What is pathology?

- Processing of biological material
  - any tissue removed from the living or dead body
- Determining the diagnosis and disease / causes of death
- Determining the nature and causes of the disease
- The effect on the choice of an adequate therapeutic approach

THE ROLE OF PATHOLOGY

- Collection of biological material
  - biopsy
  - autopsy
- Biopsy (collection)
  - organs and tissues
  - excisions
  - core-cut (core needle)
- Biopsy: invasive method
- Cytopathology
  - smears, cytology (brush, spatula, ...)
  - cytology of body fluids (serum, pleural effusion, sputum, stool, ...)
  - lavage (bronchi, ...)
- Cytopathology: non-invasive (minimally invasive) method
- The role of intervention
  - aspiration with a fine needle (FNAC)
  - Non-invasive (minimally invasive) method
  - Screening, not always representative

COLLECTION OF BIOLOGICAL MATERIAL
BIOPSY

BIOLICAL MATERIAL
- Processing and subsequent material evaluation
  - With the least possible damage to the structure of the cells and tissues
- To prevent cellular degradation and autolytic process
- Immediately after the collection of tissue samples it is necessary to fix them
  - Kill microorganisms and fix the cells
  - Avoid autolysis and microbial damage
- Accurate labeling
- Fast delivery to the laboratory for further processing

COVER LETTER
- Source of important information

FIXATION

BIOLOGICAL MATERIAL
1. Fixation associated with loss of biological function of structures
   - Chemical, physical methods
2. Fixation without loss of biological function of structures
   - Freezing
**FIXATION AND FIXATION AGENTS ASSOCIATED WITH LOSS IN BIOLOGICAL FUNCTION**

- Aldehydes
  - Formaldehyde, glutaraldehyde, ...
- Oxidising agents
  - (formaldehyde, glutaraldehyde, ...) (osmium tetroxide)
- Protein-denaturing agents
  - Ethanol, methanol, acetic acid, ...
- Physical methods
  - Heat, microwaves, ...

**MECHANISM OF FIXATION**

- Cross-linking
- Change of tertiary structure
- Without changing of the antigenicity
- Without changes in spatial relationships

**FIXATION AND FIXING AGENTS - FACTORS**

- **Formalin 10%, glutaraldehyde 3%**
  - pH (physiological, pH 5.5 gastro-mucosa, pH 6.5 catecholamines)
  - Standard is phosphate buffered formaldehyde fixation solution
  - Physiological pH important for eventual genetic analysis
  - If no buffered solution is available, dilute formaldehyde with tap water (not distilled) - formaldehyde spontaneously decomposes to formic acid
  - Temperature (laboratory, 0-4 °C, higher temperature)
    - Standard is laboratory temperature, do not store the samples in the refrigerator unless it is required by the laboratory
  - Penetration of the fixing agent (material size)
  - Concentration (3% glutaraldehyde, 10% formalin)
  - Duration of fixation (formalin 24 hours)

If the fixation is prolonged, there is a risk of tissue damage, wrinkling and damage enzymatic and antigenic reactivity!!

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**LABORATORY PROCESSING**

**MACRO PROCESSING**

Sampling
**PROCESSING**

**AIM:** It is necessary to put the tissue into a solid medium which allows its subsequent cutting. If necessary, the tissue must be pre-treated (e.g. decalcification).

**EMBEDDING MEDIA**

- **Paraffin**
  - cheap, solid, melting point 40-70 °C, fast setting
  - additives (gum, beeswax, ...)

- **Resin**
  - electron microscopy, thin sections, high resolution microscopy, bones

- **Agar**
  - small fragments of tissue

- **Gelatin**
  - frozen cuts

**TISSUE PROCESSING**

Tissue in fixative (10% formalin)

Tissue impregnated with embedding medium
Tissue in fixative (10% formalin)

**Dehydration** - removal of the fixation solution (ethanol, methanol, acetone, isopropyl alcohol, ...) in increasing concentration: 70% → 90% → 100%

**Impregnation** - embedding medium, vacuum technology

Tissue impregnated with embedding medium

**Cleaning** - the removal of the dehydrating solution substances well miscible with the embedding medium (xylene, toluene, chloroform, ...)

**Impregnation** - embedding medium, vacuum technology

Tissue impregnated with embedding medium

**DEHYDRATION AND CLEANING**

- 10% formalin
- 70% alcohol
- 95% alcohol
- 100% alcohol
- 100% alcohol
- 100% alcohol
- 100% alcohol + Xylene
- xylene
- xylene
- paraffin
- paraffin
FROZEN SECTIONS PROCESSING

- Freezing as a form of "fixation" of fresh tissue
- Condition: Speed (slow freezing – i.e. ice crystals formation and cell destruction)
- Fast diagnosis (lack of time for other fixation)
- Enzyme histochemistry when enzymes are labile
- Some immunofluorescence methods
HISTORY OF CLINICAL CYTODIAGNOSIS
1846 V.D. Lambl
“Urinary bladder cancer cells found in urine”

1936 J. Trapl
“Women estrus evaluated in vaginal cells”

CYTOLOGY

The advantages of cytology are:
- Speed of diagnostics
- Opportunity to take and evaluate samples from hard-to-reach locations
- Economic aspect

The disadvantages of cytology are:
- The impossibility of accurate classification and typing of tumors
- Impossible to consider an invasion
- Possibility of false negativity and false positivity

Today, cytology is used to diagnose tumors of all organ systems. Cytology makes it possible to carry out screening tests at vulnerable and risky populations with low financial costs.

BASIC STAINING TECHNIQUES

ACIDOPHILIA:
- Staining with acidic dyes
  - Eosin, Azure, Acriflavine, Syto Blue
  - Substrates: Cytosol, intercellular substances, ...

BASOPHILIA:
- Staining with alkaline dyes
  - Methyl blue, Toluidine, Hematoxylin
  - Substrates: Chromatin, Ribonucleic, ...

METAL IMPREGNATION:
- Methods by Golgi and Cajal
  - Silver and Gold Salts
  - Neurons and glial cells

HISTORY OF CLINICAL CYTODIAGNOSIS
1943 G. N. Papanikolaou
“Diagnosis of the uterus cancer by the vaginal smear”

after 1950 - development of non-gynecological cytology diagnostics
- lung, oral cavity, urinary bladder, GIT
  - later fine needle aspiration cytodiagnostics
    - breast, parenchymal organs, body cavities ...

BASIC PRINCIPLES OF STAINING
BASIC STAINING METHODS

HEMATOXYLIN AND EOSIN

MALLORY HEMATOXYLIN

RETICULIN

LIPIDS – FAT TISSUE (HE)
HISTOCHEMICAL METHODS

OIL RED

PERIODIC ACID SCHIFF

ALCIAN BLUE

GRAM STAINING

STAINING BK (ZIEHL–NEELSEN)
ENZYME HISTOCHEMISTRY

- Preserving the enzyme activity during fixation and embedding
- Cryostat sections are preferred

ACID PHOSPHATASE - GOMORI METHOD

Acidic phosphatase

Substrate

Intermediate product

Precipitate

Reactant

ACIDIC PHOSPHATASE - GOMORI METHOD

PROSTATE

IMMUNOHISTOCHEMISTRY

- The use of immunology methods in disease diagnostics - allows, in addition to morphological evaluation of diseases, to show the expression of different proteins
- At the level of detection of the presence of specific proteins

Significance in:
- