DIAGNOSTIC CYTOLOGY

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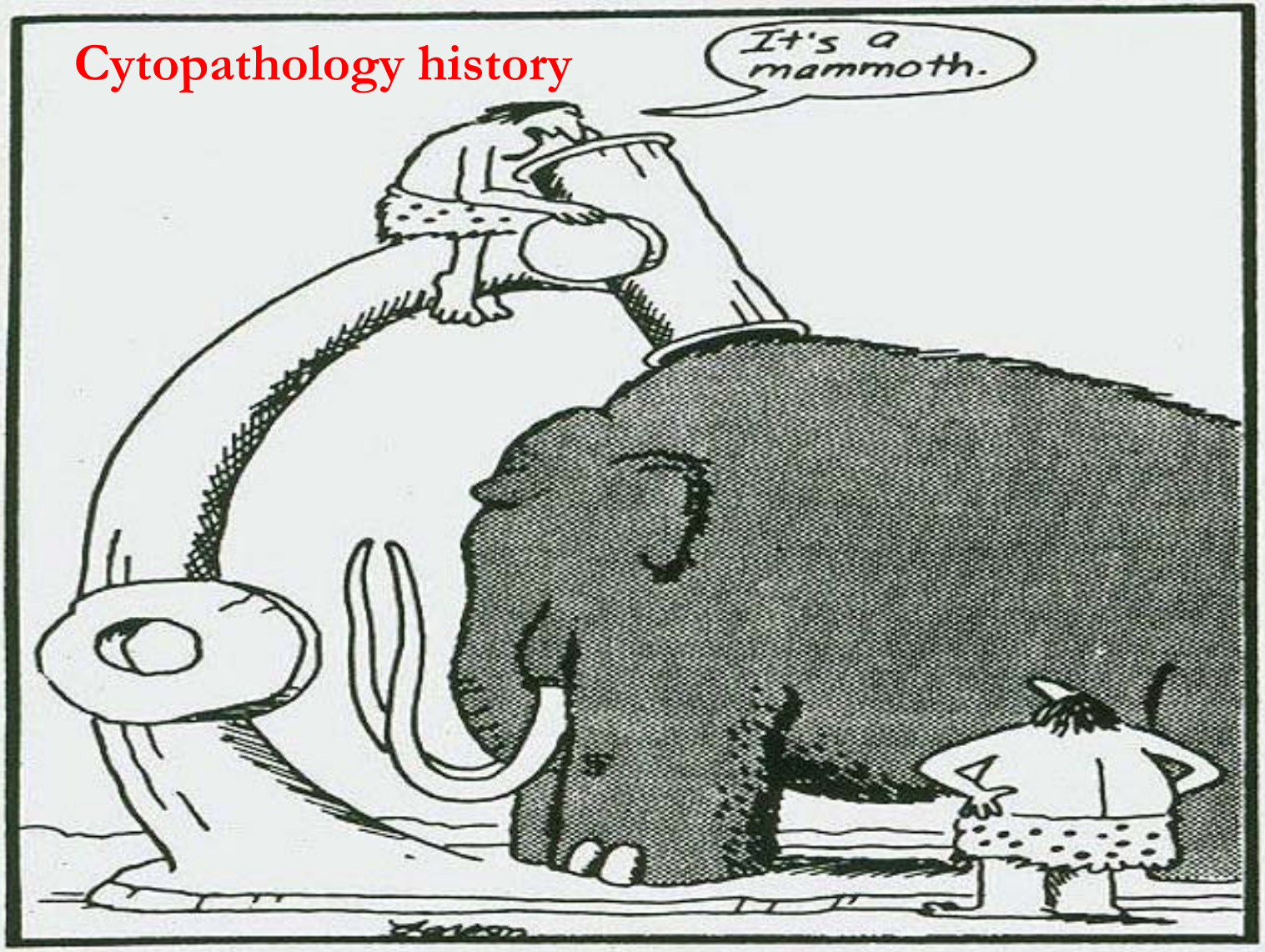
I AM CHARLY

Diagnostic Cytology

- Introduction
- Adventages and disadventages
- Samplings
- Stains
- Fluids
- FNAs
- Summary

Cytopathology refers to diagnostic techniques that are used to examine cells from various body sites to determine the cause or nature of disease

Cytopathology history



Early microscope

Cytopathology History

- The First Era – 19th century

- The Second Era – development and expansion

Father of cytopathology Dr George Papanicolaou

- The Third Era – consolidation

Dr Leopold Koss Diagnostic Cytology

- The Fourth Era – The Bethesda System for Reporting Cervical/Vaginal Cytology Diagnoses

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Advantages of Cytopathology

- Samples can be:

  collected easily and qiuckly

  prepared, stained and interpreted quickly

- Inexpensive
- Little or no risk to the patient

- Cytologic examinations identify disease process
  - neoplasia vs inflammation
  - specific vs nonspecific inflammation
- Direct therapy
- Form prognosis
- Determinate next diagnostic procedures

Disadvantages of Cytopathology

- **IT IS NOT ALWAYS POSSIBLE TO:**

- ✓ localize neoplastic lesion
- ✓ distinguishe preinvasive of invasive cancer
- ✓ distinguishe reactive of dysplastic and neoplastic changes
- ✓ determine tumor type


32nd European Congress of Cytology
Venice
October 1 - 4, 2006
Congresso Nazionale
SIAPEC-IAP
Venezia 2006

Advantages of Histopathology

- Microscopic examination usualy is much less demanding
- Ability to evaluate architecture
- Ability to cut additional section for special stains

Disadvantages of Histopathology

- Time required to create sections
- Identification of certain type of cells – small cell carcinoma vs lymphoma

Always use histopathology !!!!!!!!!!!!!

- To examine margines of resection
- To examine stromal invasion and deep of invasion
- Gross/cytopathology discrepancies

Cytopathology should not be compared to histopathology!!!

Used together will provide rapid and most accurate diagnosis!!!!

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Cytopathology Methods

1. **Exfoliative cytology** – spontaneosly shed cells in body fluids
2. **Abrasive cytology** – dislodges cells from body surfaces
3. **Fine needle aspiration cytology** – FN, FNA, FNAB, FNAC

Cytopathology Methods

1. **Exfoliative cytology** – spontaneosly shed cells in body fluids

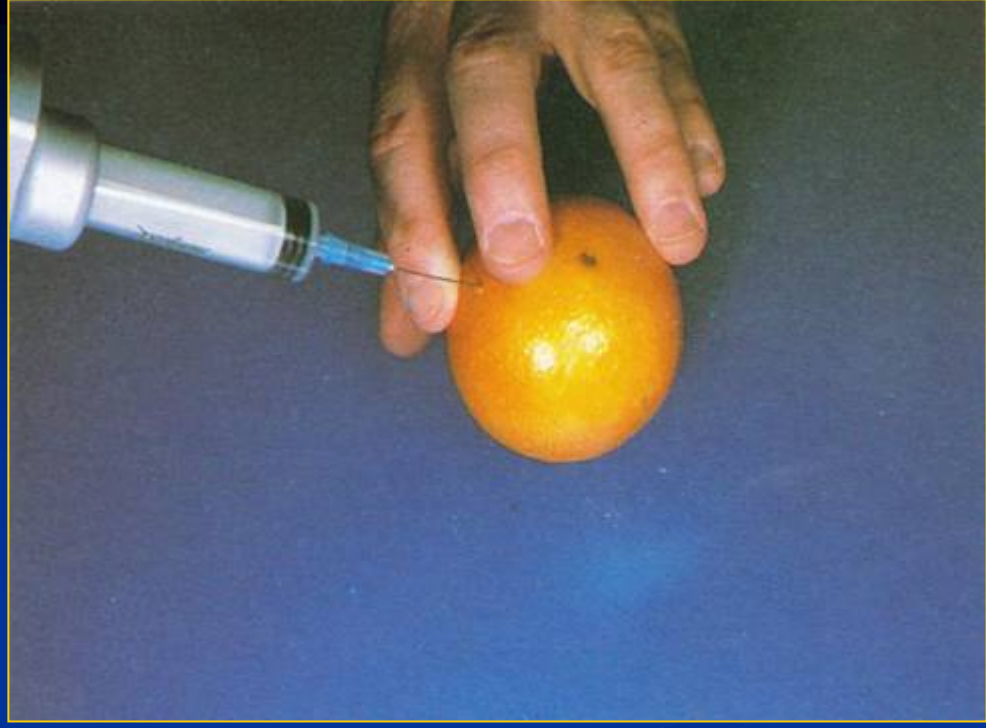
- Urine
- CSF
- Sputum
- Effusions in body cavities (pleura, pericardium, peritoneum)

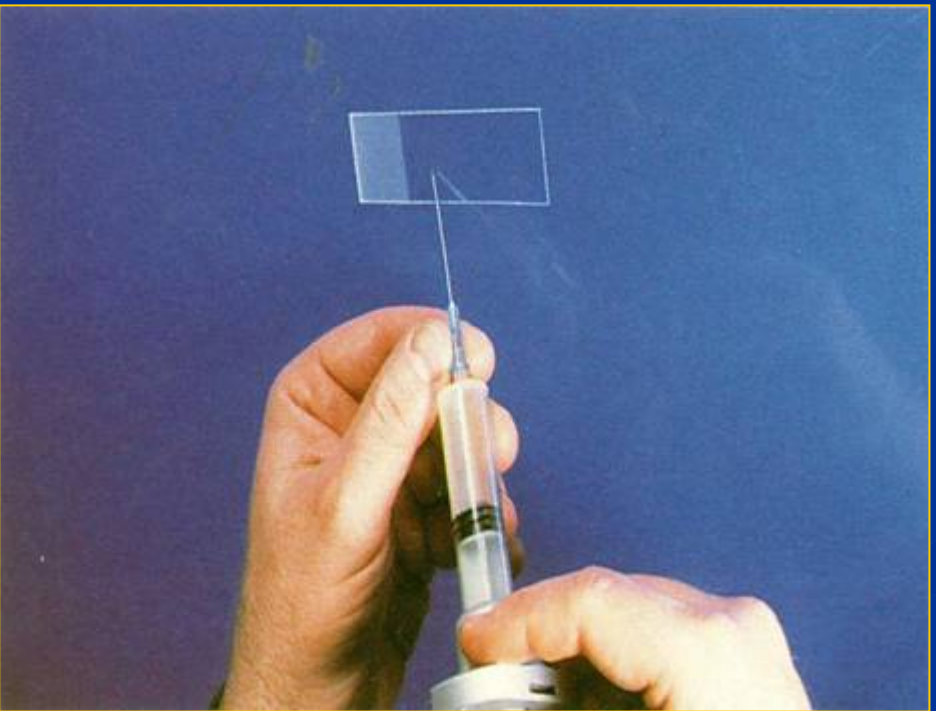
2. Abrasive cytology – dislodges cells from body surfaces

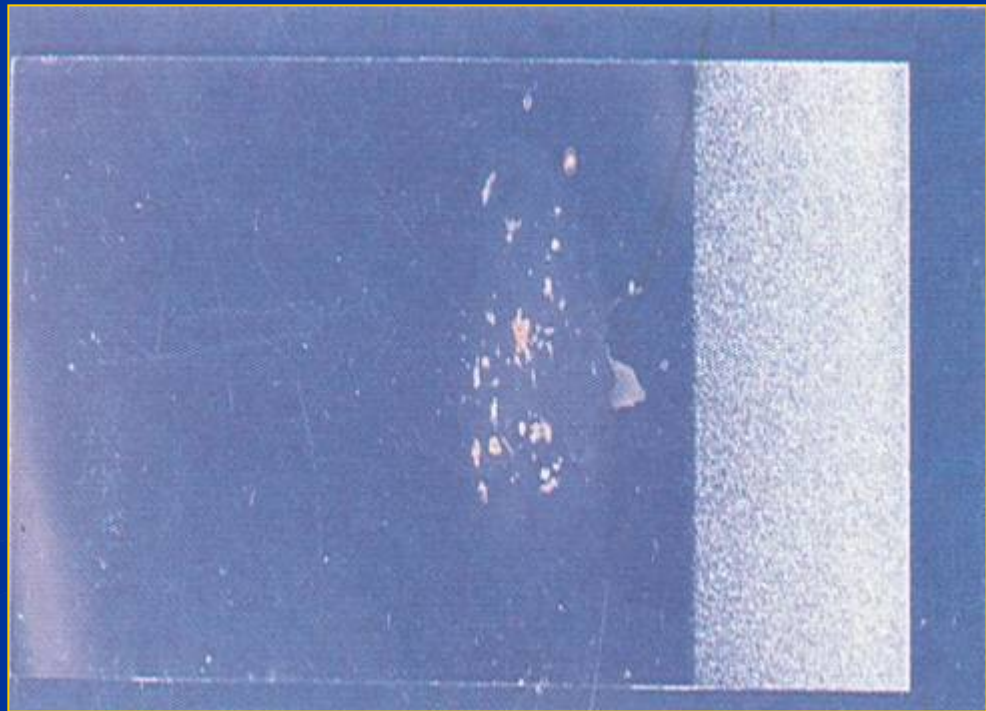
- Imprint
- Scraping
- Endoscopic brushing of mucosal surfaces
- Washing (lavage) of mucosal or serosal surfaces
- Swab

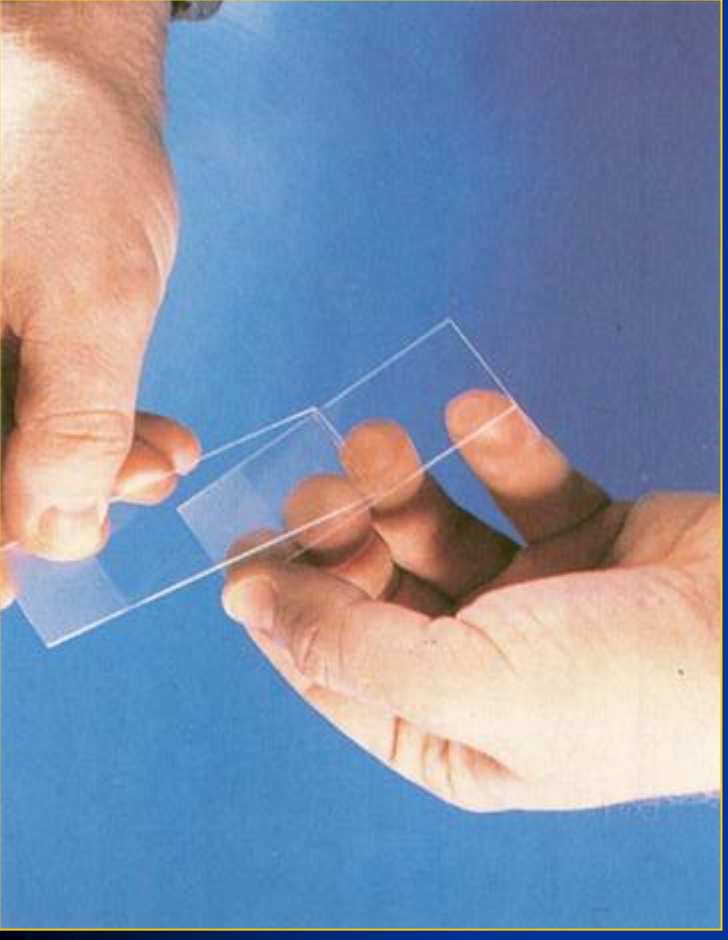
3. Fine needle aspiration cytology – FN, FNA, FNAB, FNAC

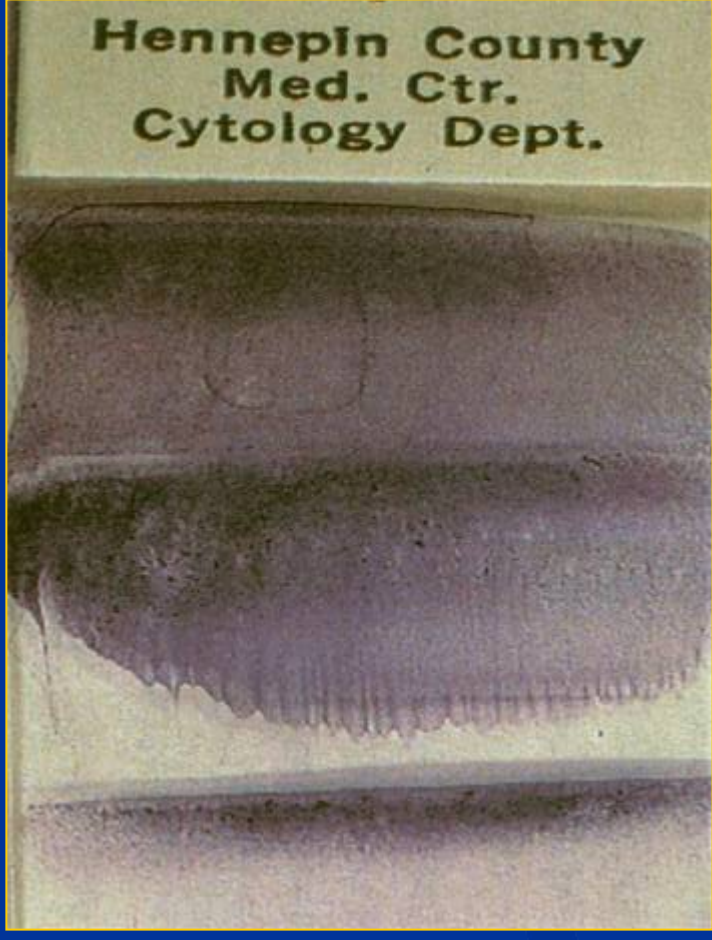
- Superficial nodules and organs - easily targeted
- Deep organs – guidance of CT, US

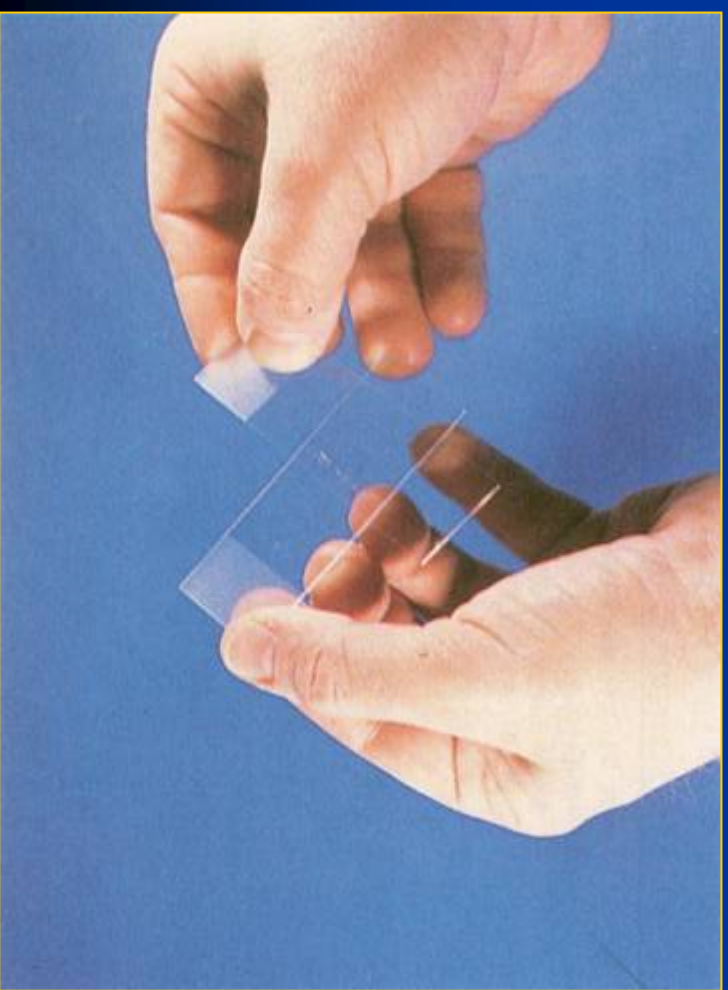


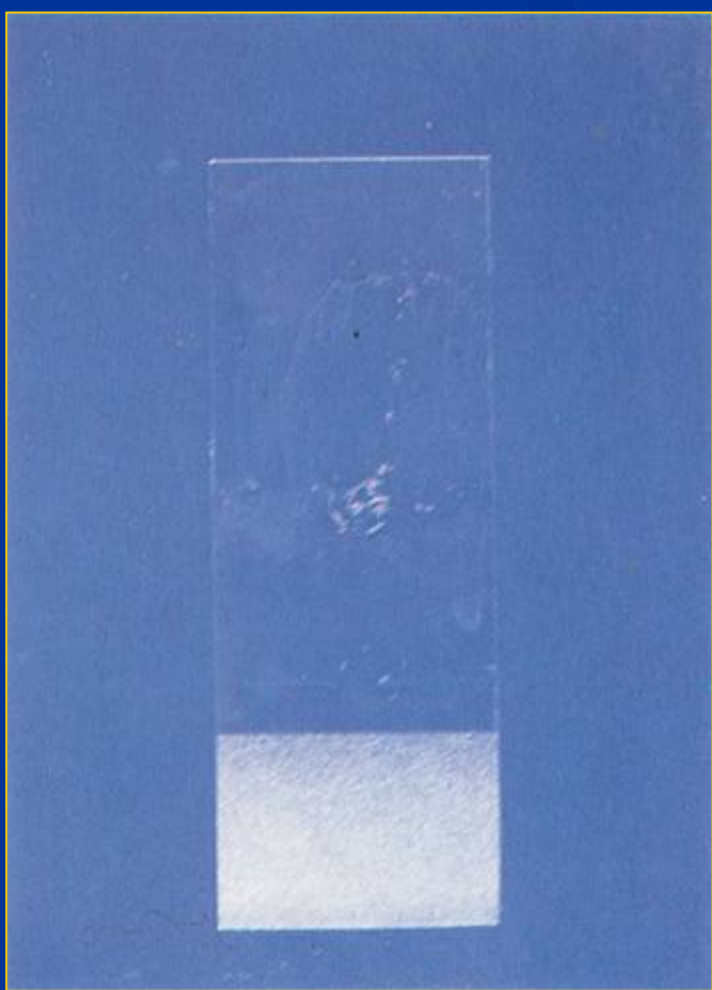







Hennepin County
Med. Ctr.
Cytology Dept.





