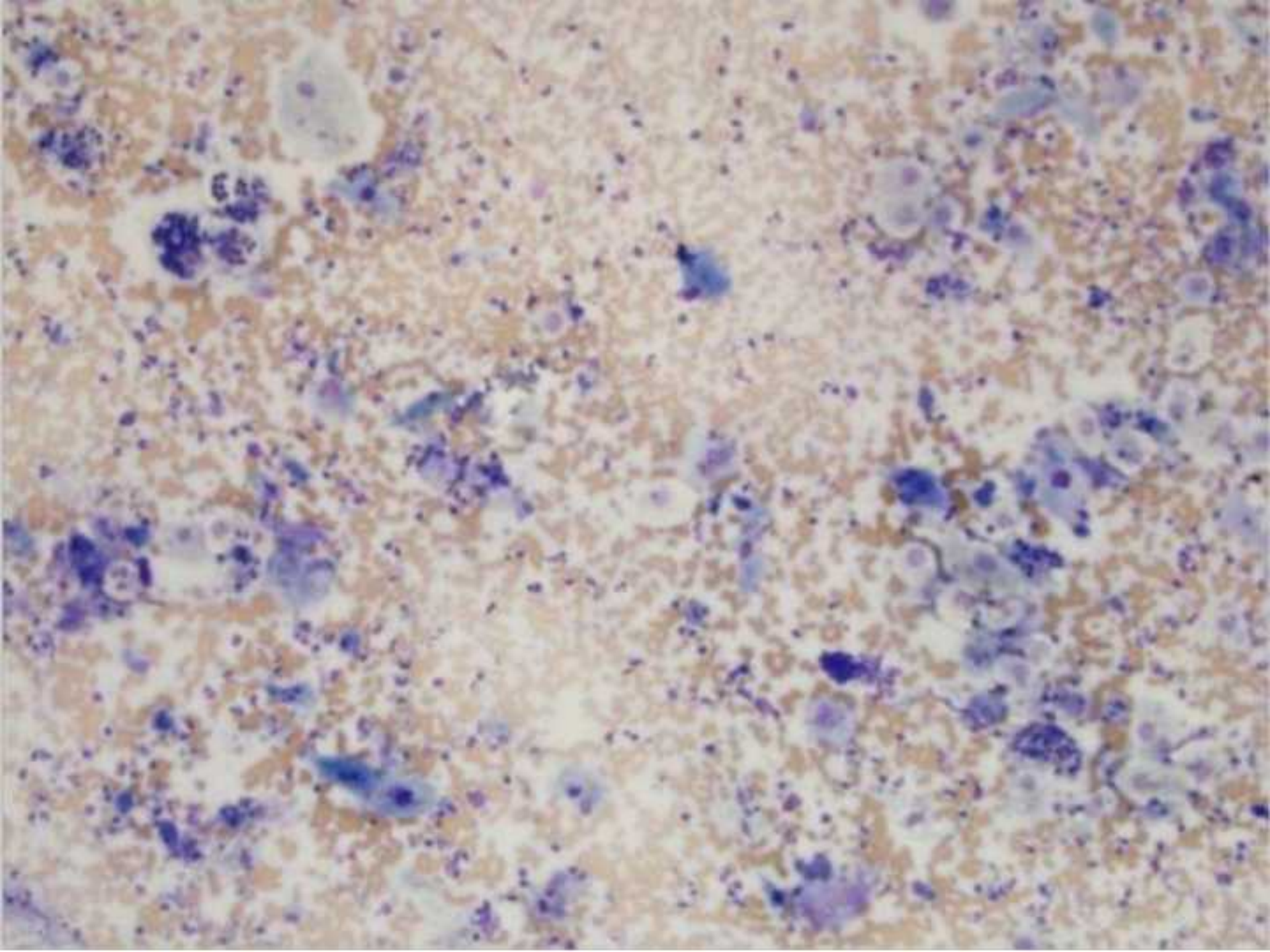


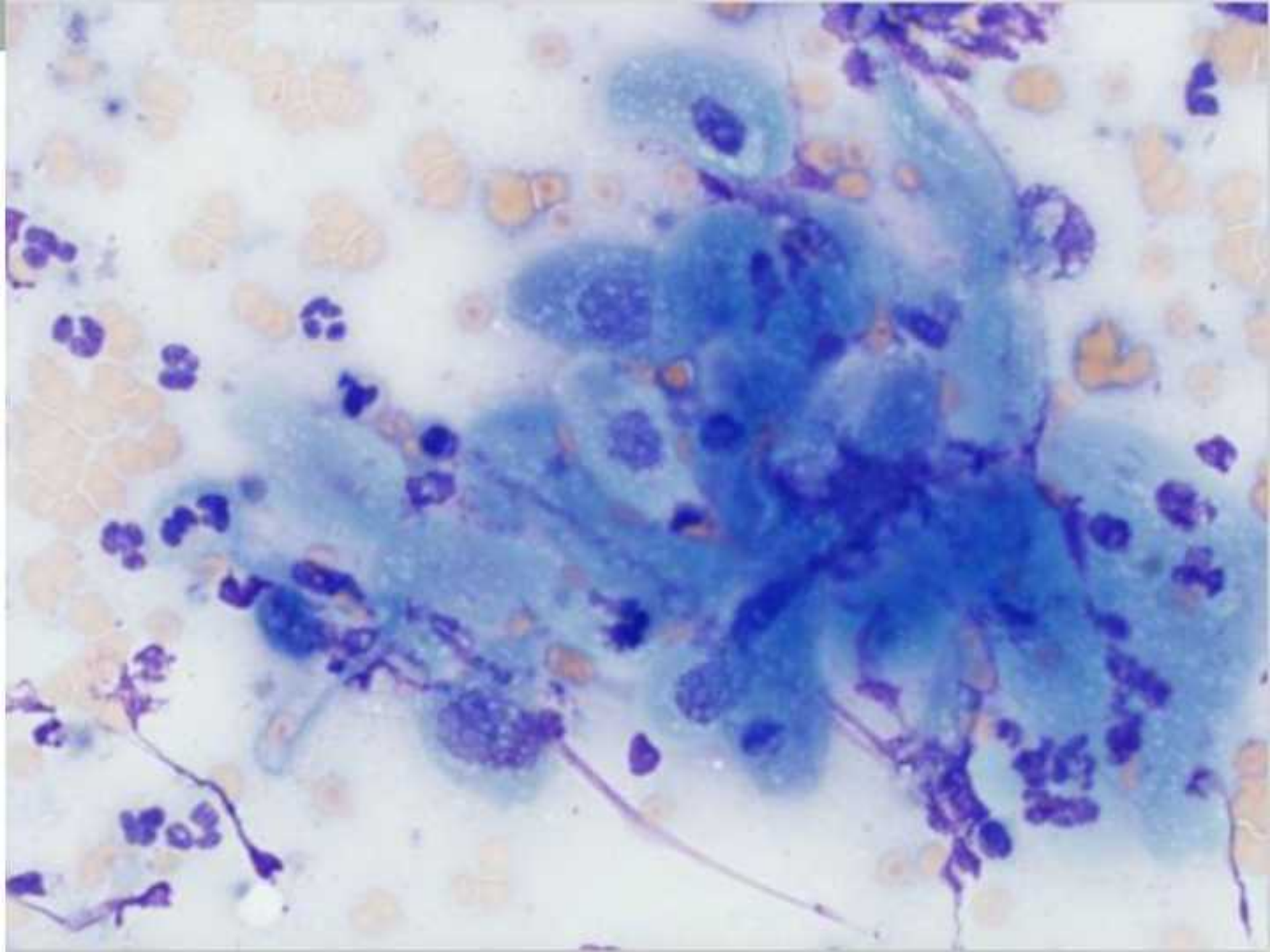


- Highly cellular
- cells present individually & dense aggregates
- round to spindloid cells predominate
- low N:C
- marked anisocytosis and anisokaryosis
- abundant blue-grey cytoplasm
- occasionally contains abundant melanin granules
- variably placed round to ovoid nuclei
- very coarse chromatin
- multiple, prominent and pleomorphic nucleoli
- bi and multinucleated cells present
- light blue vacuolated background containing rare RBC, rare rod-shaped bacteria, low numbers of neutrophils, rare keratin scroll.

