DIAGNOSTIC CYTOLOGY

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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

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
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

Determinate 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 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** – 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
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

![Immunocytochemistry Images](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
- Add 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 $<2.5 \text{g/dl}$
- TNCC $<1500 \text{ cells/\mu g}$

MACROPHAGES
TRANSUDATE

- MACROPHAGES
- MESOTHELIAL CELLS
mesothelial cells
TRANSUDATE

- MACROPHAGES
- MESOTHELIAL CELLS
- LYMPHOCYTES
lymphocytes
MODIFIED TRANSUDATE

- Moderate protein concentration 2.5-7.5 g/dl
- Moderate cellularity 1000-7000 cells/µg

- Cardiovascular disease
- Neoplastic disease
- FIP
- Rupture of urinary bladder
- Hepatic disease
EXUDATE

- High protein concentration $> 3,0 \, \text{g/dl}$
- High TNCC $> 7000 \, \text{cells/\mu g}$

- 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 cells

Cytopathology:
- Eosinophilic background
- Large number of neutrophils
- Lesser number of macrophages, mesothelial cells, lymphocytes, and plasma cells
SPECIFIC EXUDATIVE

Lupus erythematosus SLE
Malignant Effusion

- Primary tumors:

MESOTHELIOMA
LYMPHOPROLIFERATIVE DISORDERS
acute lymphoblastic leukemia

non-Hodgkin lymphoma

multiple myeloma

large cell lymphoma
Secondary tumors

METASTATIC TUMORS
adenocarcinoma

liposarcoma

adenocarcinoma

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 (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
squamous

mesenchymal

glandular

neuroendocrine
The first important decision is:

INFLAMMATION VS NEOPLASIA
Cellular Changes
Cellular changes induced by Herpes simplex virus infection
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
Second important decision is:

BENIGN VS MALIGNANT
BENIGN TUMORS
adenoma

papilloma
Criteria of Malignancy

- **Nuclear features**
  - Hyperchromasias
  - 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
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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