Intraoperative Cytopathology

Intraoperative Cytopathology

- Accurate
- Fast
- More complete sampling
- Preserves tissue for permanent sections

Slide Preparation

- Conventional preparation
- Liquid based preparation
- Cell block

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Stains

- **Romanowsky type stains** (for air dried slides)

- **Papanicolaou stains** (for immediate fixated slides)

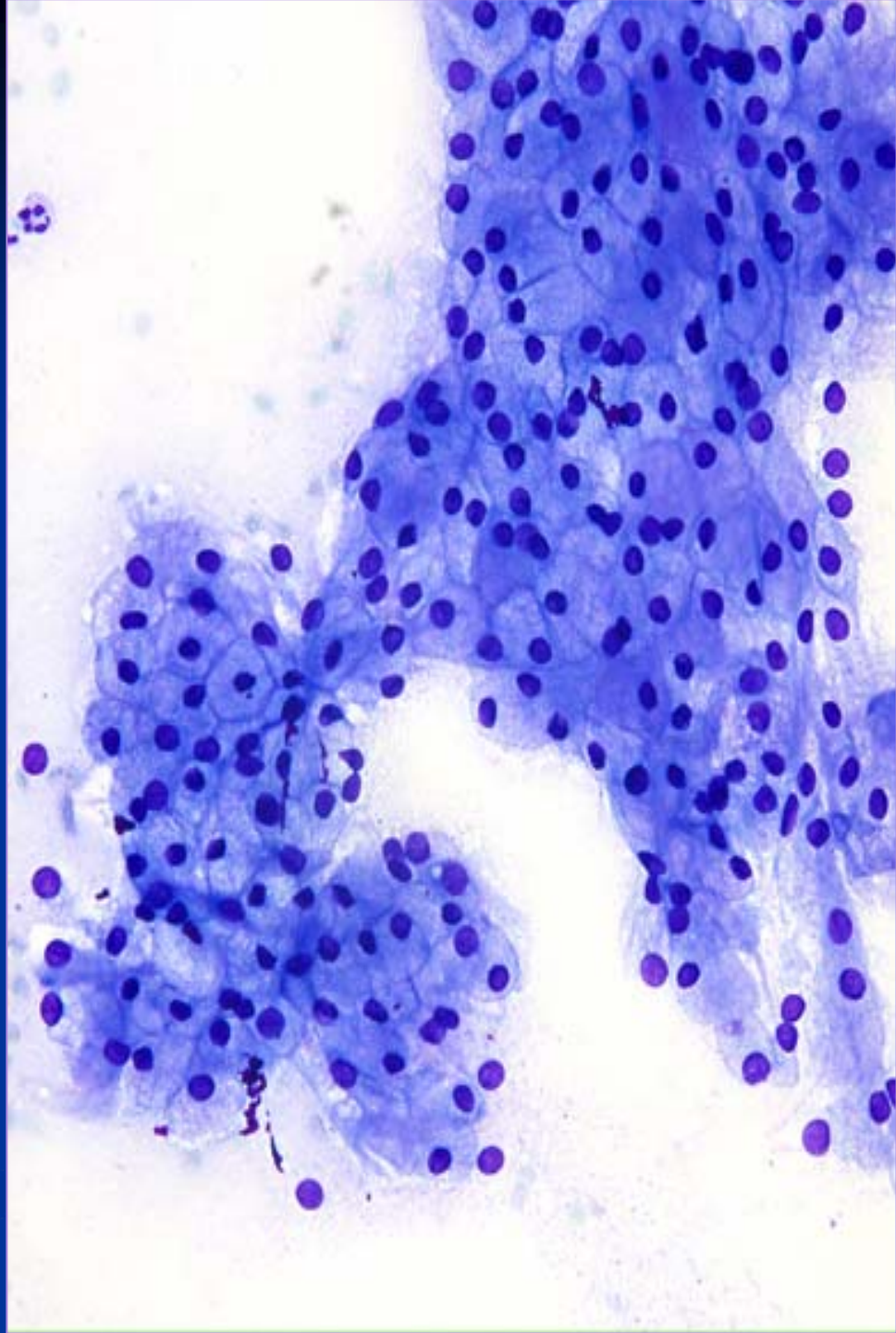
Stains

- **Romanowsky type stains** (for air dried slides)
  - Wright's stain
  - Giemsa stain
  - Wright's Giemsa stain
  - May Grunwald Giemsa stain
  - Diff- Quik stain

Diff-Quik stain

Nuclear and nucleolar features are less preserved

Cytoplasmatic features are better preserved



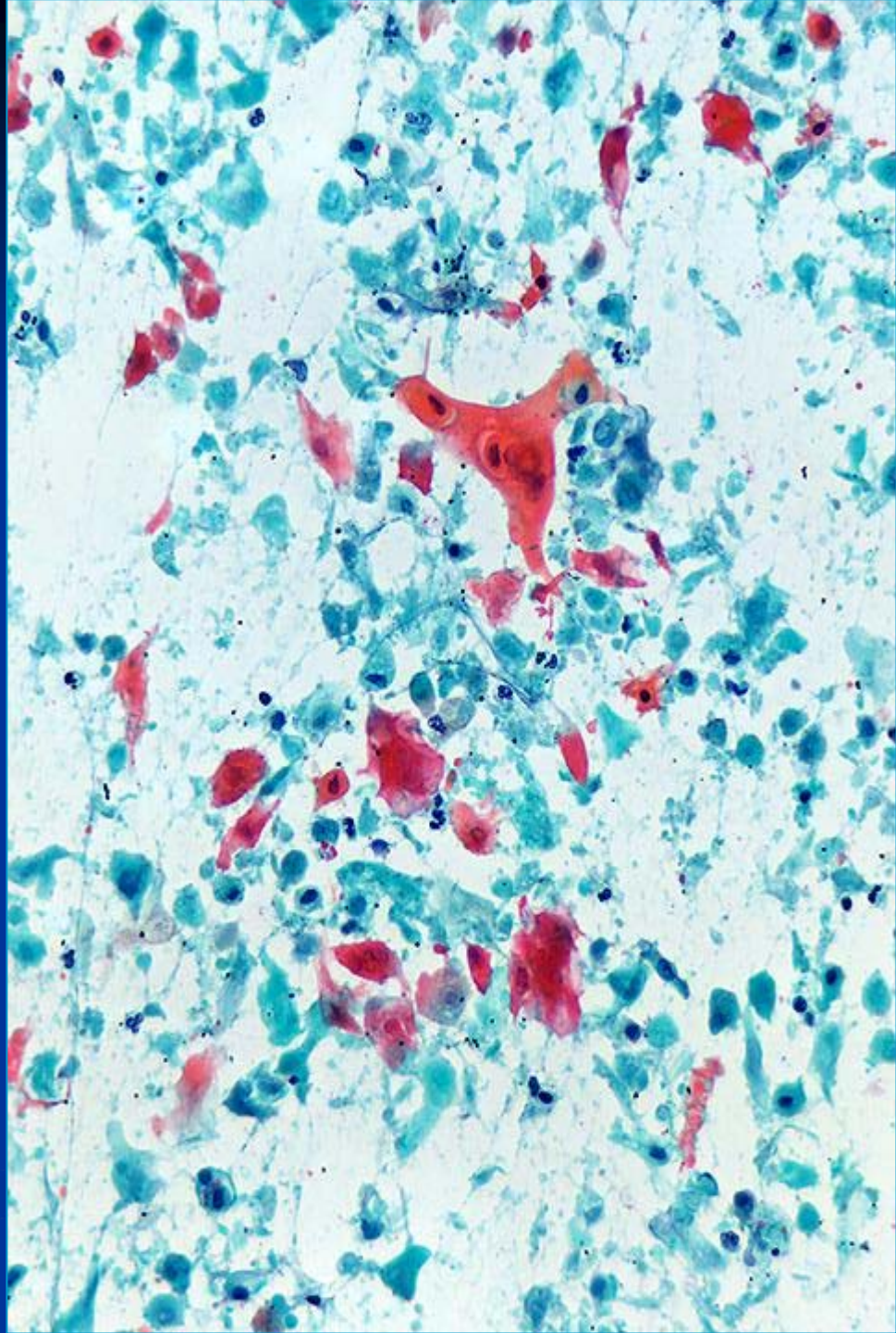
- **Papanicolaou stains** – for immediate fixated slides

considerable time!!!

Papanicolaou stain

Nuclear and nucleolar features are better preserved

Cytoplasmic changes and microorganisms are not demonstrated

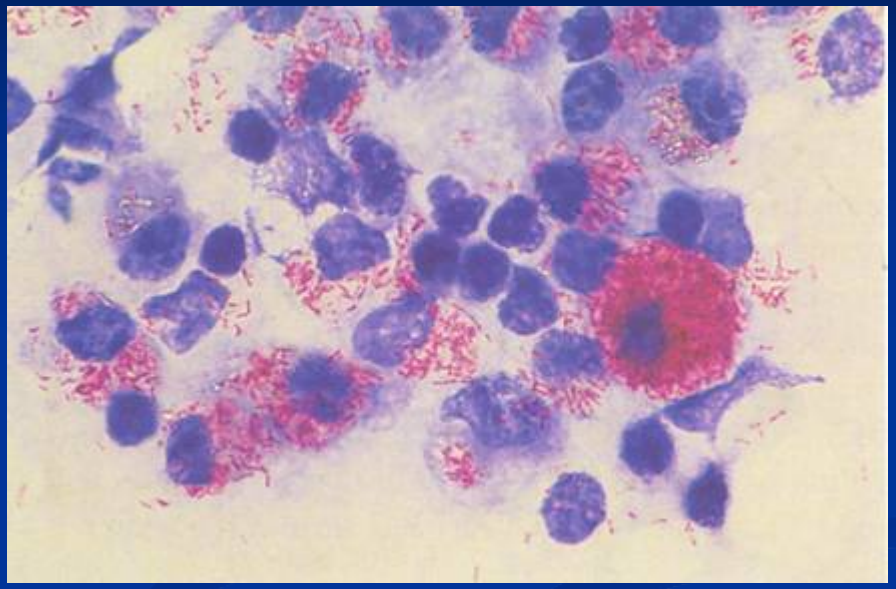


Fixation and Staining Effects

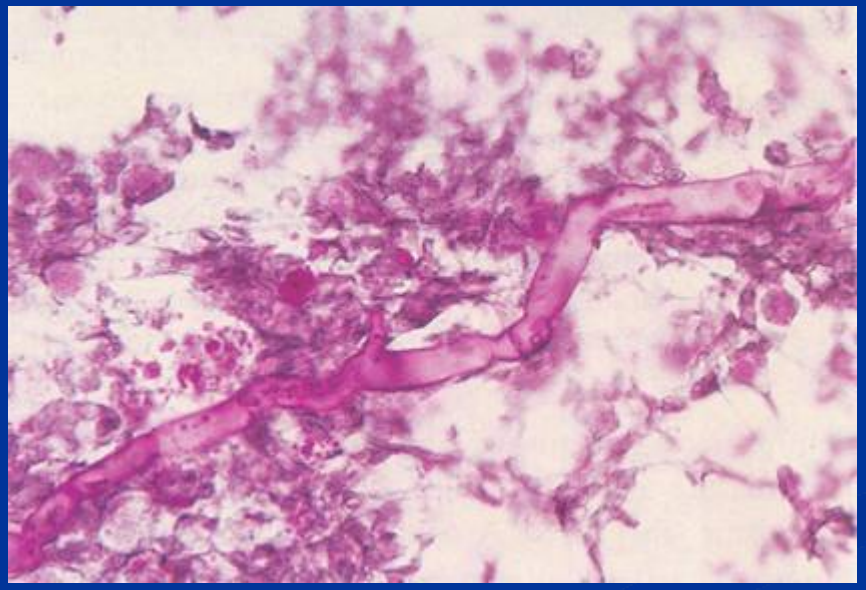
- Artifact
- Nuclear/ cytoplasmic ratio
- Chromatin pattern and color
- Nucleolar appearance
- Cytoplasmic features
- Extracellular matrix visiability and color

Additional Stains

- Cytochemistry

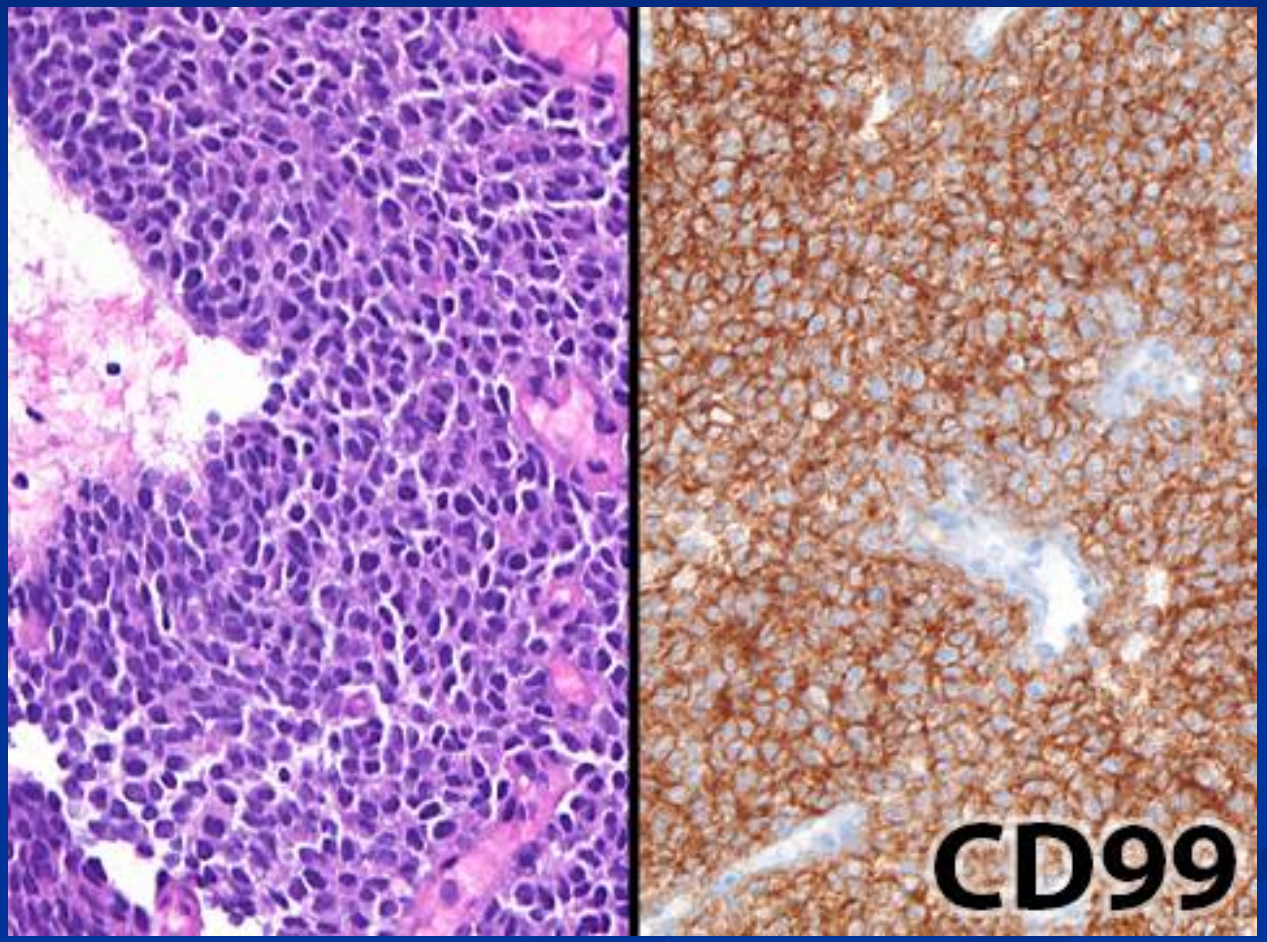


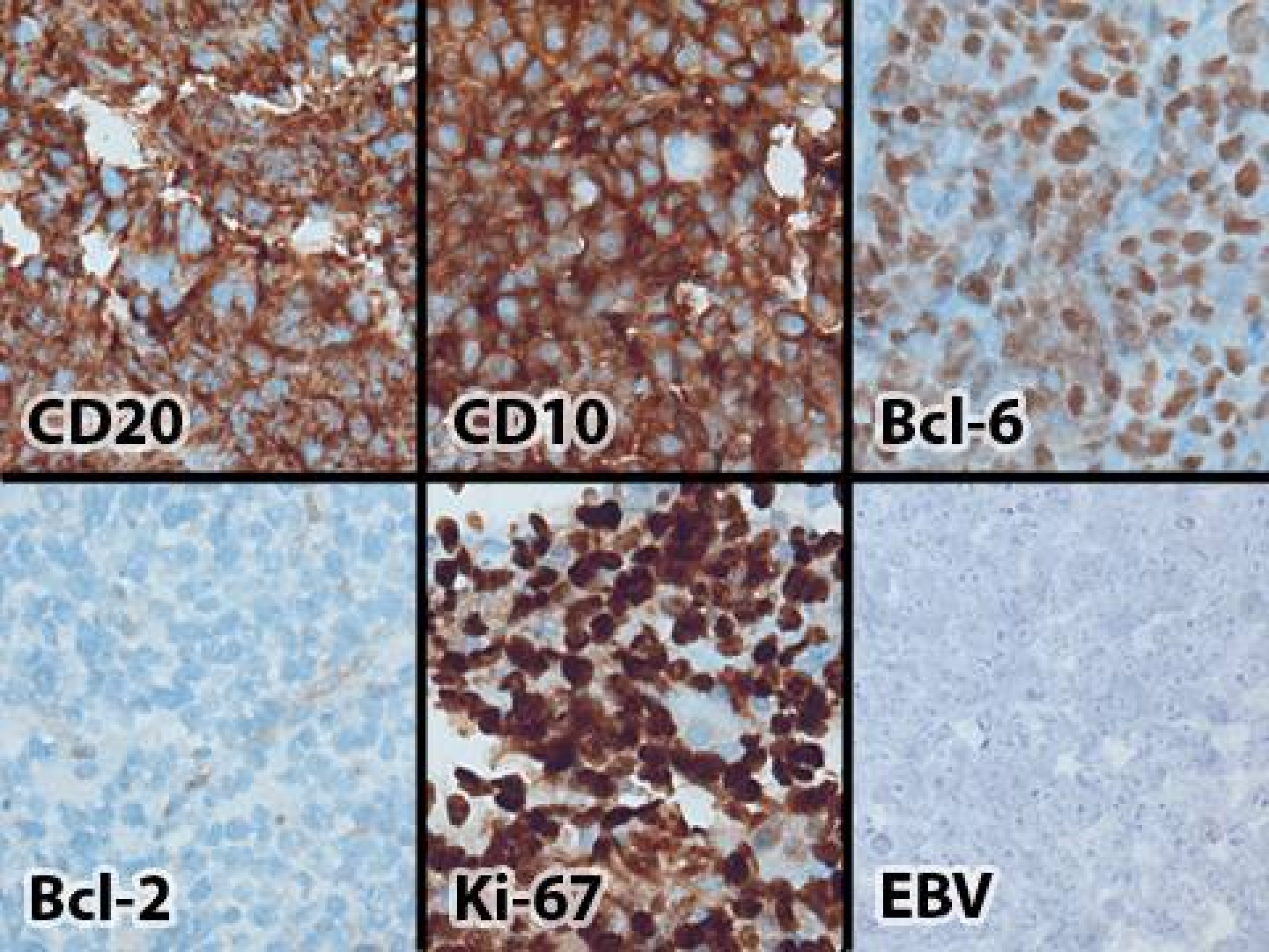
Ziehl-Neelsen stain



PAS stain

- Immunocytochemistry




CD20
CD10
Bcl-6
Bcl-2
Ki-67
EBV

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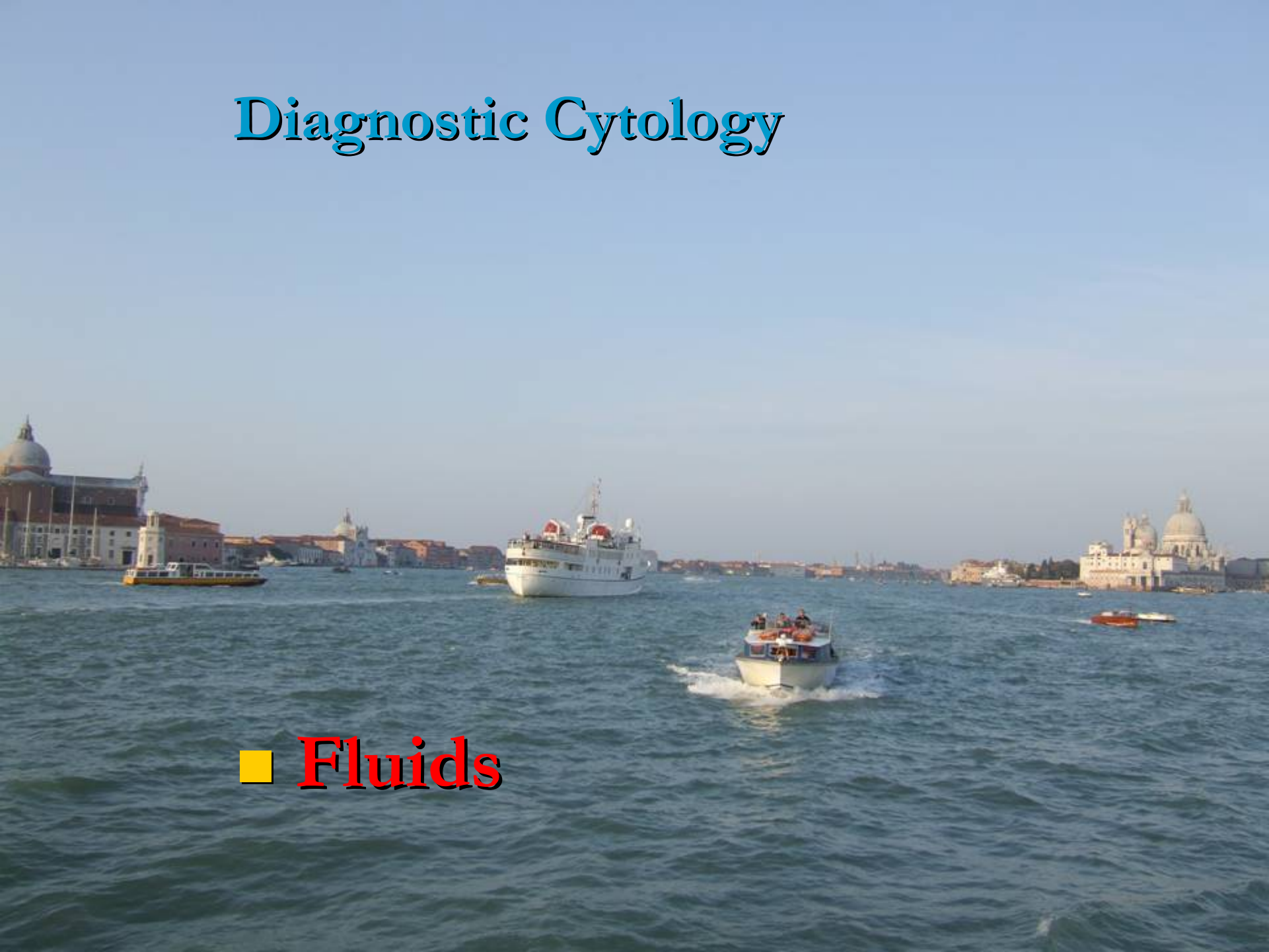


