DIAGNOSTIC CYTOLOGY

ŽIVKA ERY
MILANA PANJKOVIĆ
Institute of Pulmonary Diseases, Sr. Kamenica
School of Medicine,
University of Novi Sad
Diagnostic Cytology

- **Introduction**
- Advantages and disadvantages
- 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
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
Diagnostic Cytology

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Advantages of Cytopathology

- Samples can be: collected easily and quickly, 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
- Deternimate next diagnostic procedures
Disadvantages of Cytopathology

IT IS NOT ALWAYS POSSIBLE TO:

- localize neoplastic lesion
- distinguish preinvasive of invasive cancer
- distinguish reactive of dysplastic and neoplastic changes
- determine tumor type
Advantages of Histopathology

- Microscopic examination usually 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 **margins** of resection
- To examine stromal **invasion** and deep of invasion
- **Gross/cytopathology** discrepancies
Cytopathology should not be
cmpared to histopathology!!!

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

1. **Exfoliative cytology** – spontaneously 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** – spontaneously 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
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
Cytoplasmatic features are better preserved.

Nuclear and nucleolar features are less preserved.

Diff-Quik stain
- 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 visibility and color
Additional Stains

- Cytochemistry

  - Ziehl-Neelsen stain
  - PAS stain
- Immunocytochemistry

CD99
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Diagnostic Cytology

- Fluids
Cavity Fluids

- Abdominal
- Pleural
- Pericardial
- Synovial
- CSF
Cavity Fluids

- Sampling techniques
  - 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, especially if visible 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 < 2.5 g/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 bladder
- Hepatic disease
EXUDATE
EXUDATE

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

- NONSPECIFIC
- SPECIFIC
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 cells

**Cytopathology:**
- eosinophilic background
- large number of neutrophils
- lesser number of macrophages, mesothelial cells, lymphocytes and plasma cells
SPECIFIC EXUDATE

Lupus erythematosus SLE
Malignant Effusion

- Primary tumors:

MESOTHELIOMA
mesothelioma

EMA stain - mesothelioma
LYMPHOPROLIFERATIVE DISORDERS
- acute lymphoblastic leukemia
- non-Hodgkin lymphoma
- multiple myeloma
- large cell lymphoma
Secondary tumors
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 (erythrocytes, 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**
The first important decision is:

INFLAMMATION VS NEOPLASIA
Infected 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
Tumor Cell Types

- Spindle to stelate small to medium cells – mesenchymal tumors
Tumor Cell Types

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

- Cytology is a diagnostic method.
- Cytology is quick, inexpensive, and accurate, with a little risk to the 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