Malignant melanoma

Mass under tongue, cat

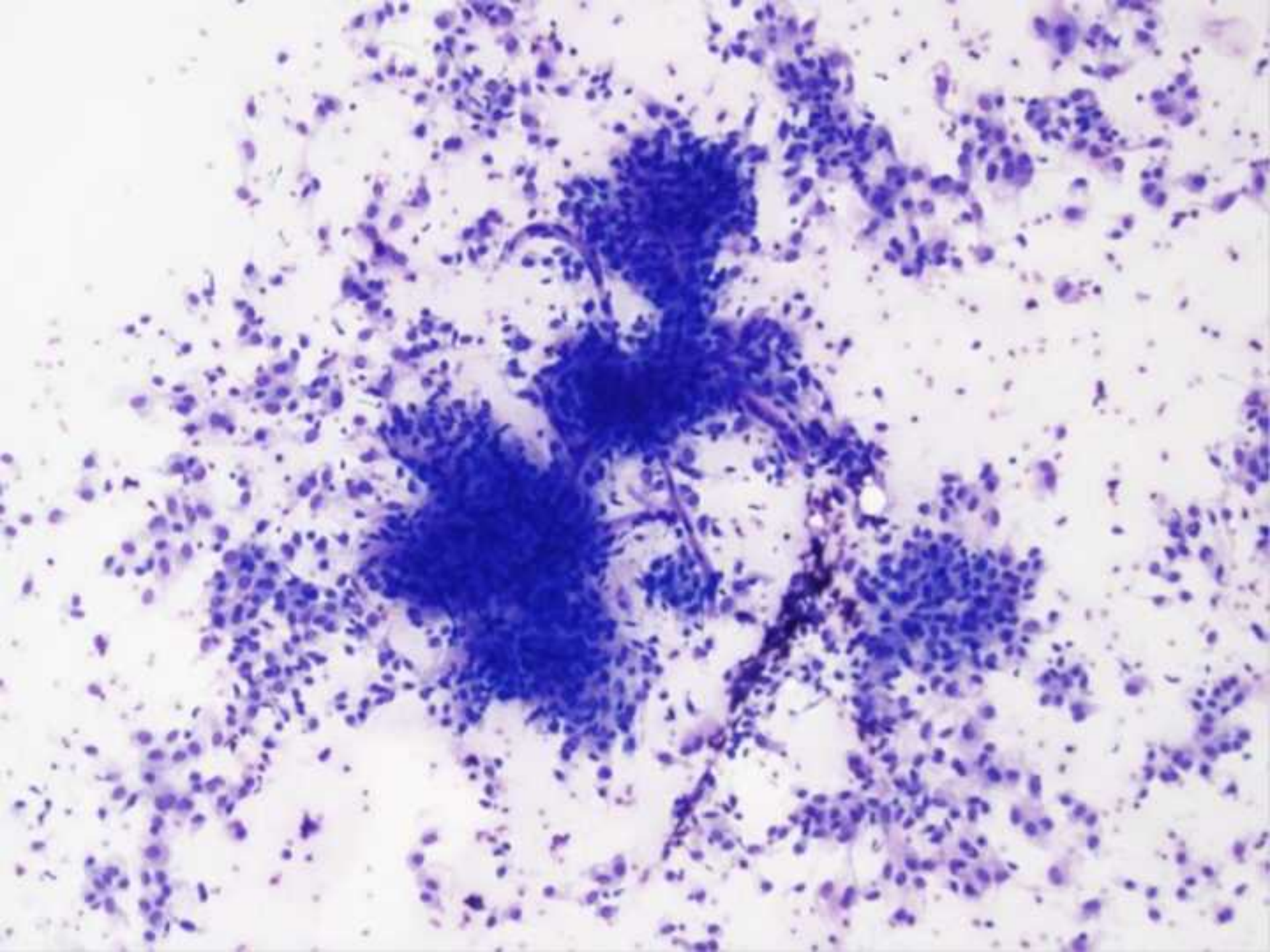


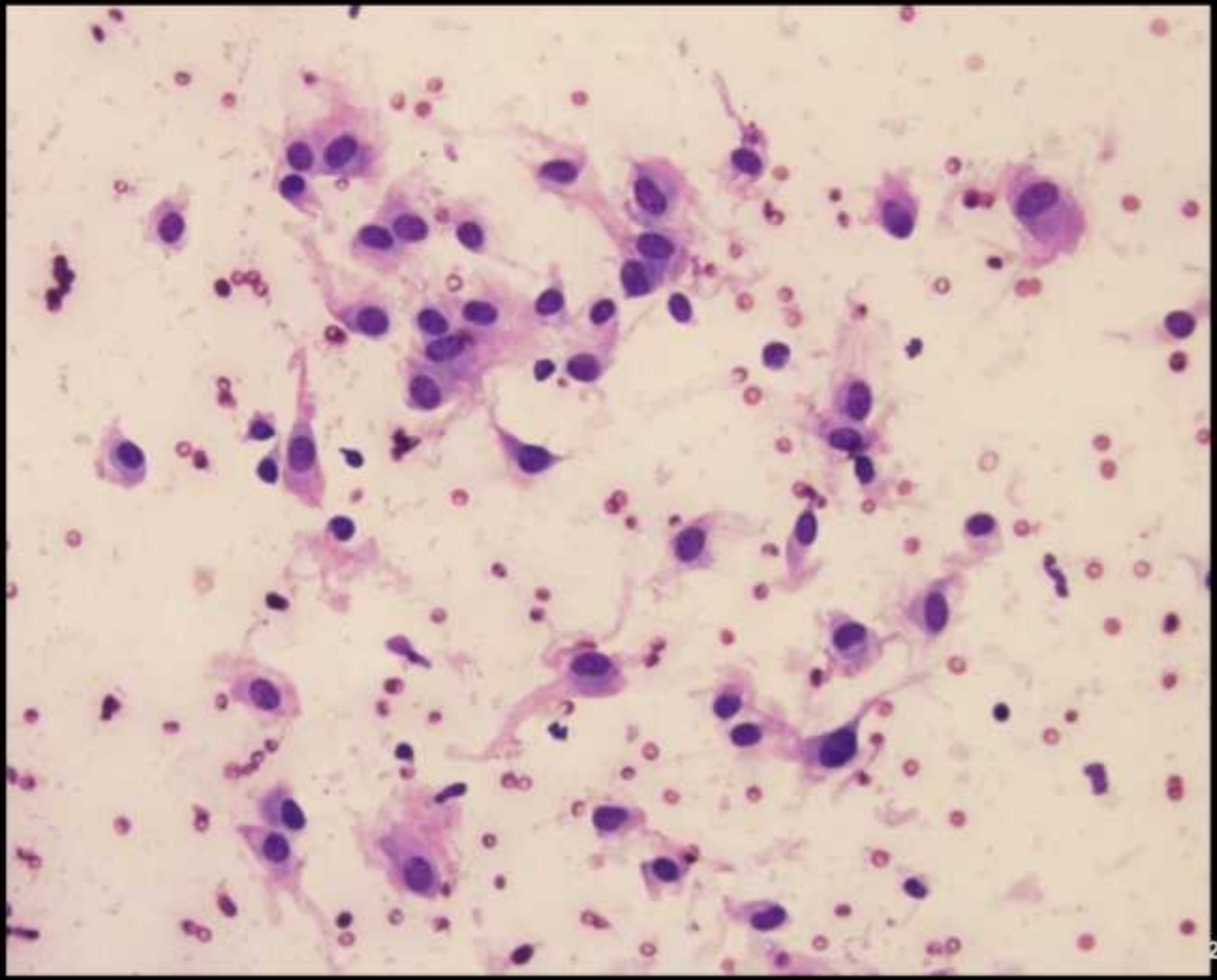


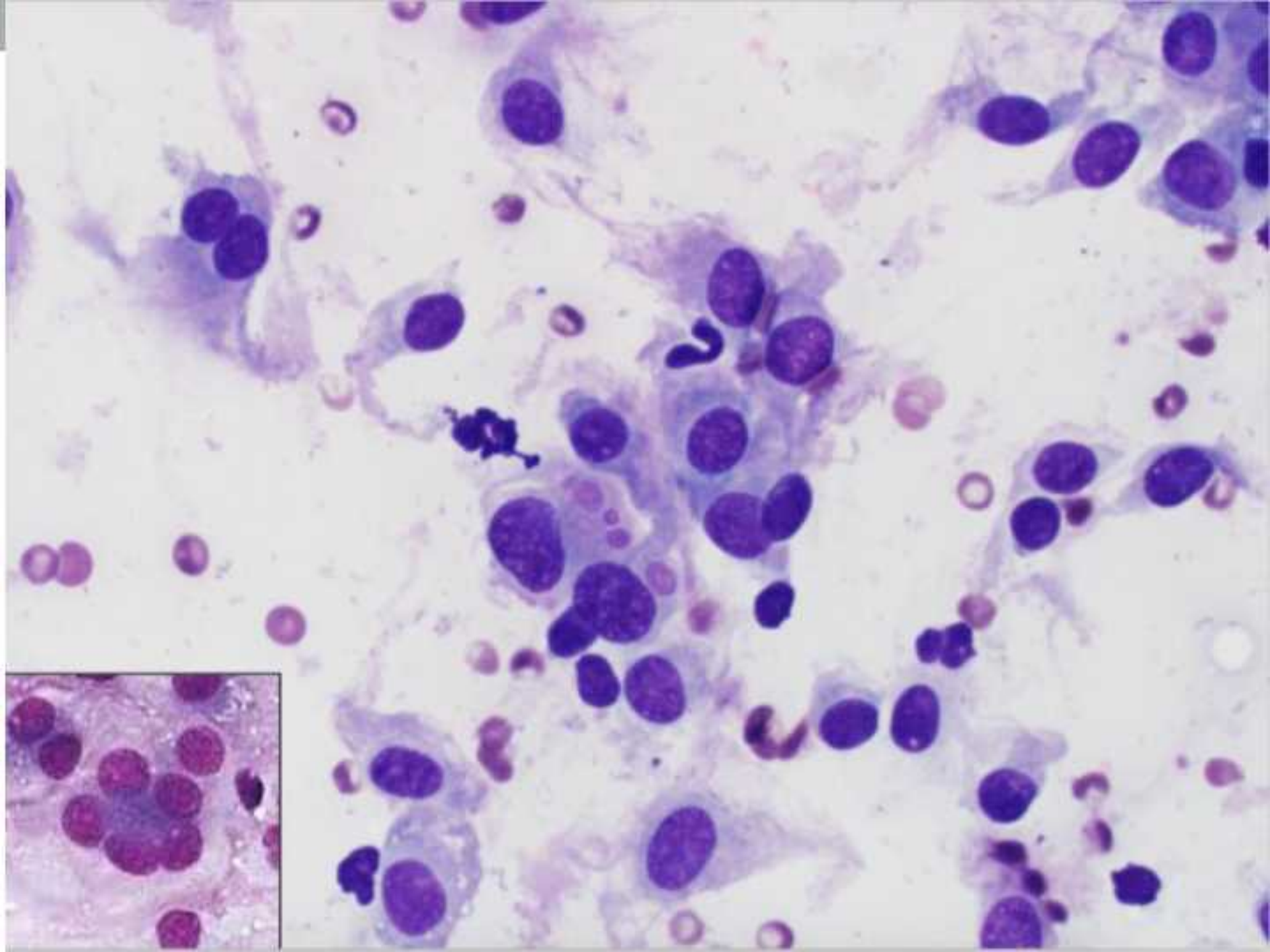
- Low to moderate cellularity
- cells present individually & dense disorganized clusters
- pleomorphic, round to angular to elongate epithelial cells
- low N:C
- marked anisocytosis and anisokaryosis
- faint blue to dark blue/sky blue keratinized cytoplasm; often vacuolated with vacuoles occasionally concentrated around the nucleus
- exhibit occasional dyskeratosis (mismatch between nuclear and cytoplasmic maturation)
- large, round, central nucleus with coarse chromatin and rarely a large, prominent nucleolus
- light blue, hemodiluted background containing small amounts of free keratin and streaming nuclear debris
- Mild neutrophlic inflammation & neutrophilic emperipolesis