- **Fluids**

Cavity Fluids

- Abdominal
- Pleural
- Pericardial
- Synovial
- CSF

Cavity Fluids

- Sampling techiques

  appearance during collection

  EDTA to prevent clotting

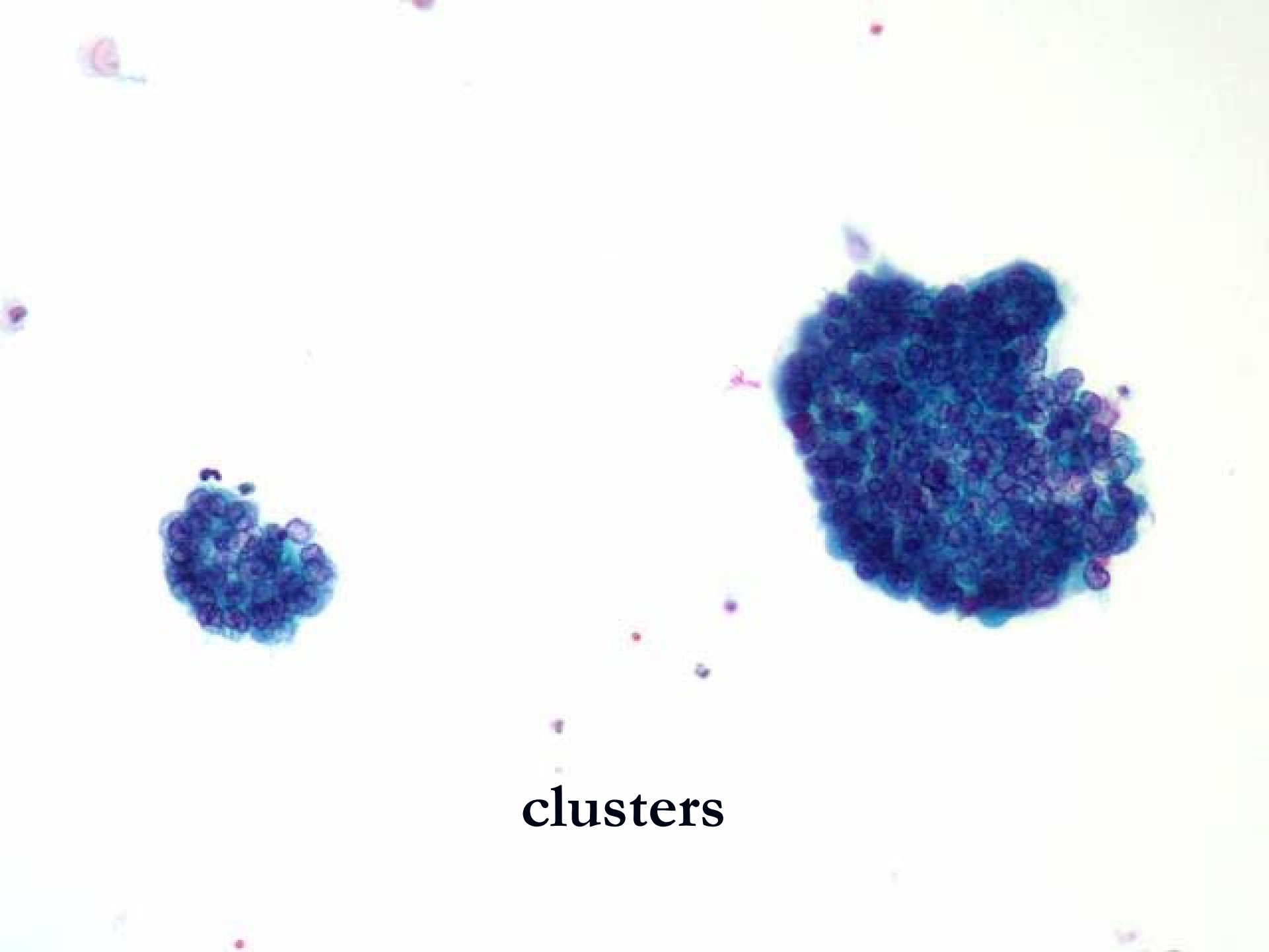
  direct smear - delayed processing
- Cell concentration
- Protein concentration

Cavity Fluids

- TRANSUDATE
- EXUDATE
- MODIFIED TRANSUDATE

Cavity Fluids

- Cell concentration
- Making slides
- Staining – (keep one or two slides in reserve, esspecialy if visiable clumps of cells)



clusters

Fluid Examination

- Gross appearance of stained slide
- Scan using low magnification
- Examine details using oil lens
- Ad special stains as indicated

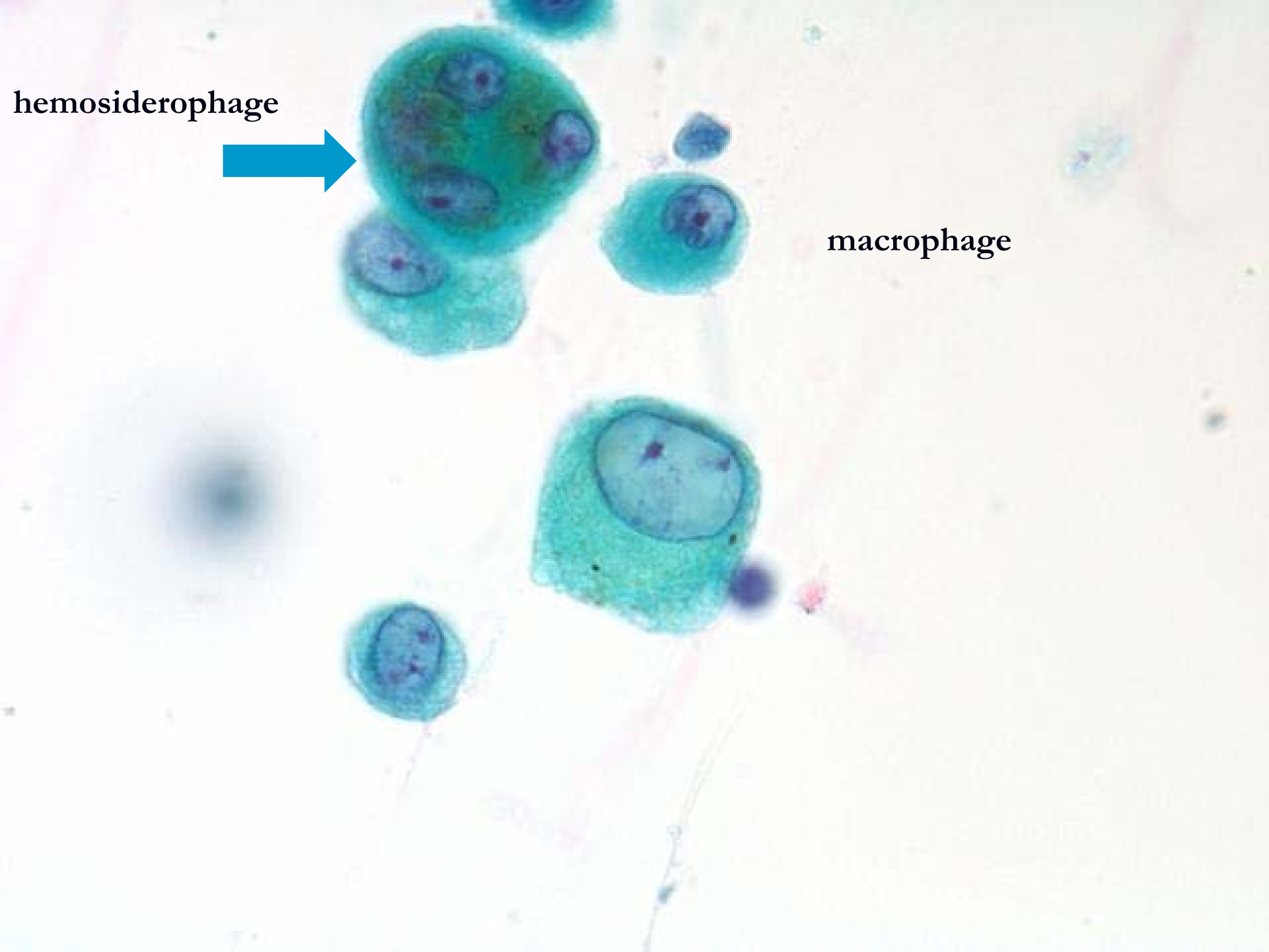
Description

- Adequacy – on site
- Background – necrotic, mucinous.....
- Cell concentration – high, low.....
- Cell preservation – lysis.....
- Inflammatory cells – which? dominant?
- Lining cells – mesothelial, epithelial.....
- Cells of interest – tumor cells.....

TRANSUDATE

- Protein concentration<2,5g/dl
- TNCC <1500 cells/µg

MACROPHAGES


hemosiderophage
macrophage

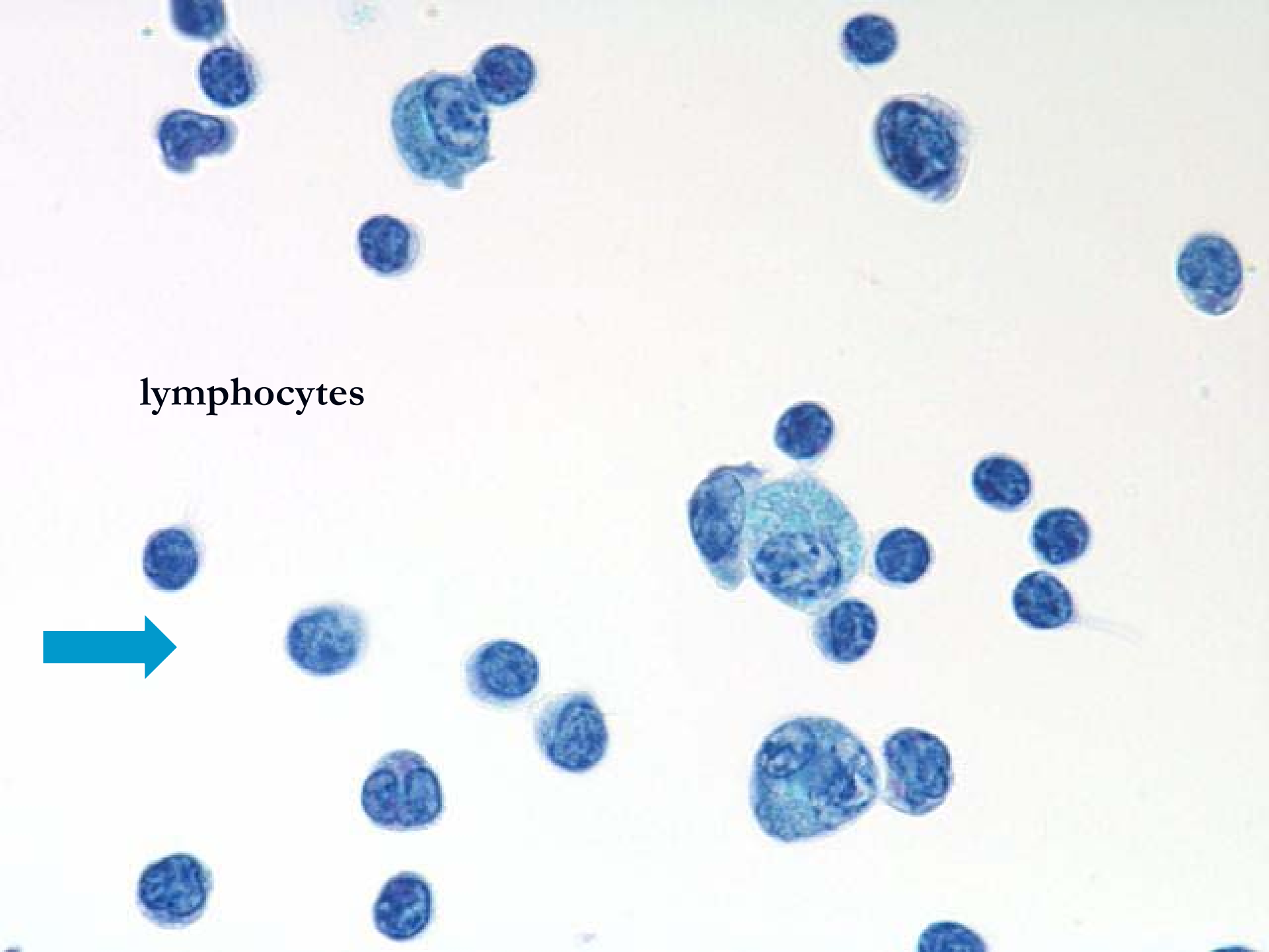
TRANSUDATE

- MACROPHAGES
- MESOTHELIAL CELLS


mesothelial cells

TRANSUDATE

- MACROPHAGES
- MESOTHELIAL CELLS
- LYMPHOCYTES


lymphocytes

MODIFIED TRANSUDATE

- Moderate protein concentration 2,5- 7,5g/dl
- Moderate cellularity 1000-7000 cells/µg

✓ Cardiovascular disease

✓ Neoplastic disease

✓ FIP

✓ Rupture of urinary bleadder

✓ Hepatic disease


EXUDATE

EXUDATE

- High protein concentration > 3,0 g/dl
- High TNCC > 7000 cells/µg

- NONSPECIFIC
- SPECIFIC


NONSPECIFIC
EXUDATE

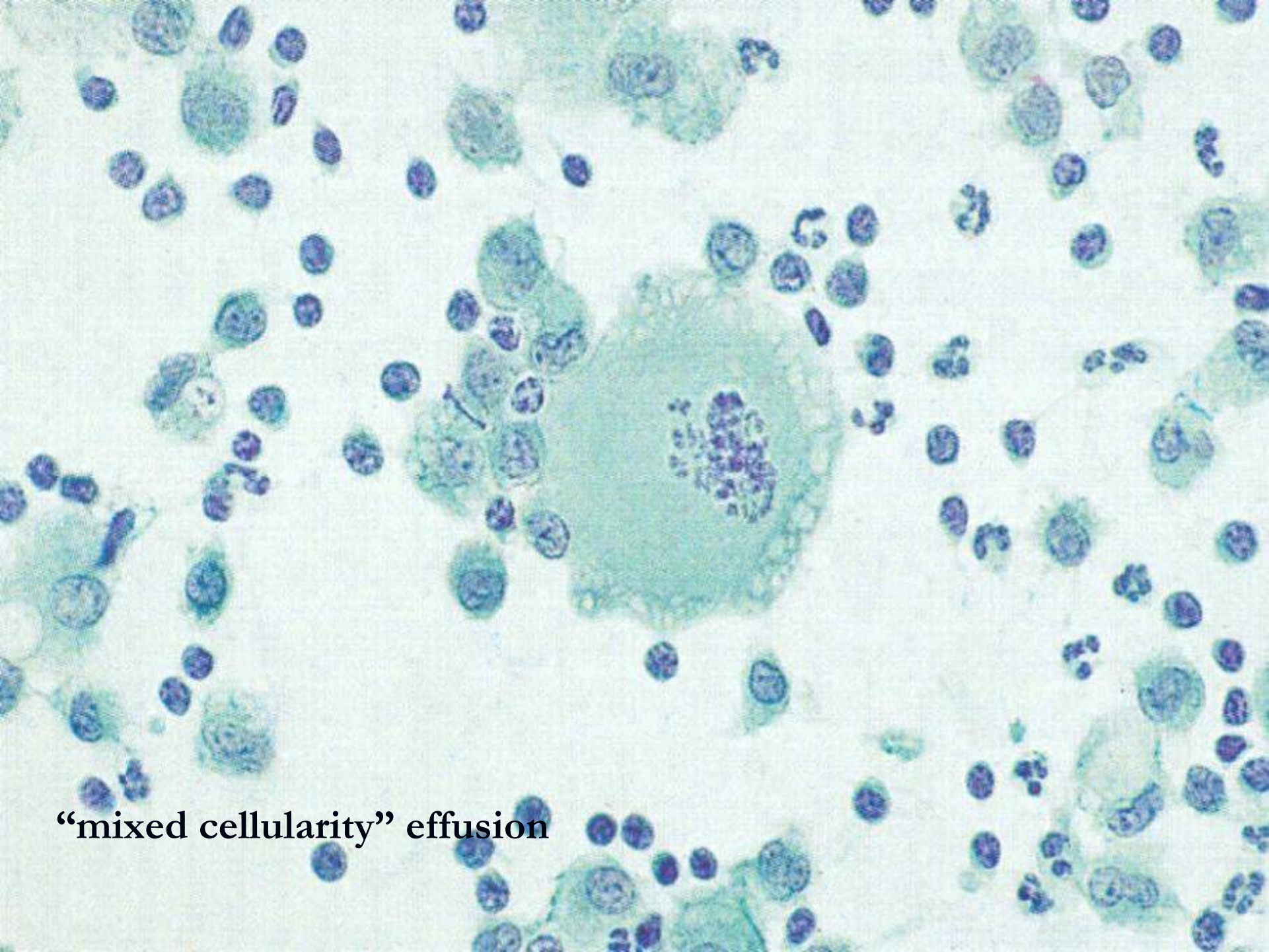
NONSPECIFIC EXUDATE

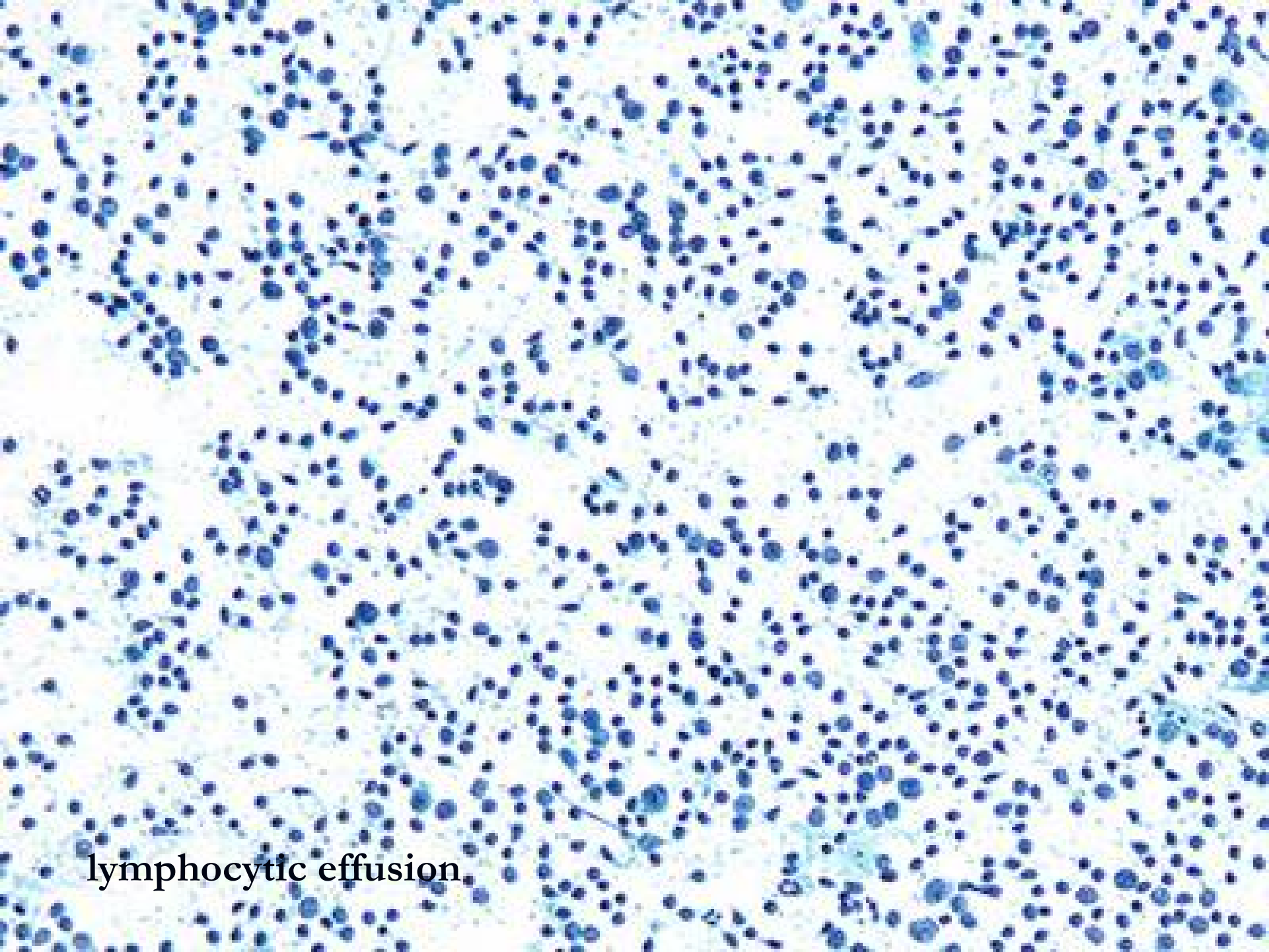
Terminology:

- acute, subacute, chronic
- predominant cells


neutrophilic effusion


eosinophilic effusion


“mixed cellularity” effusion


lymphocytic effusion

Giant multinucleated cell – Langhans type

Feline Infectious Peritonitis - FIP

- Abdominal and/or thoracic effusion in cats
- High protein concentration > 3,5
- Low-moderate number of cell
- Cytopathology:

✓ eosinophilic background

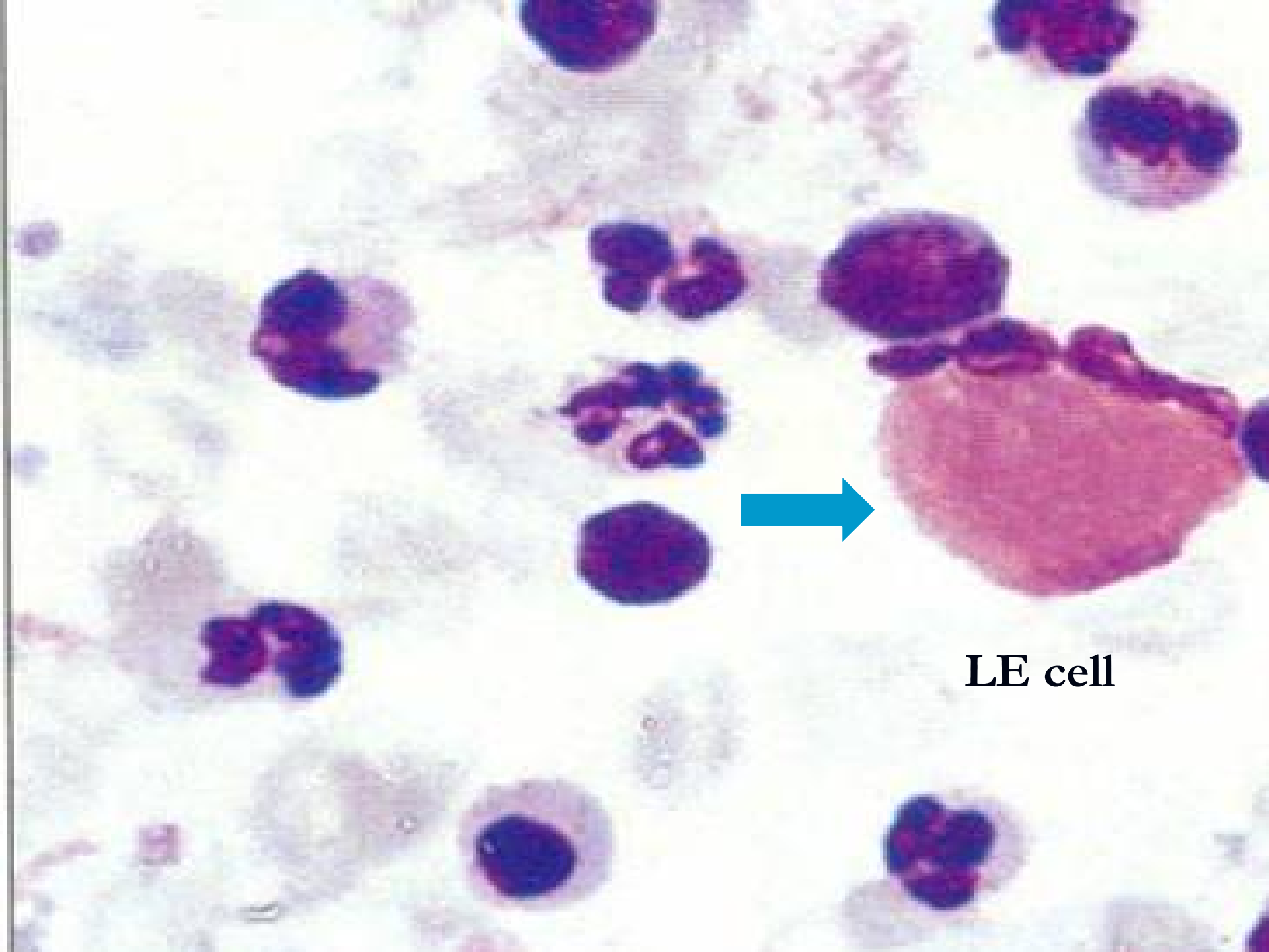
✓ large number of neutrophils

✓ lesser number of macrophages, mesothelial cells, lymphocytes and plasma cells


SPECIFIC EXUDATE

SPECIFIC EXUDATE

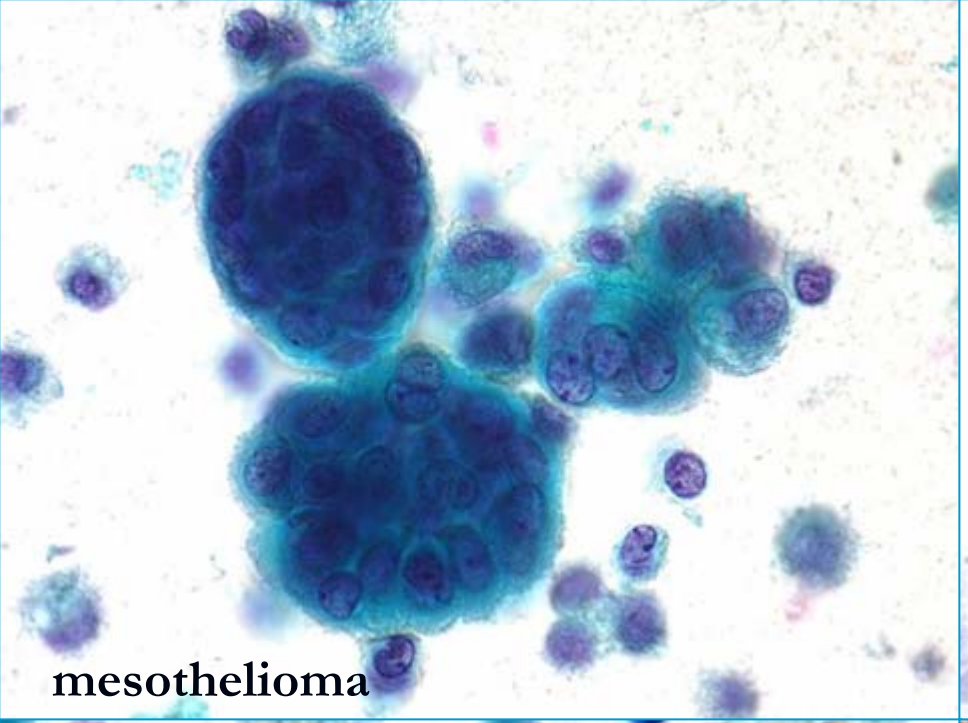
Lupus erythematosus SLE

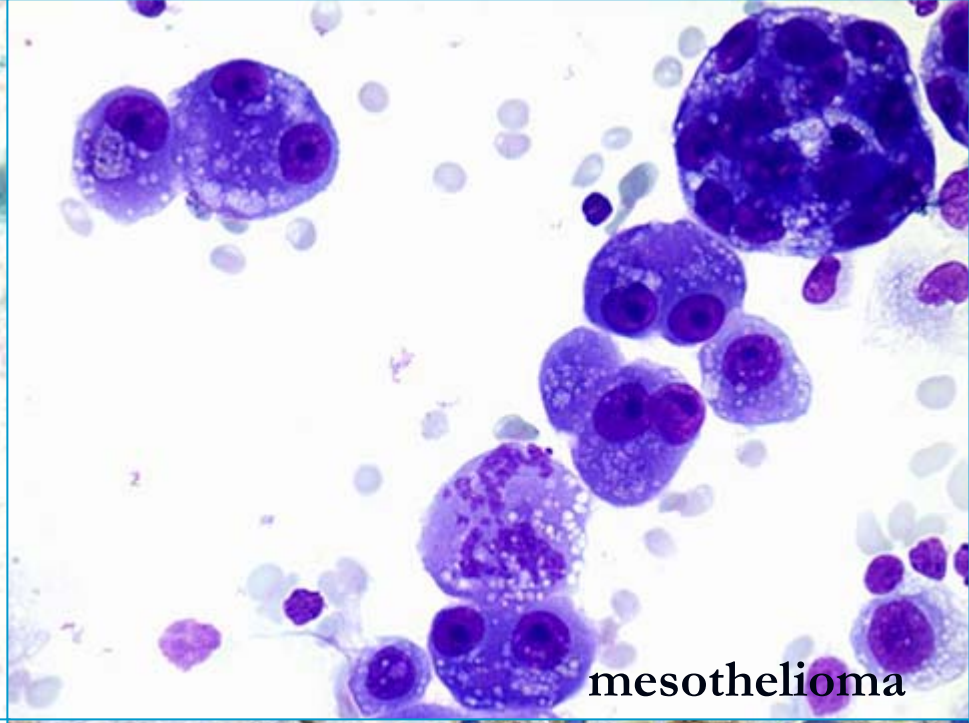

LE cell

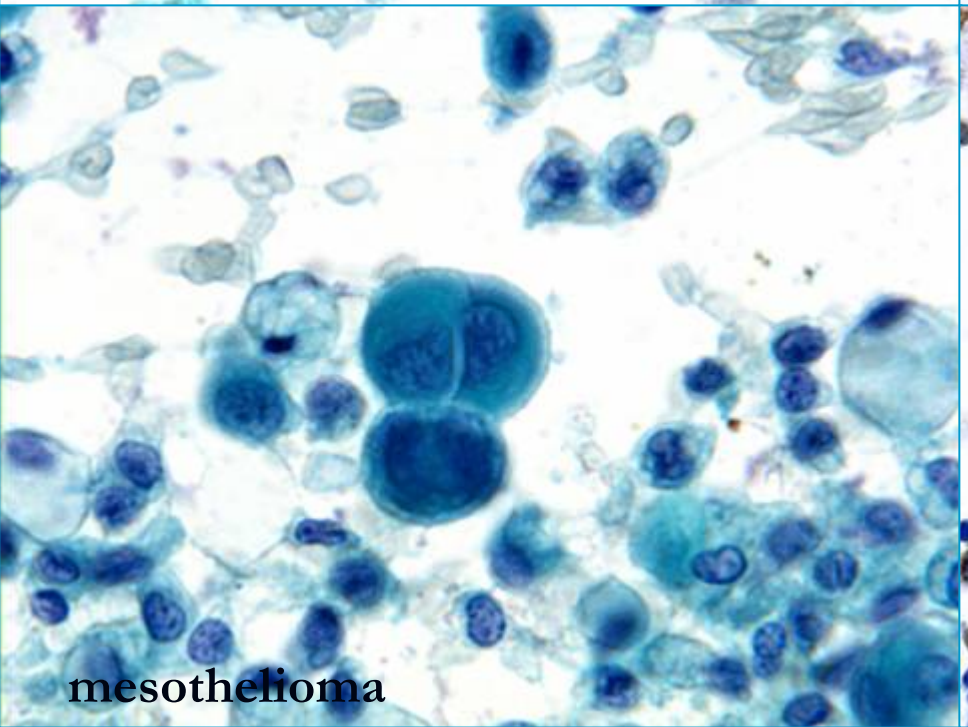
Malignant Effusion

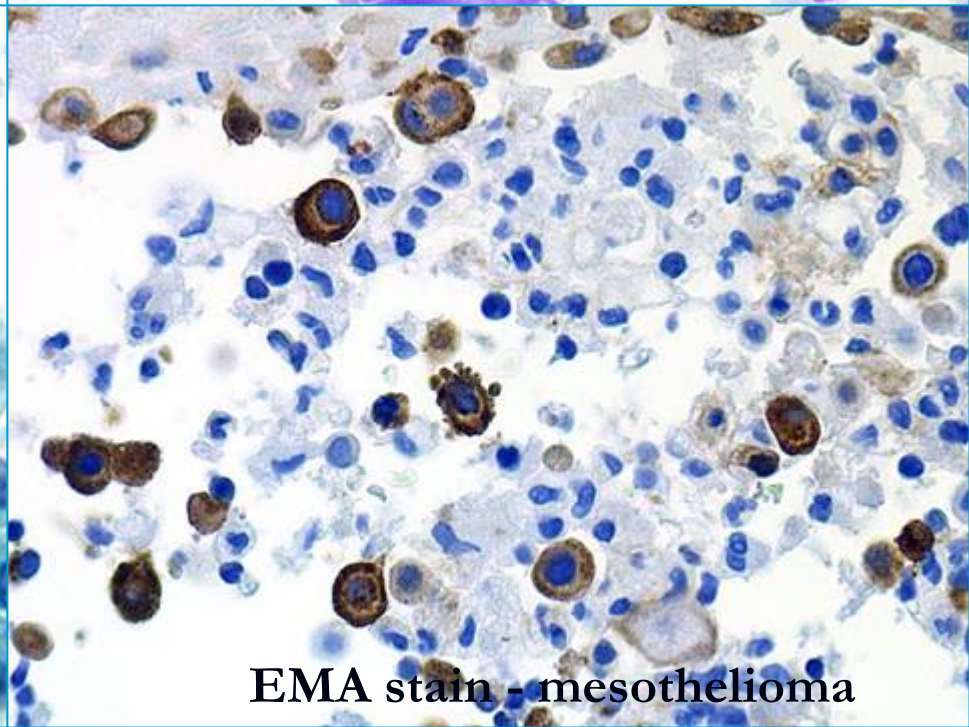
- Primary tumors:

MESOTHELIOMA

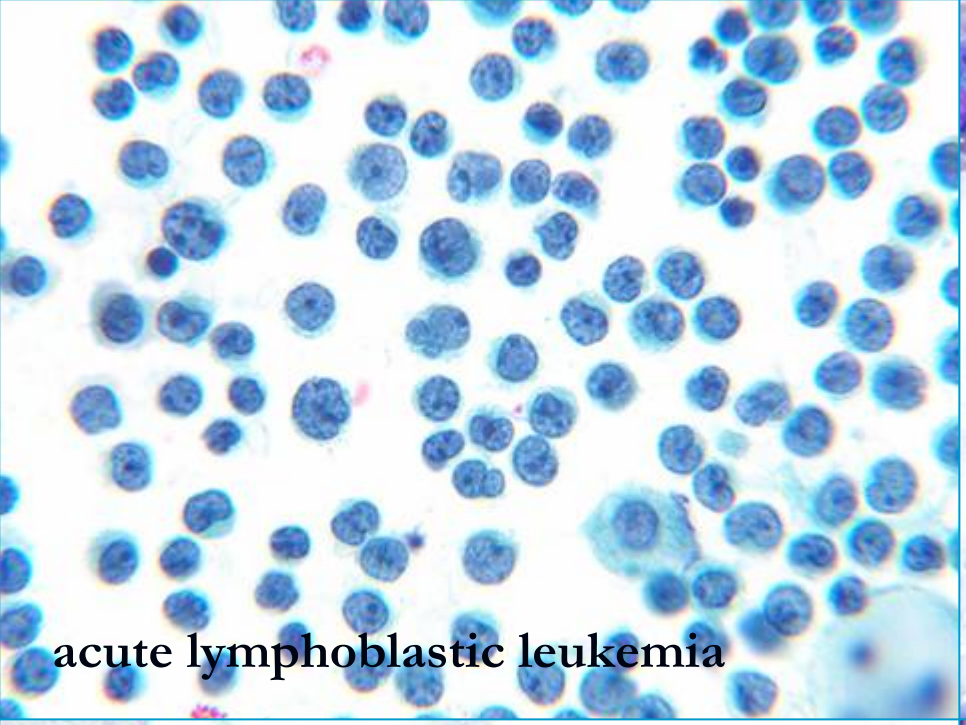

mesothelioma

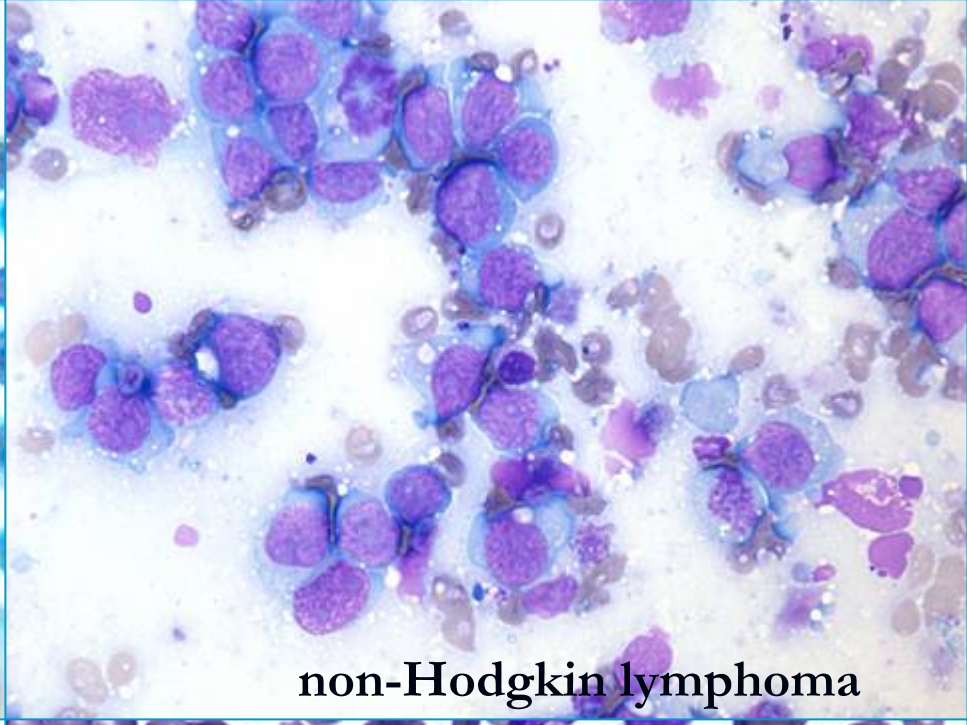

mesothelioma

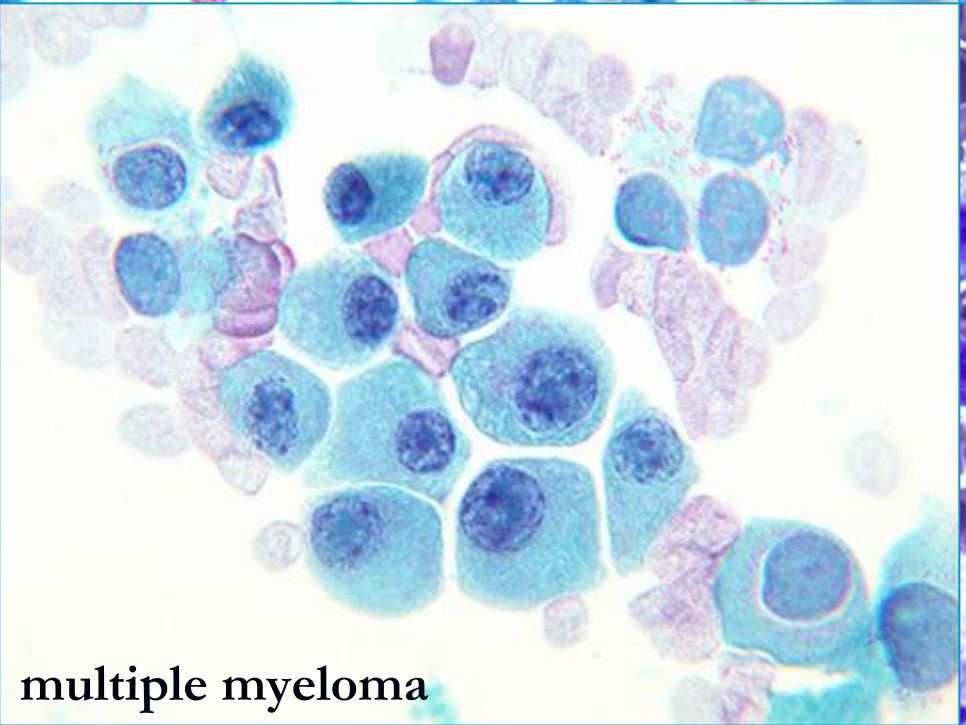

mesothelioma

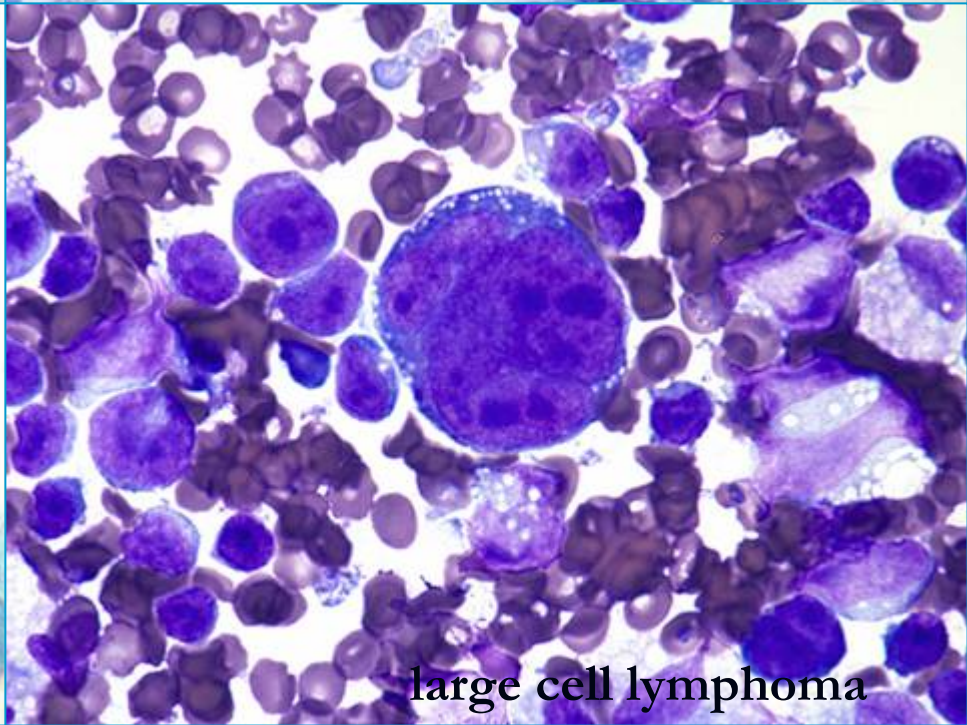

EMA stain - mesothelioma

LYMPHOPROLIFERATIVE DISORDERS


acute lymphoblastic leukemia

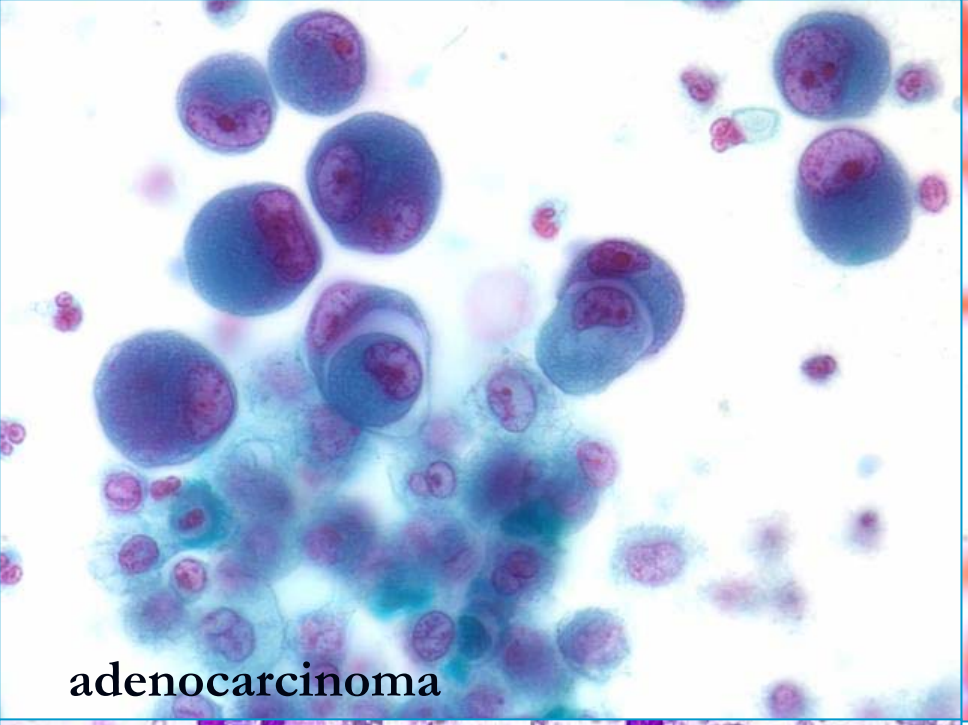

non-Hodgkin lymphoma

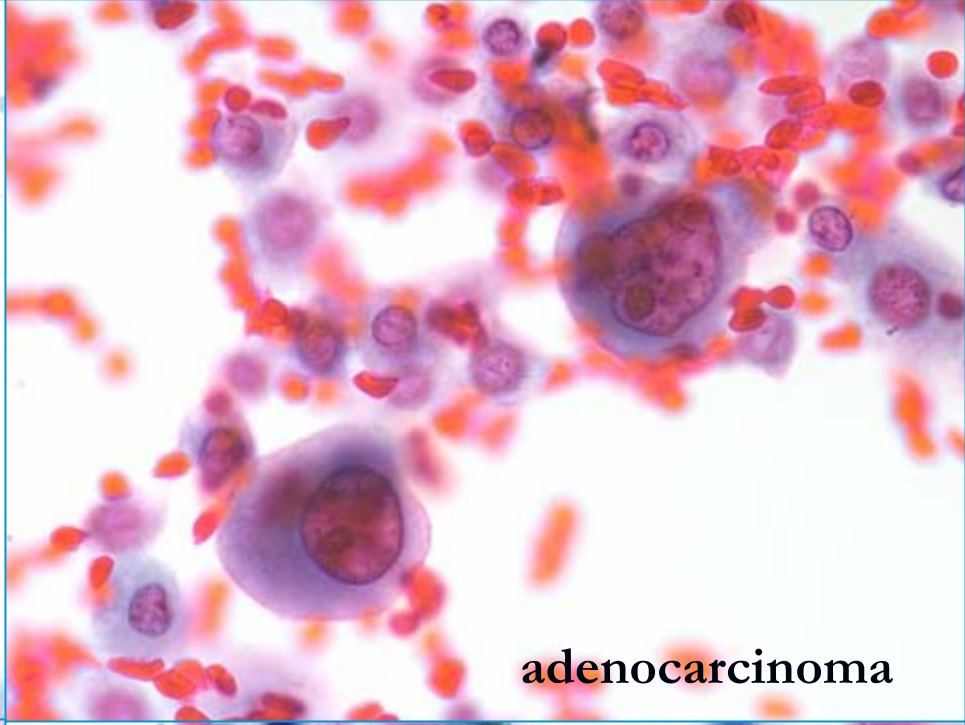

multiple myeloma

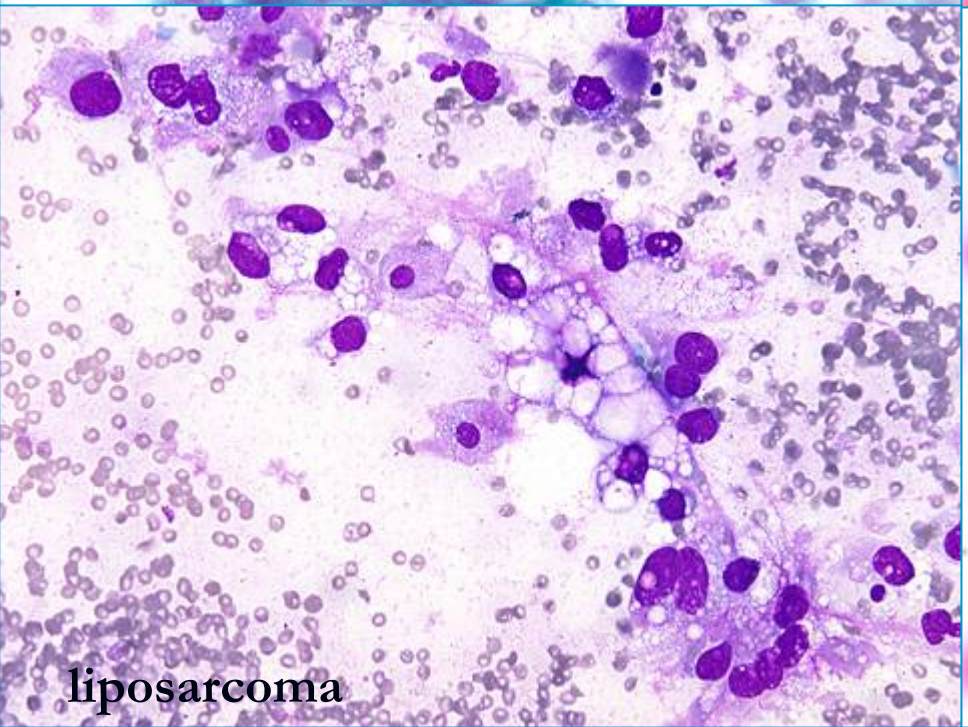

large cell lymphoma

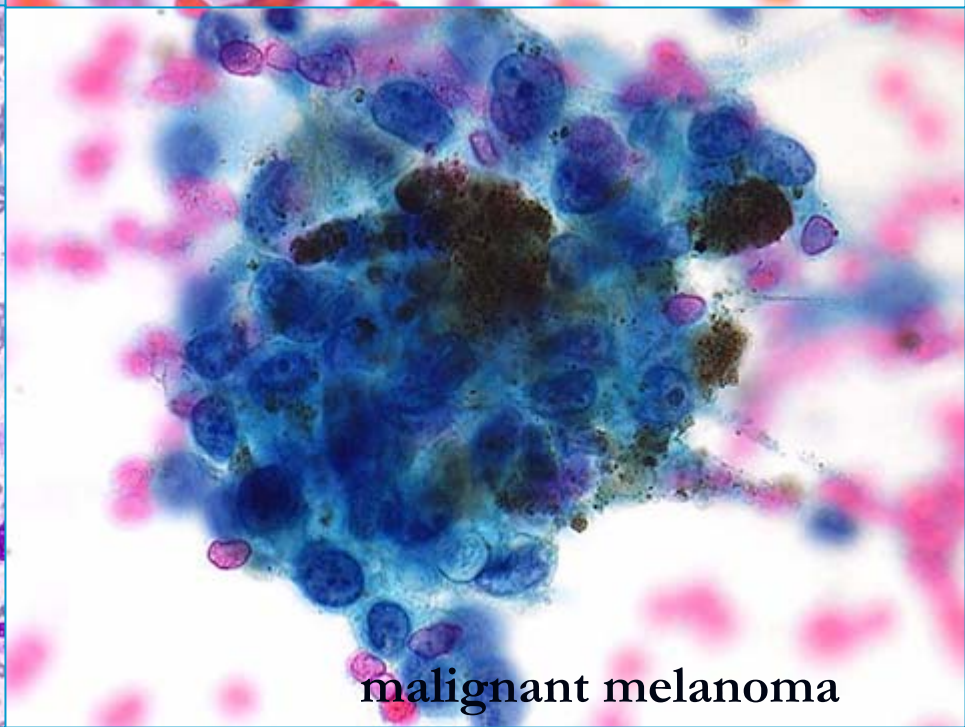
- Secondary tumors

METASTATIC TUMORS


adenocarcinoma


adenocarcinoma


liposarcoma


malignant melanoma

Positivity of fluids

60% - 70% - 90%

Hemorrhagic Effusions

- Presence of hemosiderophages
- Absence of platelets

Hemostatic defect

Trauma

Neoplasia

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Fine Needle Aspiration Cytology

- Gross appearance of the stained slide
- Scan using low magnification - **cellularity**
- Examine areas of interest:

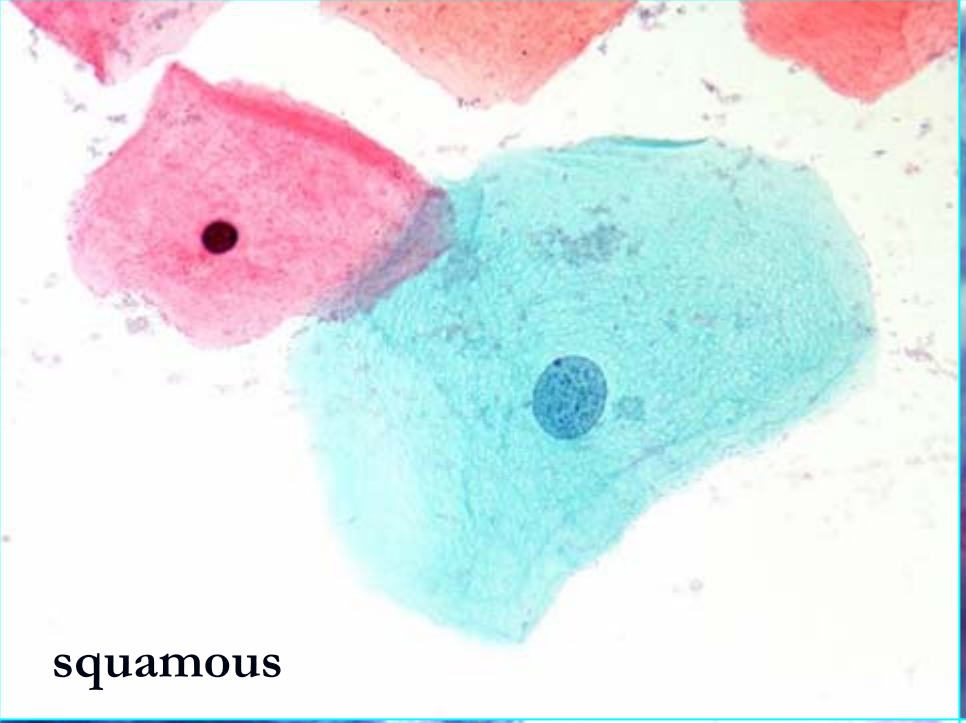
✓ **background** (erytrocytes, necrosis, preservation of cells)

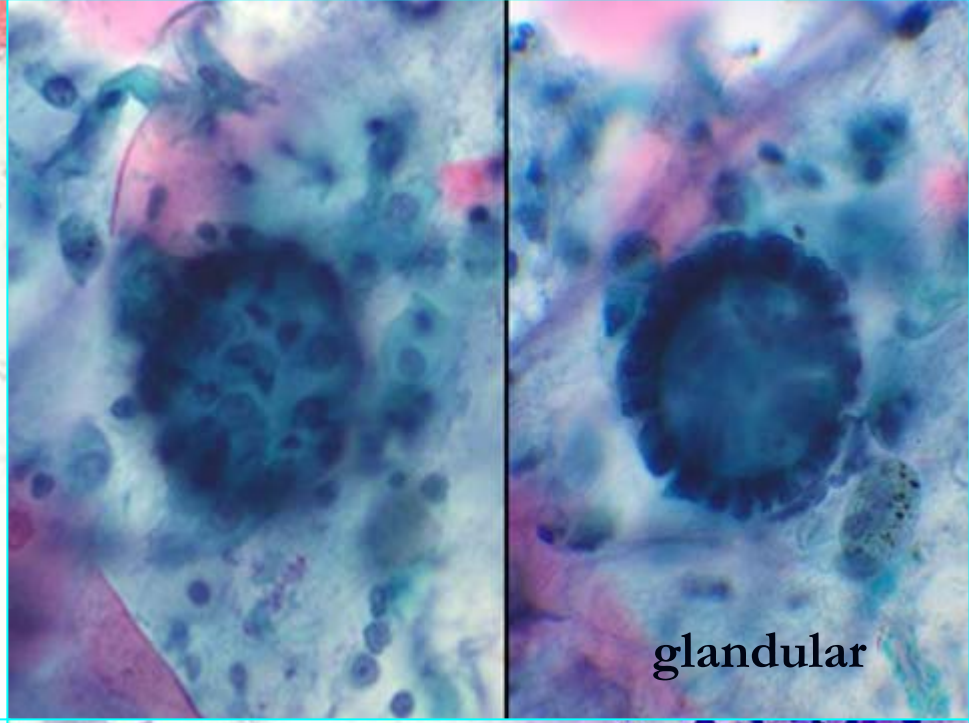
✓ **cell types, distribution, organisation**

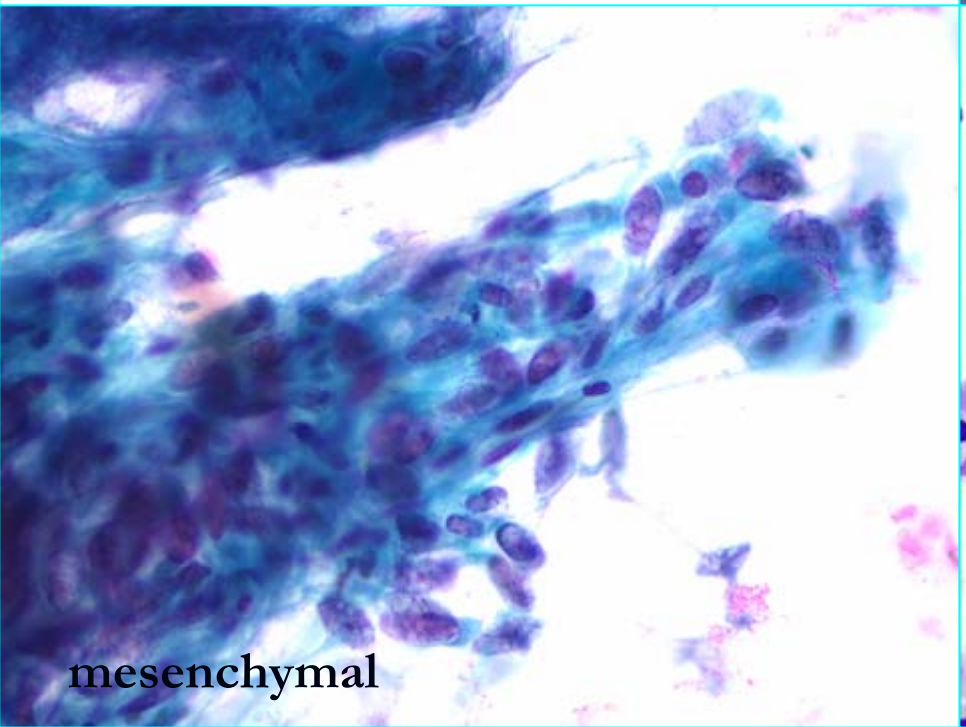
✓ request **special stains** if required

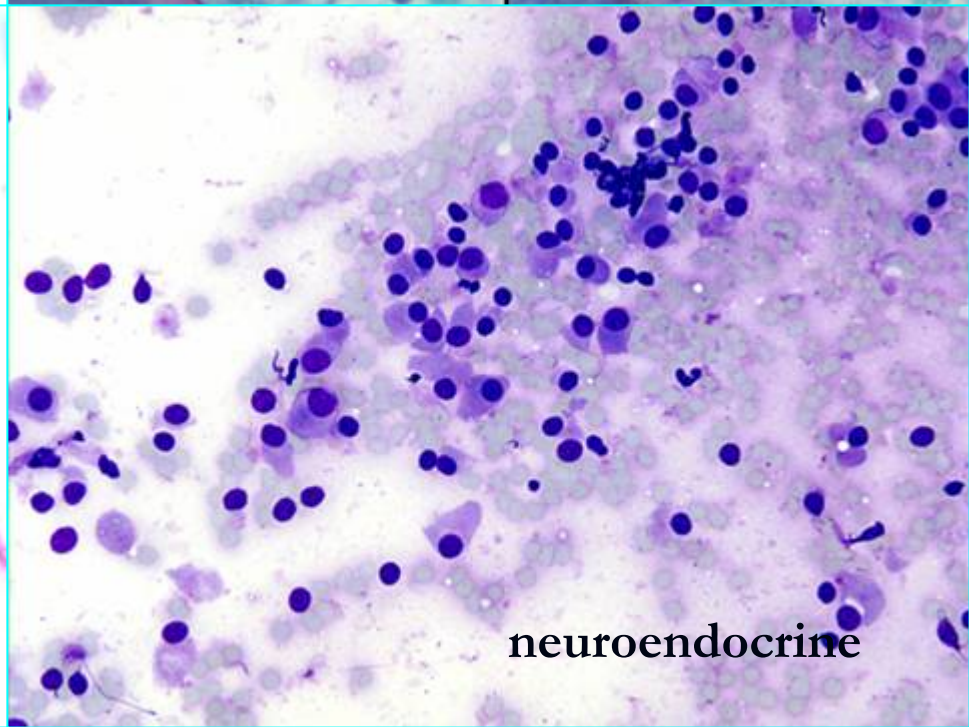
Cell Types

- Epithelial
  - ✓ glandular
  - ✓ squamous
- Stromal – mesenchymal
  - ✓ fibro
  - ✓ chondro
  - ✓ osteo
  - ✓ neuroendocrine
- Inflammatory cells


squamous


glandular

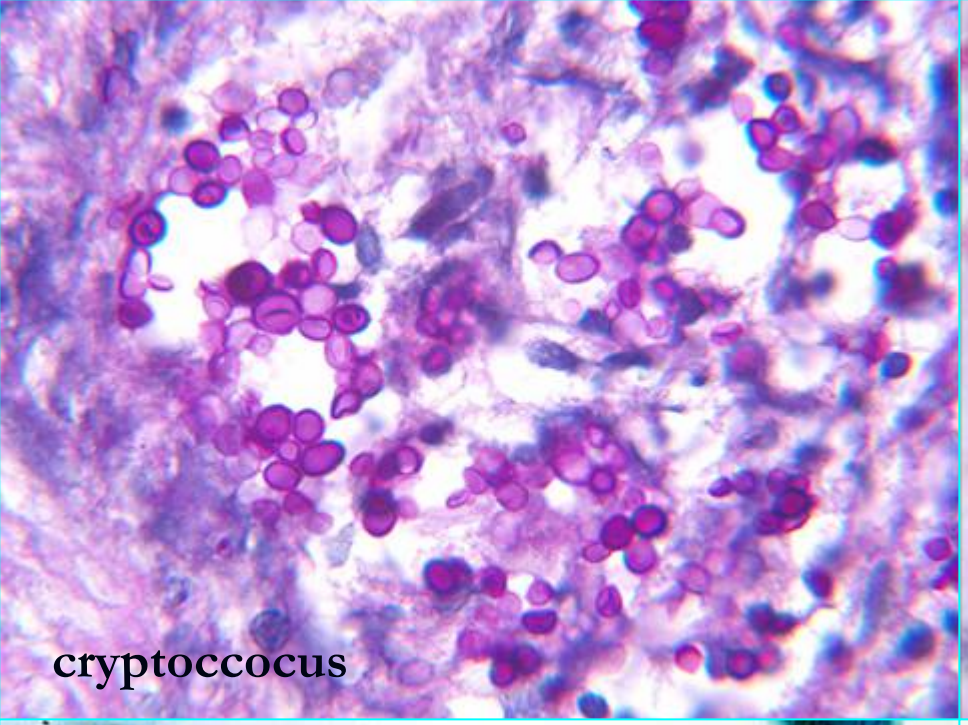

mesenchymal


neuroendocrine

The first important decision is:

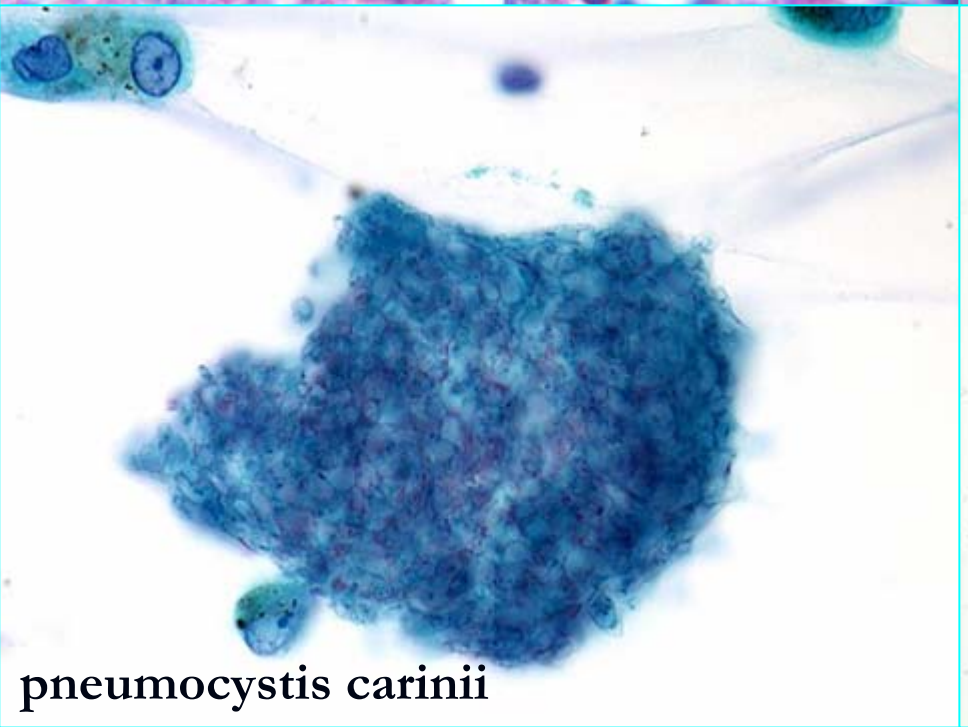
INFLAMMATION VS NEOPLASIA

Infective Agents



cryptoccocus

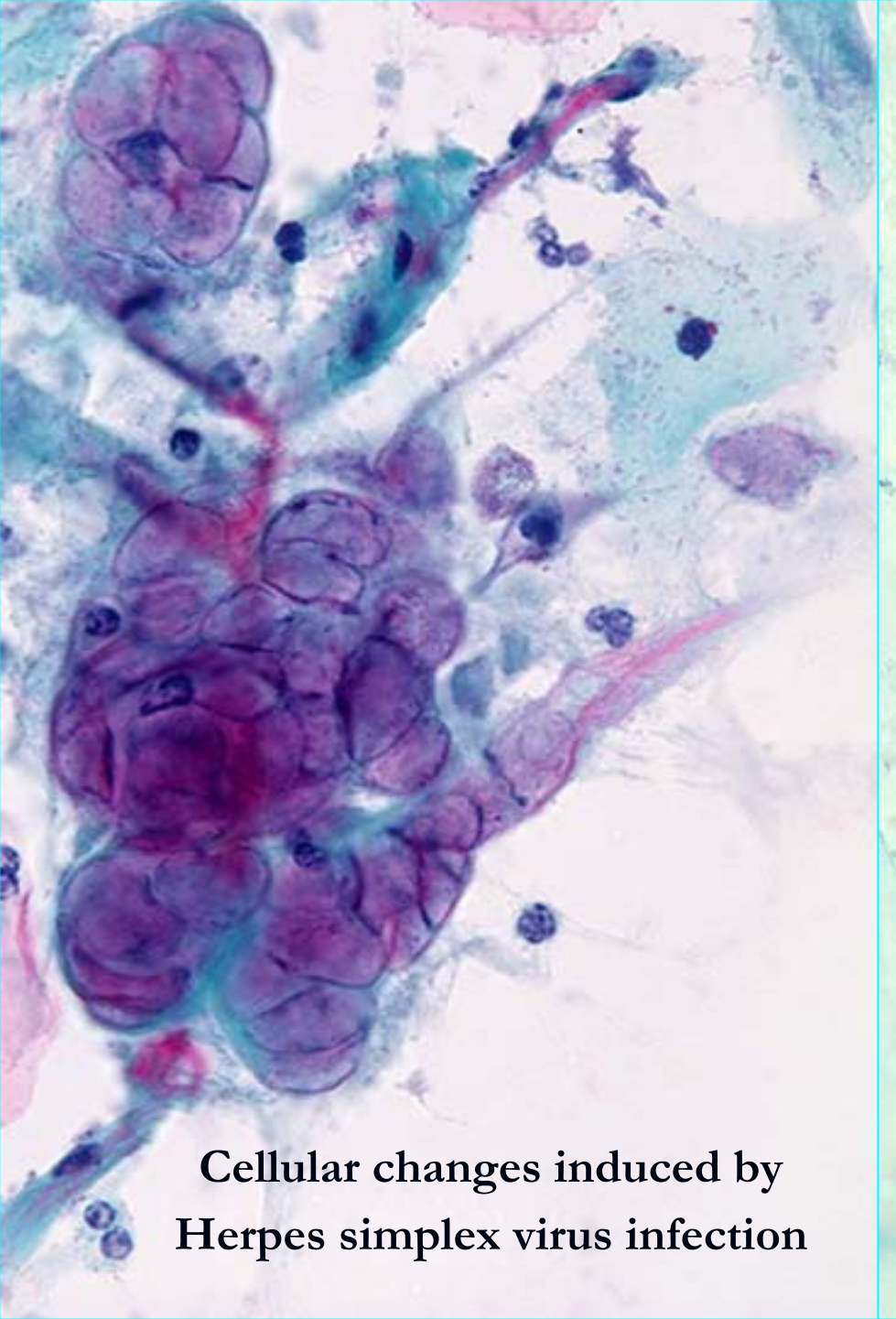
parasytic eggs



pneumocystis carinii

worm

Cellular Changes

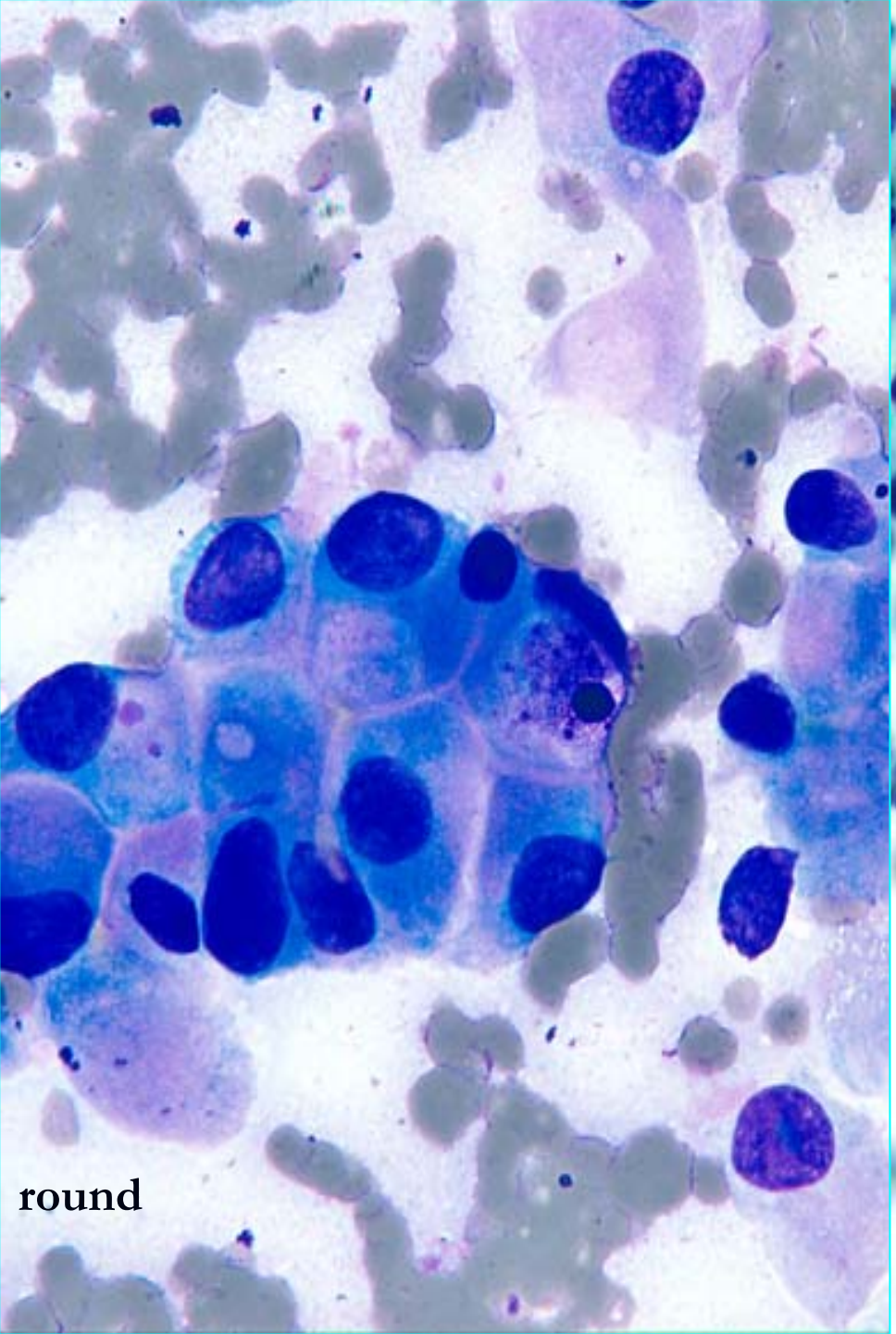


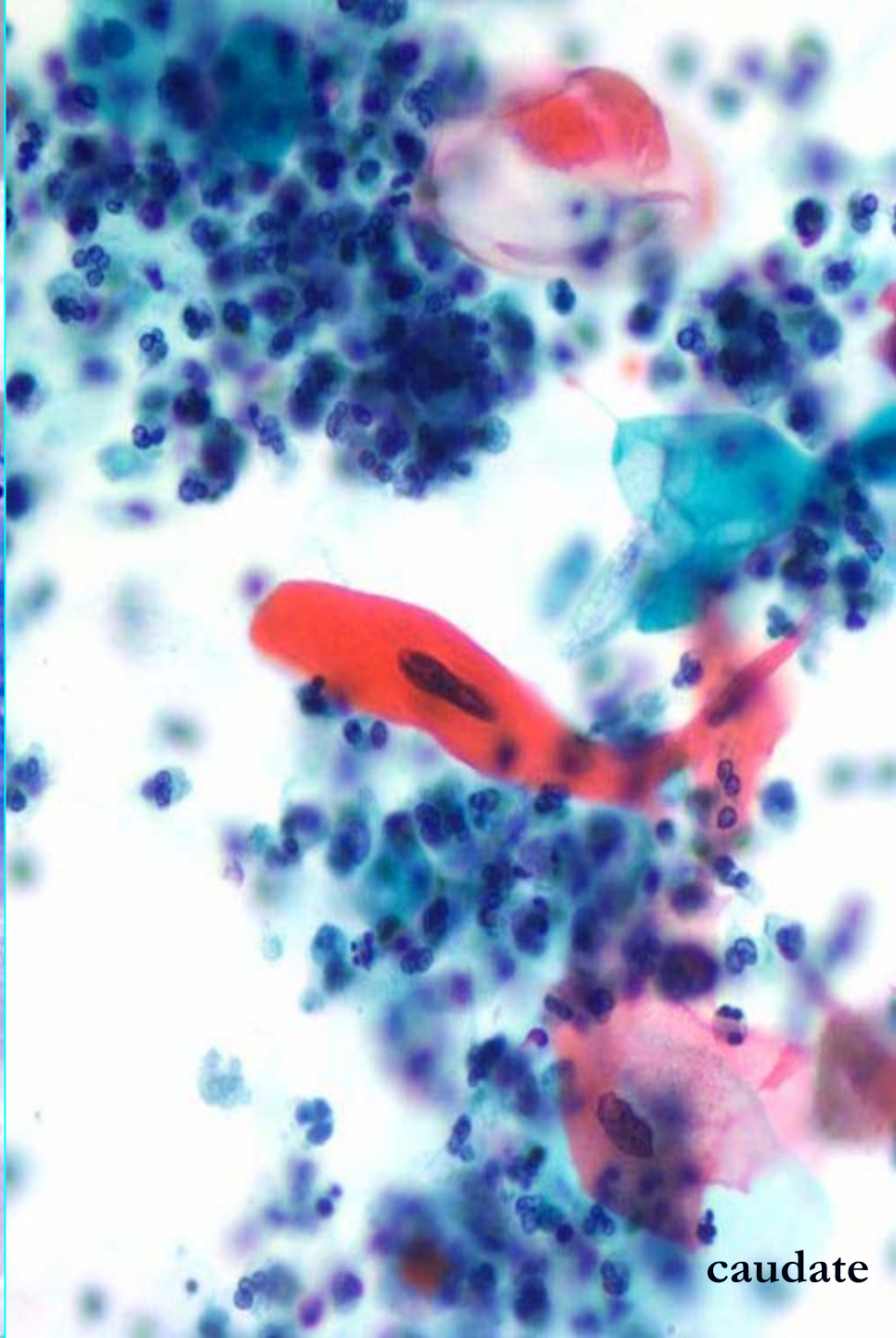
Cellular changes induced by
Herpes simplex virus infection


TUMORS

Tumor Cell Types

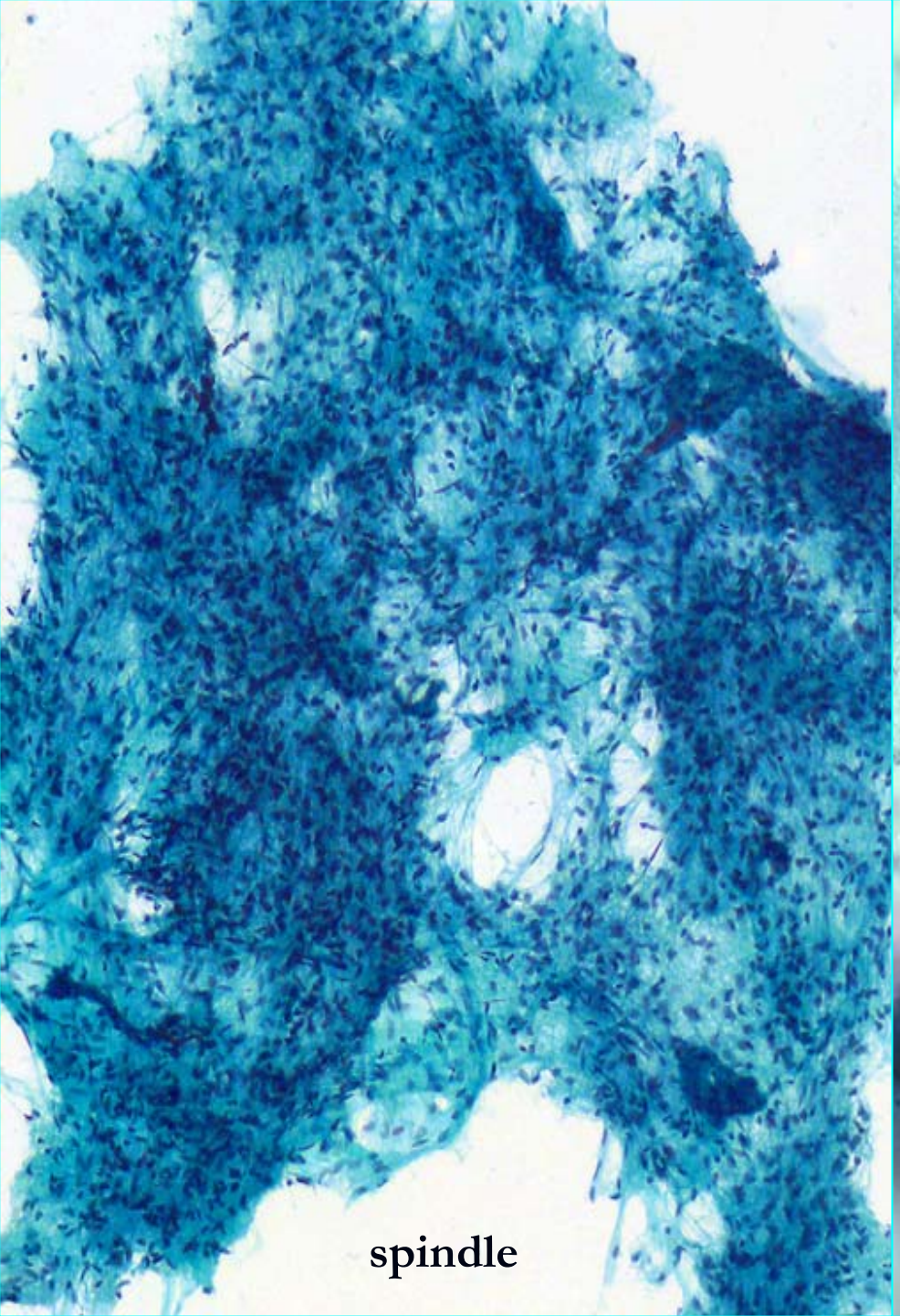
- Round to caudate large cells – epithelial tumors

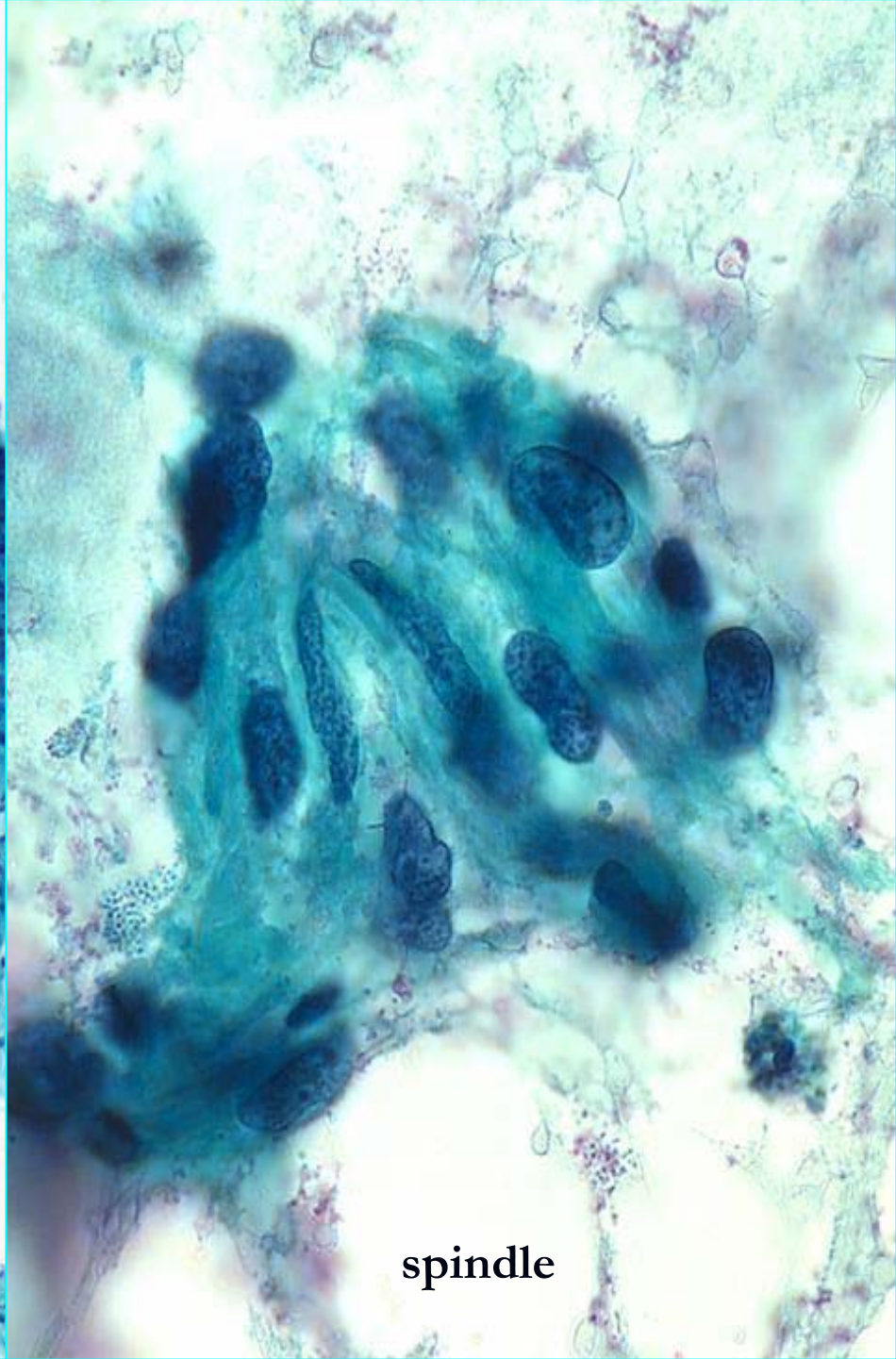

round


caudate

Tumor Cell Types

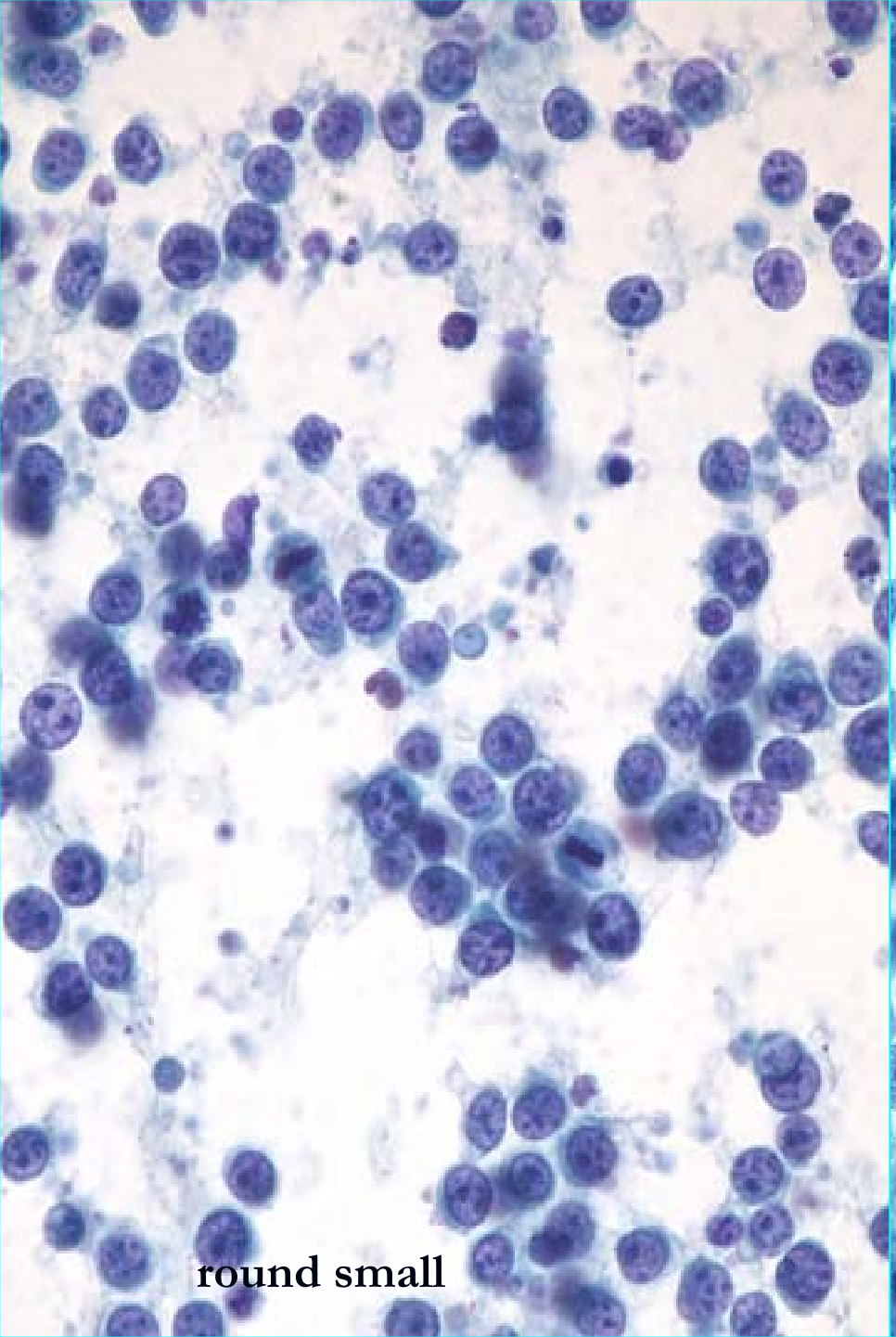
- Spindle to stelate small to medium cells – mesenchymal tumors

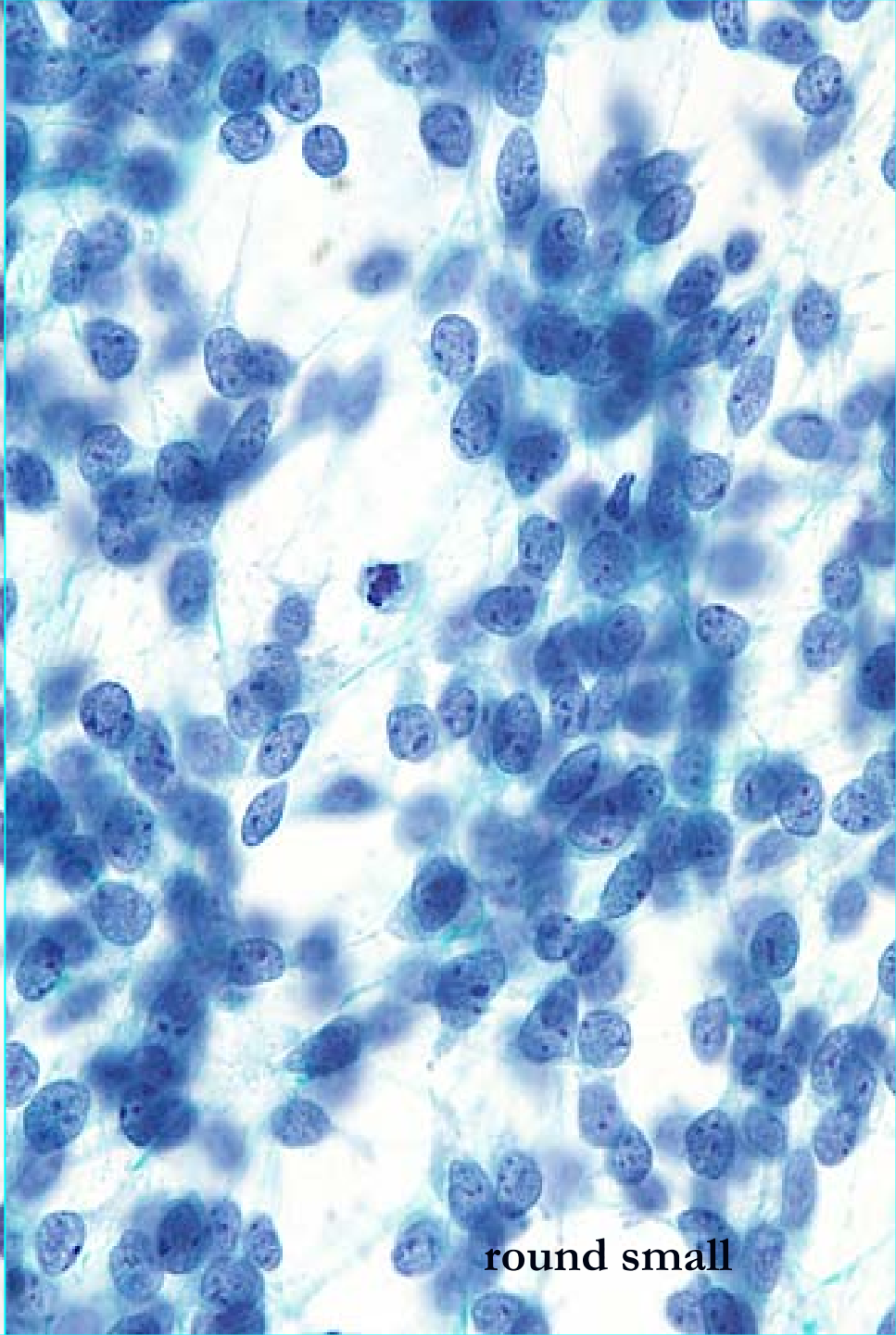

spindle


spindle

Tumor Cell Types

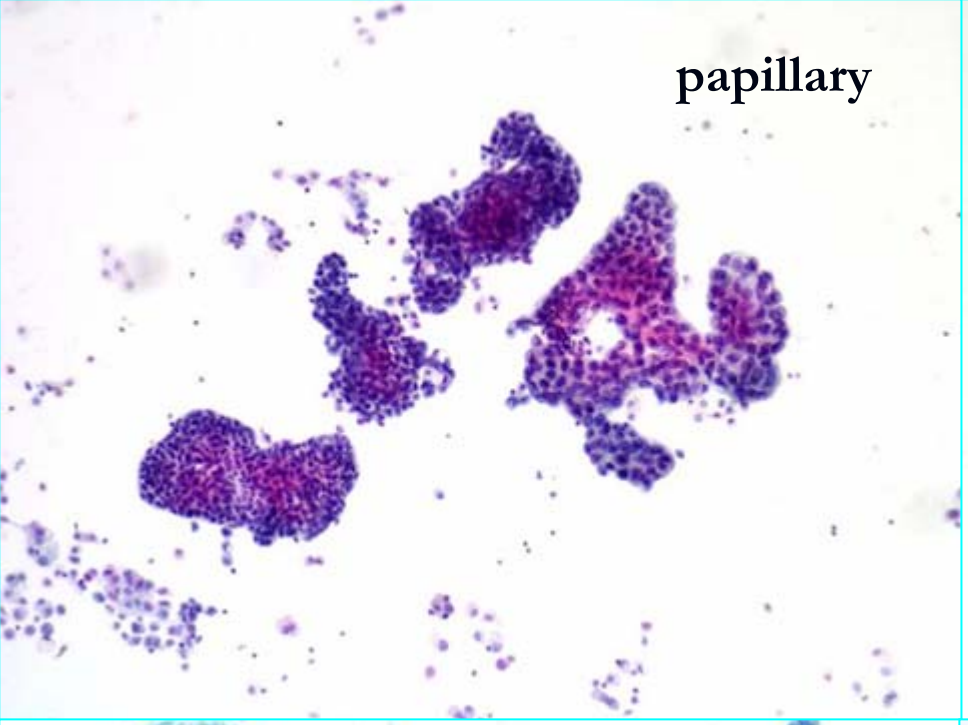
- Discrete small to medium round cells – lymphoproliferative diseases, neuroendocrine tumors, poorly differentiated tumors