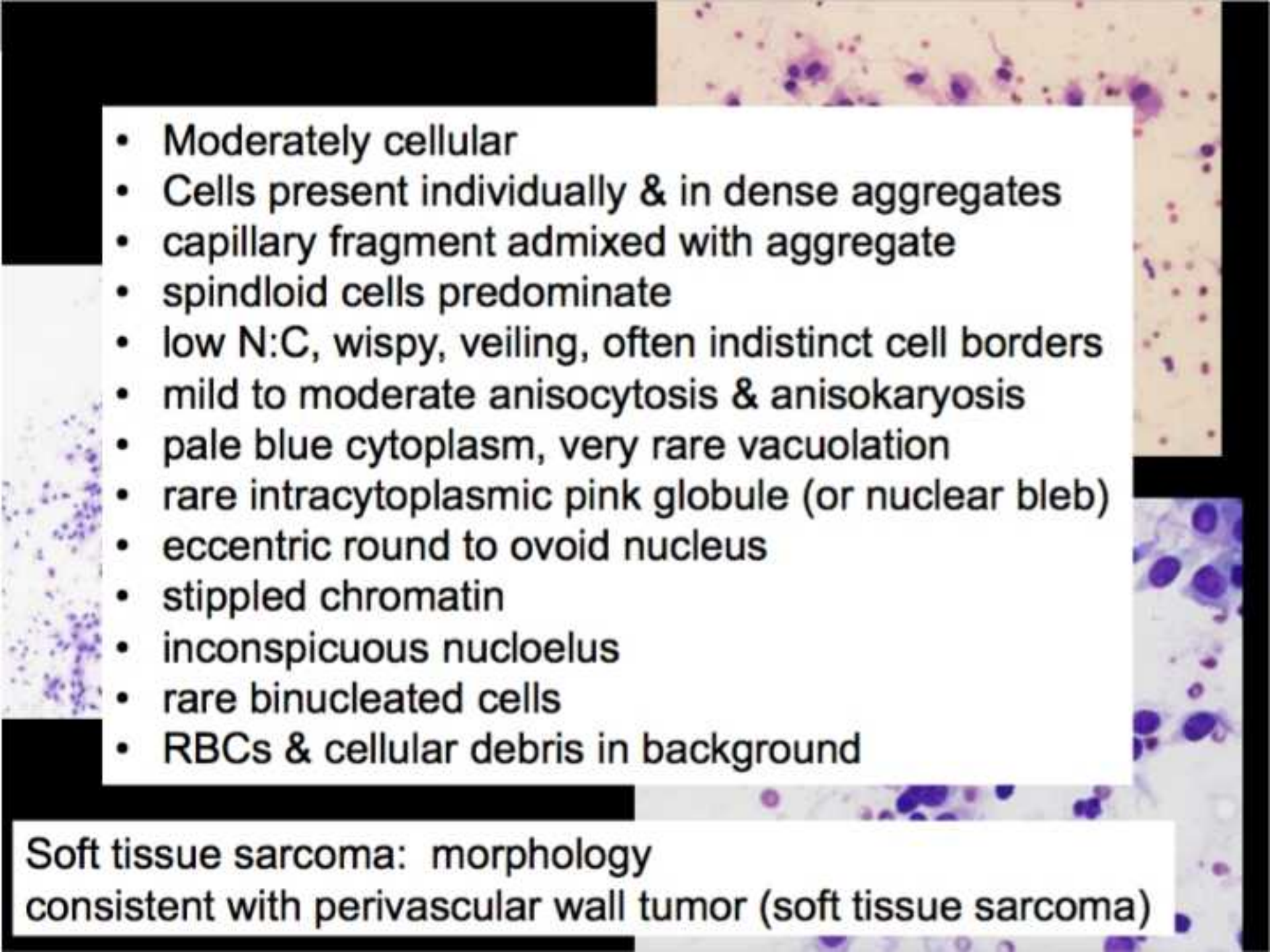
Squamous cell carcinoma

Subcutaneous mass, dog









- Moderately cellular
- Cells present individually & in dense aggregates
- capillary fragment admixed with aggregate
- spindloid cells predominate
- low N:C, wispy, veiling, often indistinct cell borders
- mild to moderate anisocytosis & anisokaryosis
- pale blue cytoplasm, very rare vacuolation
- rare intracytoplasmic pink globule (or nuclear bleb)
- eccentric round to ovoid nucleus
- stippled chromatin
- inconspicuous nucloelus
- rare binucleated cells
- RBCs & cellular debris in background

Soft tissue sarcoma: morphology
consistent with perivascular wall tumor (soft tissue sarcoma)

Acknowledgements

- Clinician's brief
- C.L. Davis DVM Foundation
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