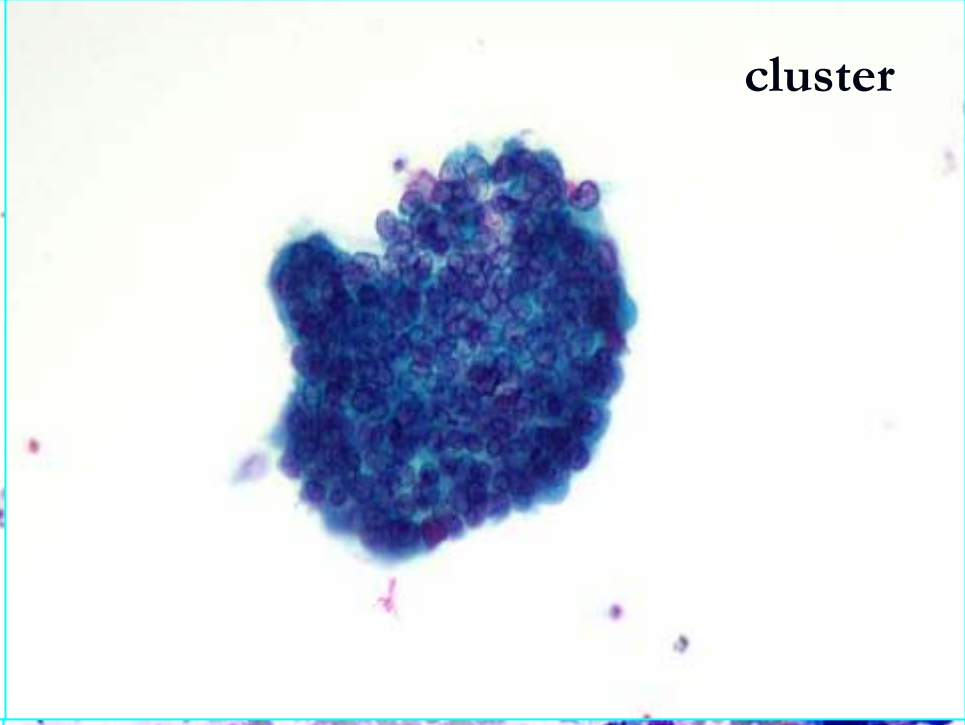

round small

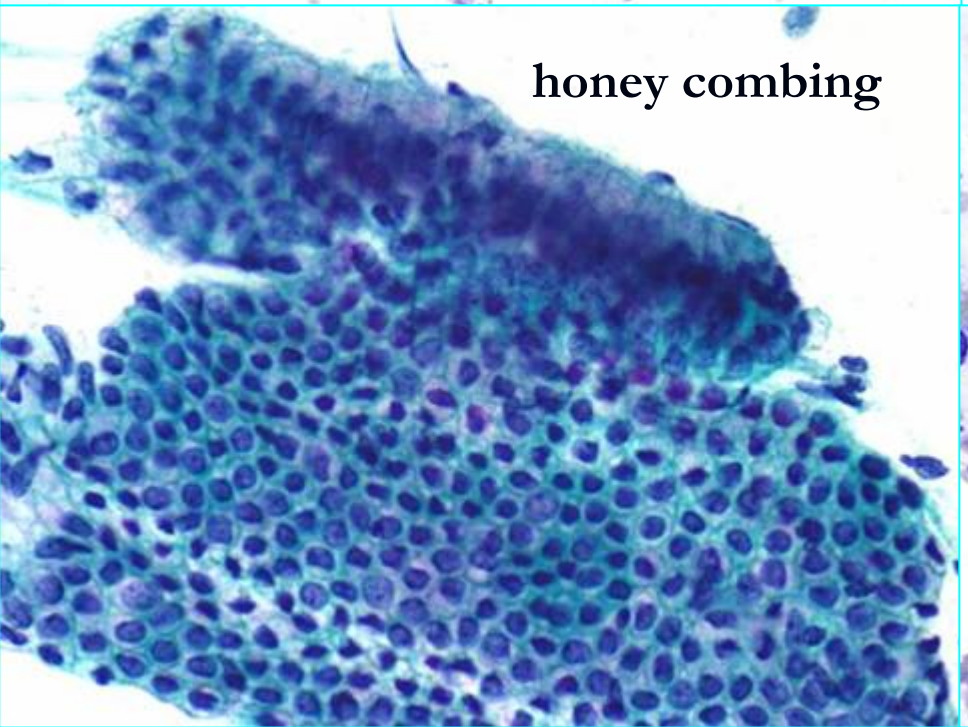

round small

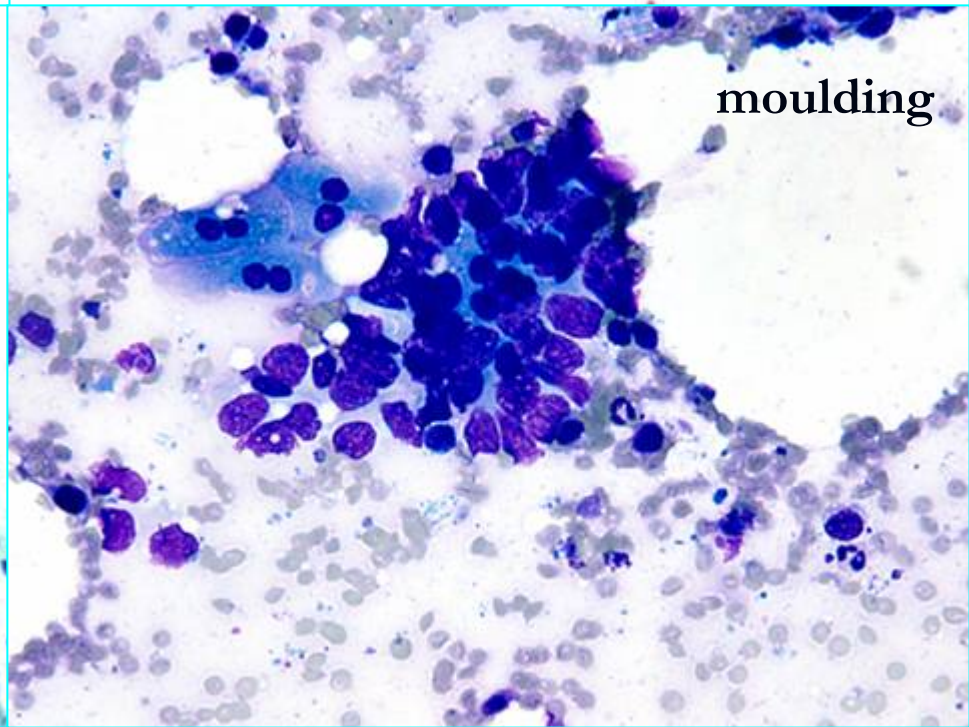
Cell Organisation

- Papillary structures
- Clusters
- Sheets
- Glandular formations
- Honey combing
- Moulding


papillary


cluster

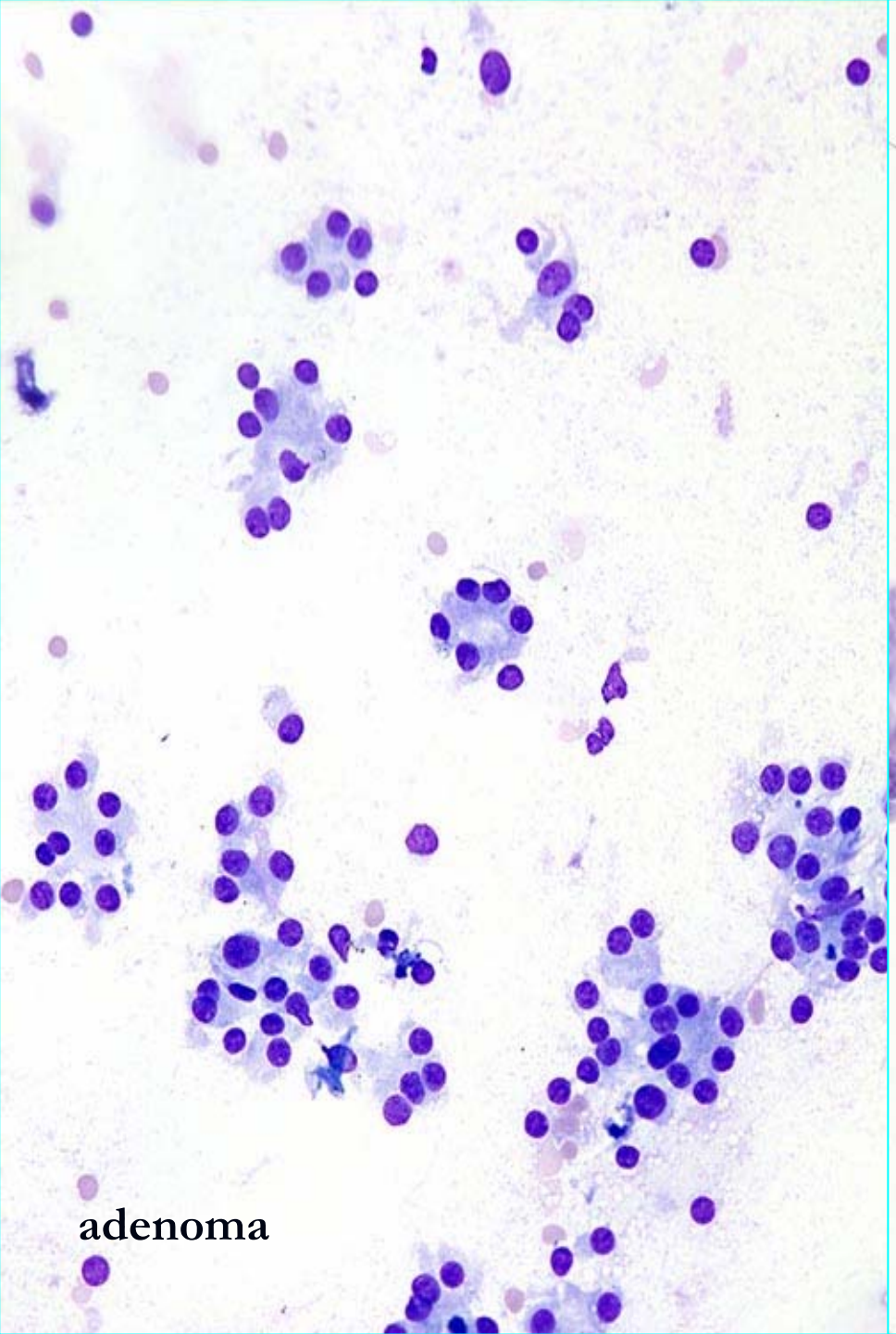

honey combing

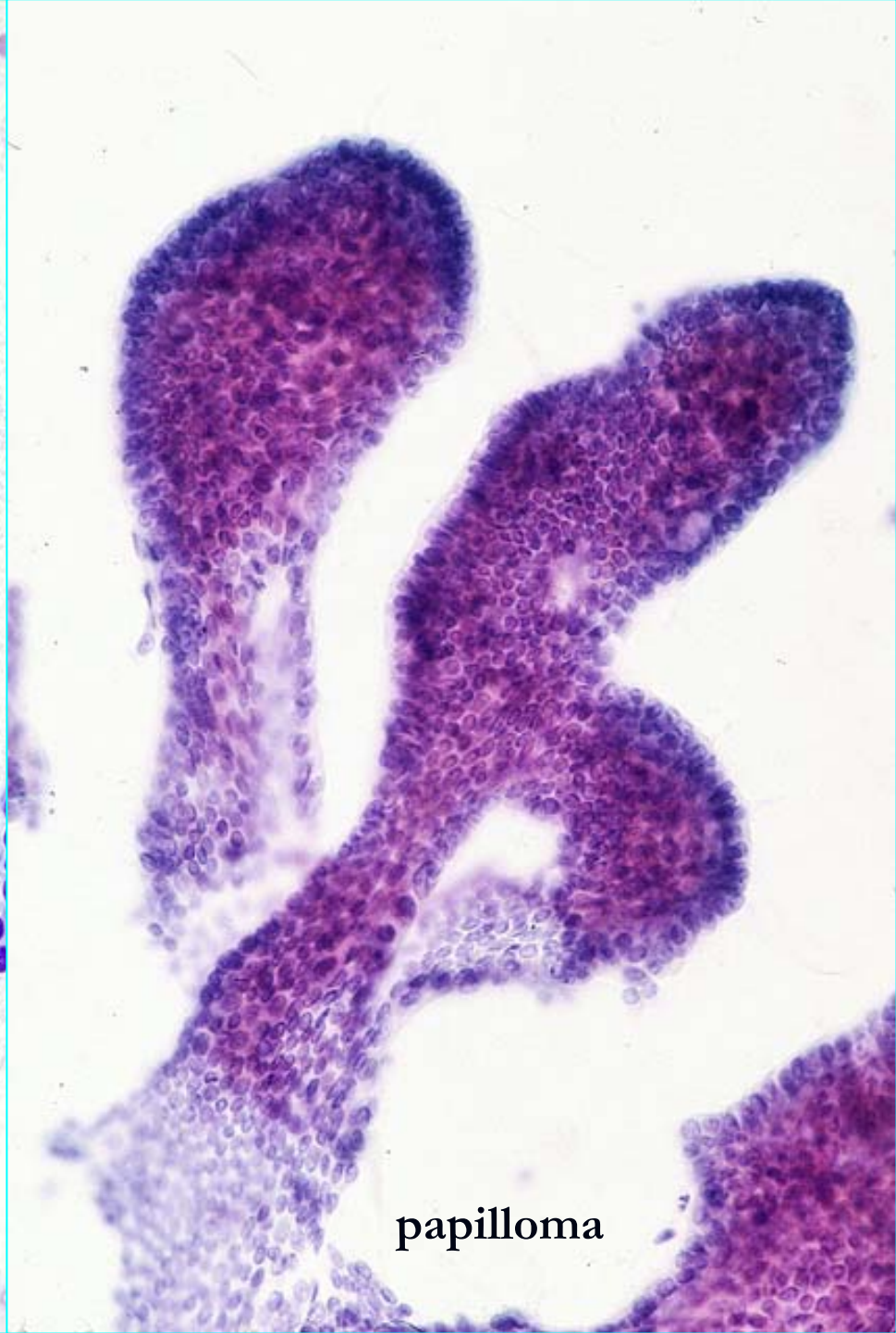

moulding

Second important decision is:

BENIGN VS MALIGNANT

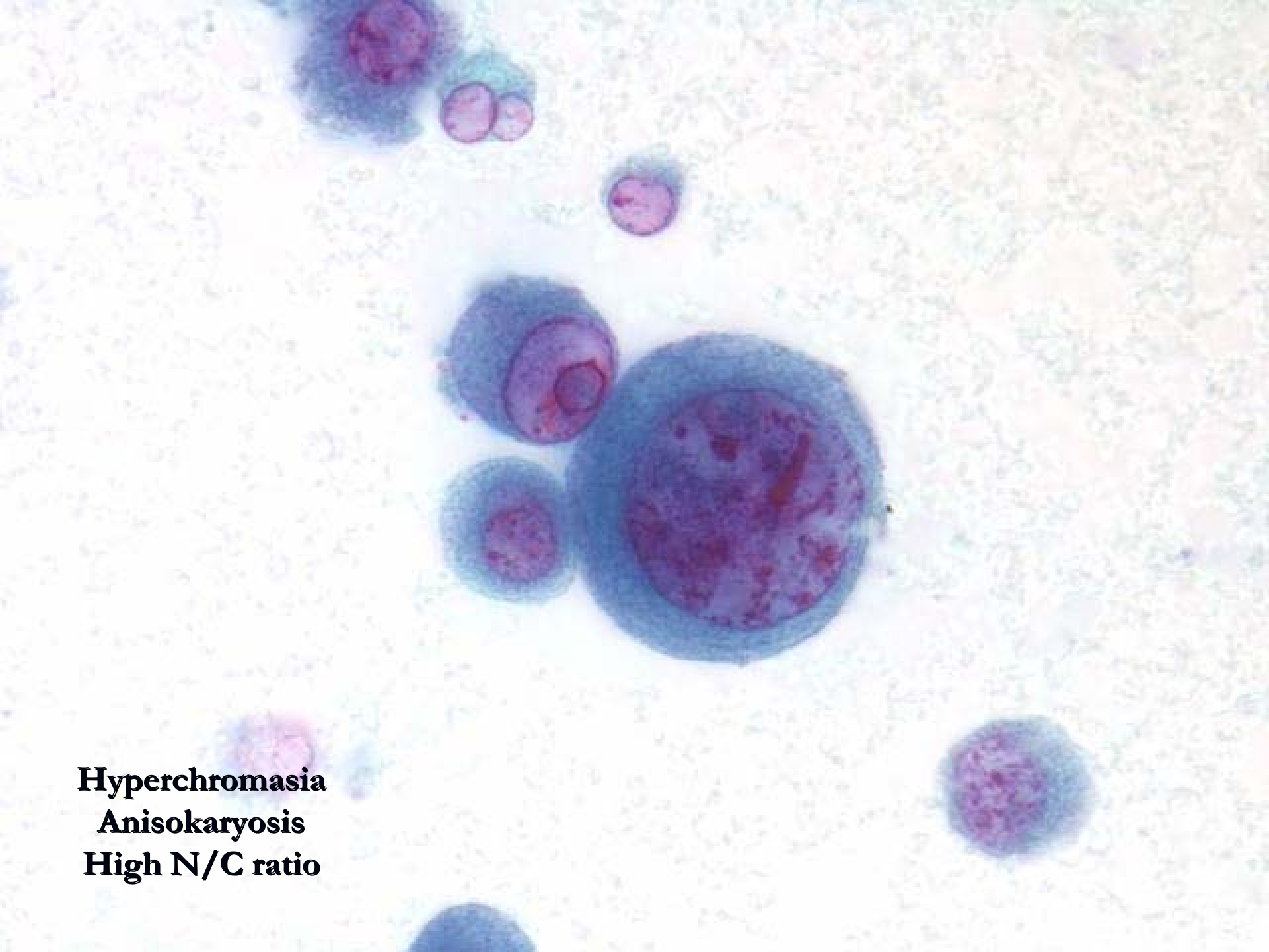
BENIGN TUMORS


adenoma


papilloma

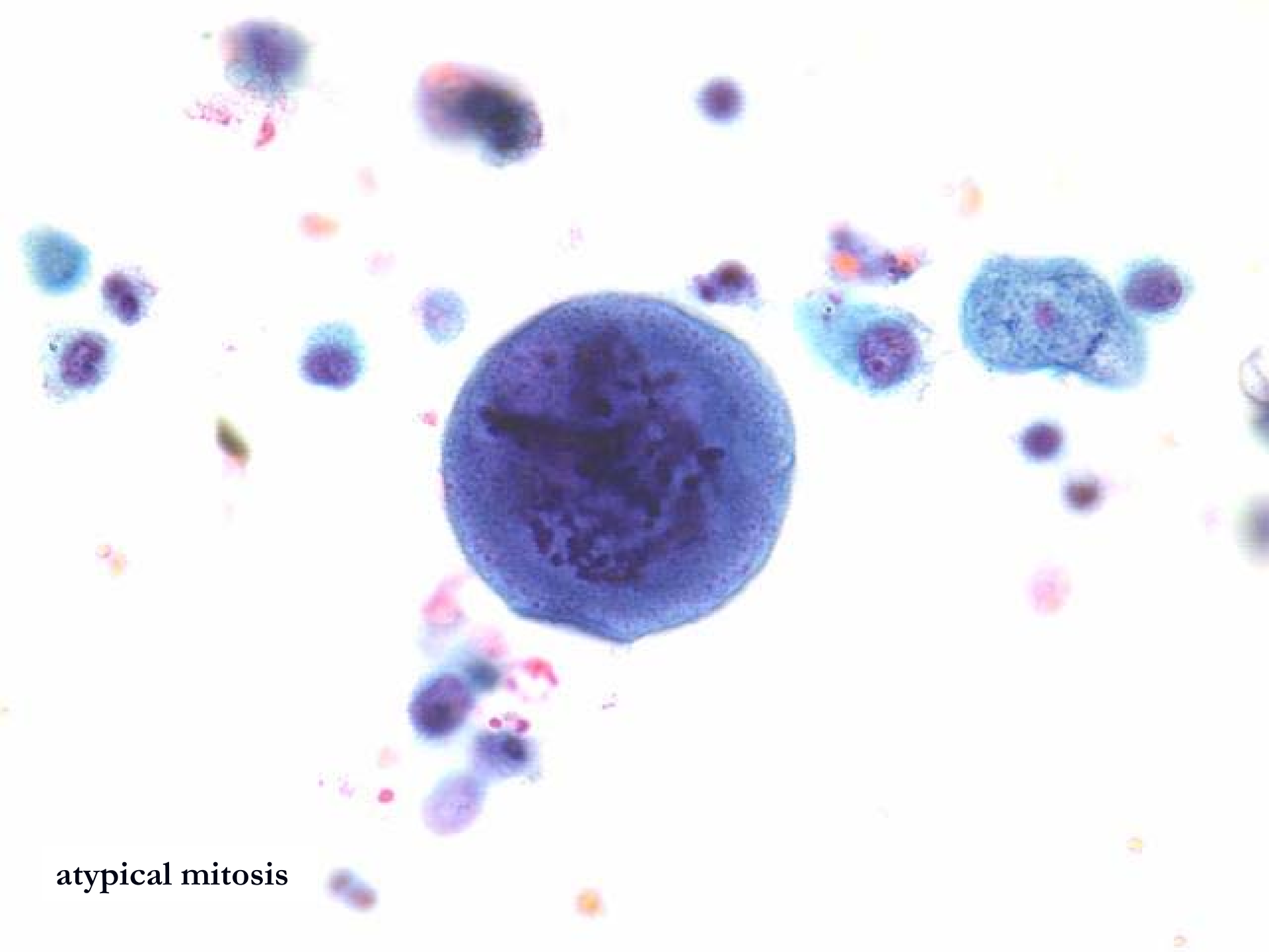
Criteria of Malignancy

- **Nuclear features**
  - Hyperchromasia
  - Anisokaryosis
  - High N/C ratio
  - Multinucleation
  - Mitotic figures – increases/abnormal
  - Nucleoli large/variable shaped

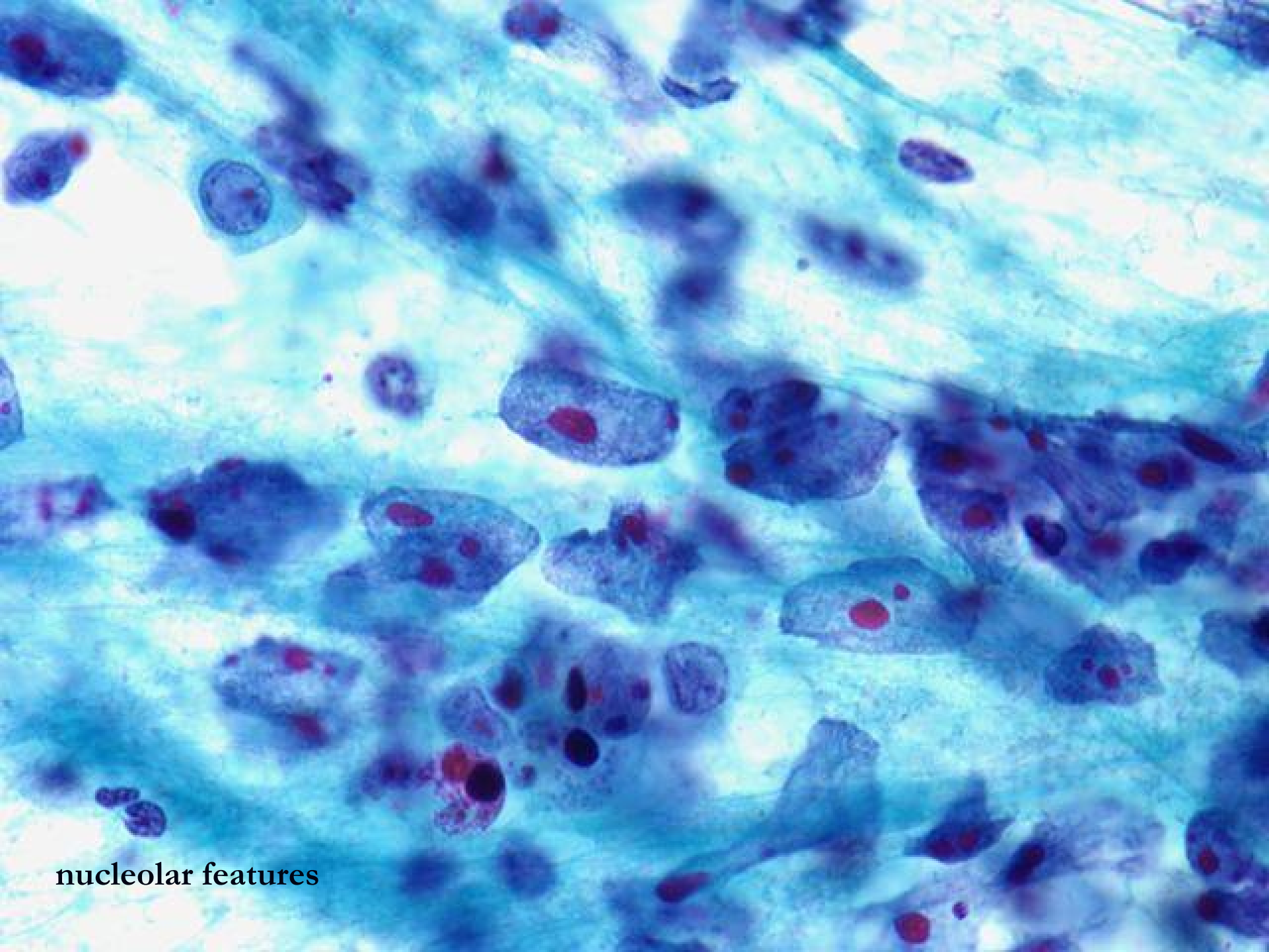


Hyperchromasia
Anisokaryosis
High N/C ratio

multinucleation

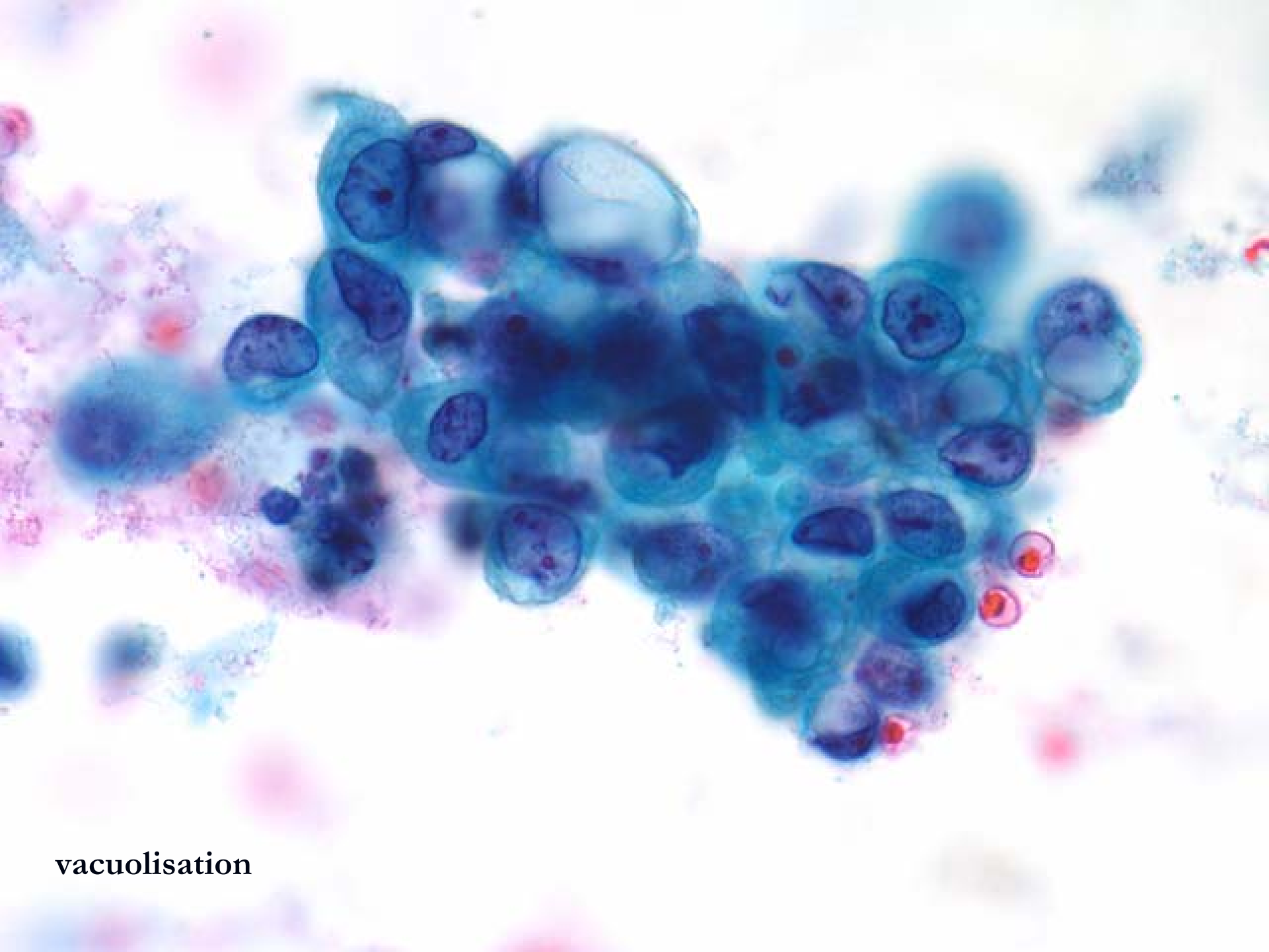


atypical mitosis


nucleolar features

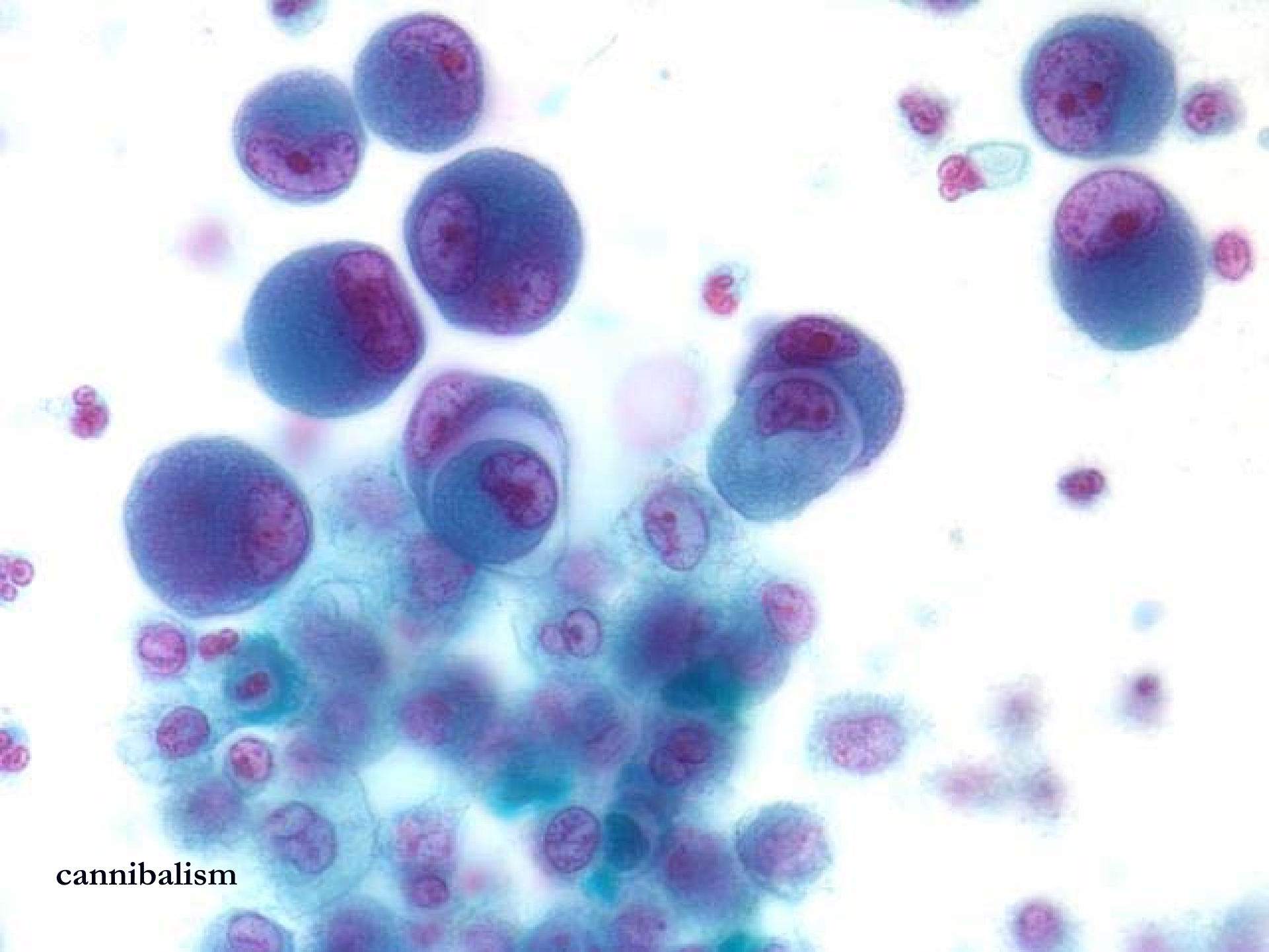
- Cytoplasmic features

✓ Vacuolisation
✓ Keratinization
✓ Cannibalism



vacuolisation


keratinization


cannibalism

FNA Positivity

90%-100%

- Our results (798 cases of lung FNA)

54% positive for malignancy

36% negative for malignancy

10% inadequate

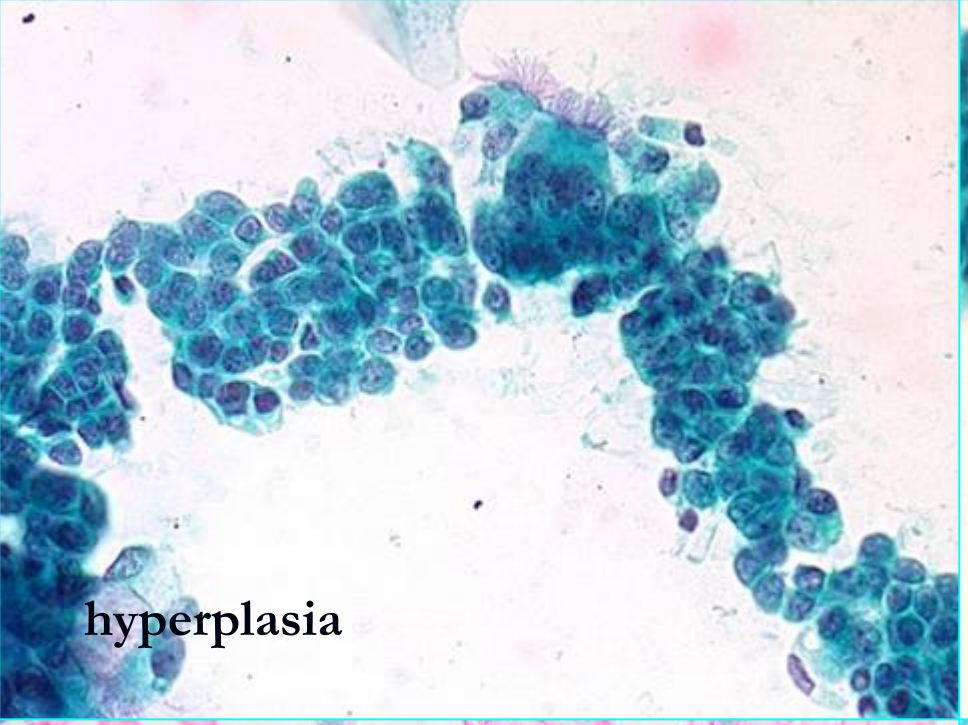
Lesions that can mimic many criteria of malignancy:

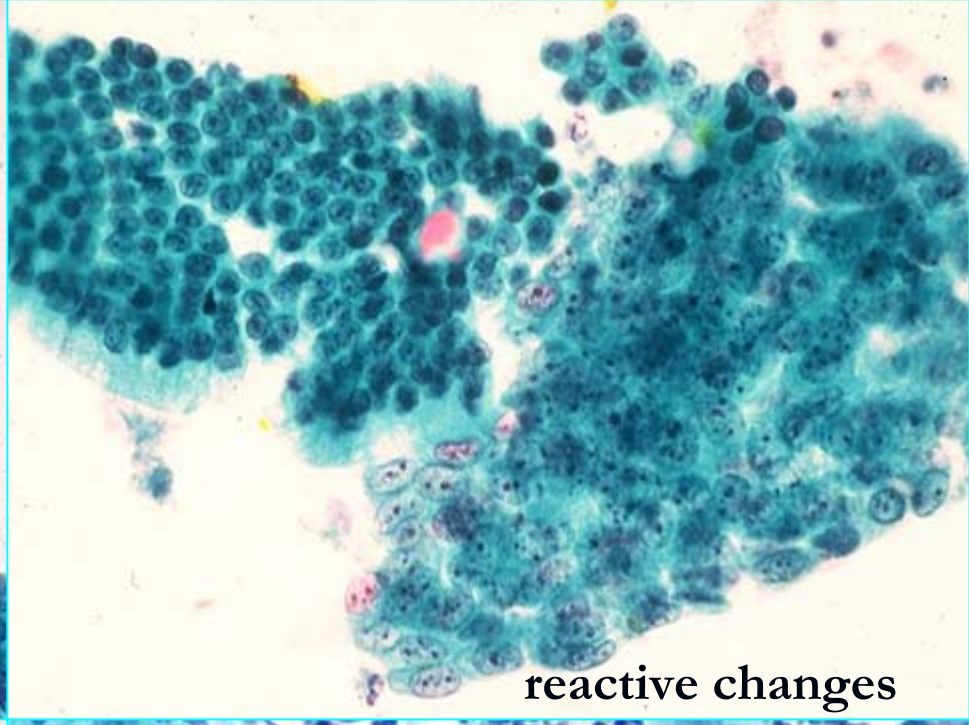
Hyperplasia

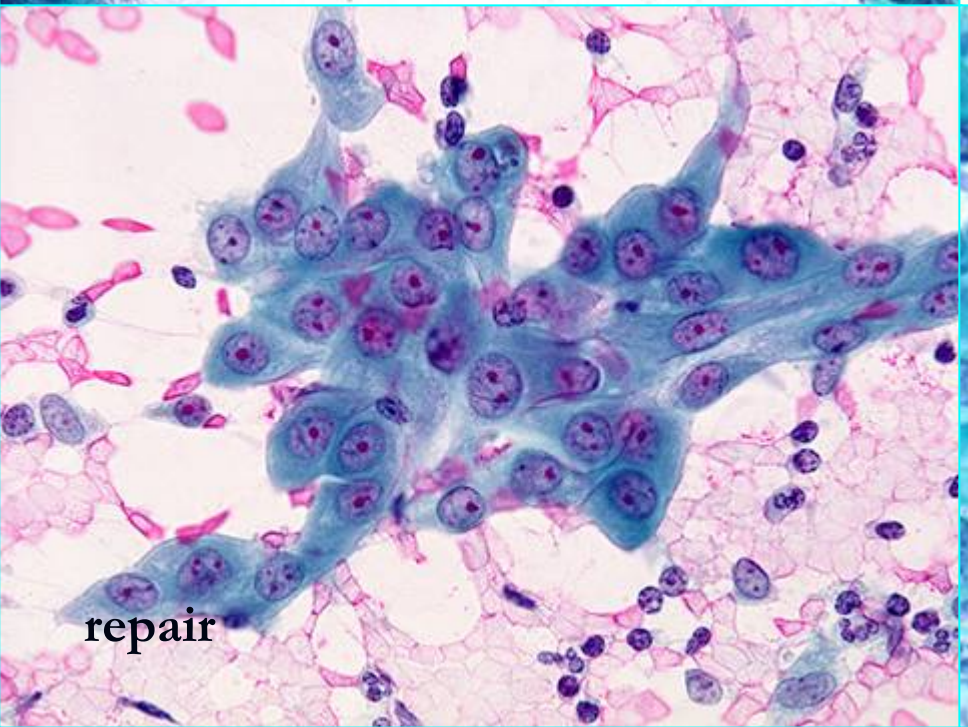
Reactive changes

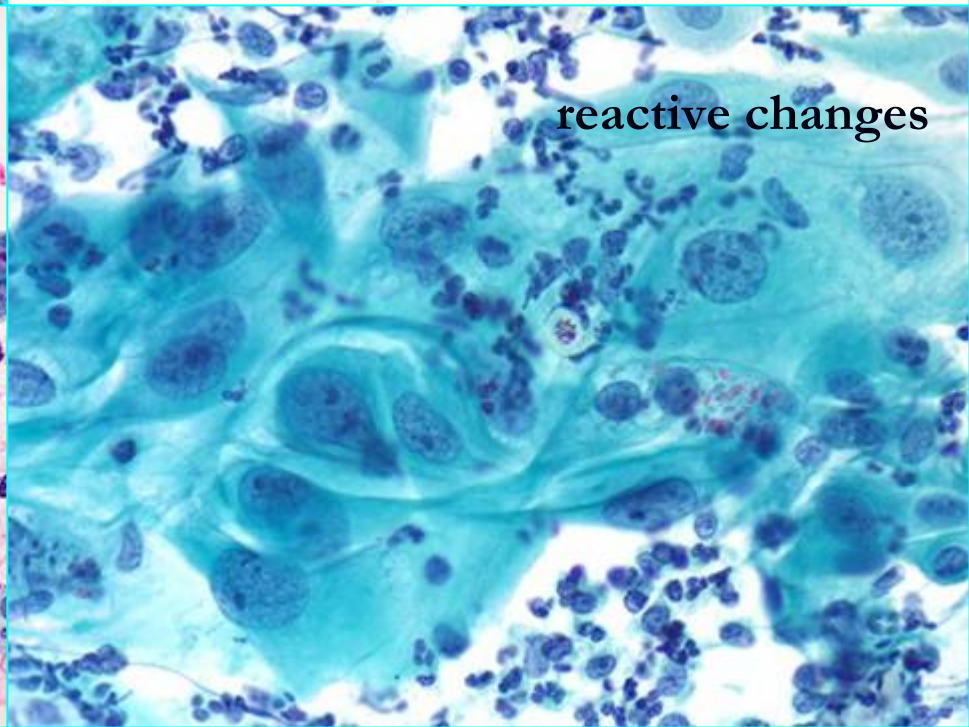
Regenerative and reparative changes


MIMIC MALIGNANCY
OR
REAL MALIGNANCY ?????


hyperplasia


reactive changes


repair


reactive changes

- **SPRIGGS I BODDINGTON (1989)**

"THERE IS NO KNOWN CRITERION NOR CONSTELLATION OF CRITERIA WHICH ARE UNIVERSALLY DIAGNOSTIC OF MALIGNANCY"

Future Challenges for Veterinary Cytopathology

- Image guided FNA cytology
- Telecytology
- Use of additional stains
  - ✓ Cytochemistry
  - ✓ Immunocytochemistry

Diagnostic Cytology

- Introduction
- Adventages and disadventages
- Samplings
- Stains
- Fluids
- FNAs
- Summary

Summary

- Cytology is diagnostic method
- Cytology is quick, inexpensive and accurate method, with a little risk to patient
- Requires good communication with clinicians and correlation with other diagnostic methods
- Requires continual learning and education
- Enjoy!!!!!!!

33th EUROPIAN CONGRESS OF CYTOLOGY
MADRID 14-17. October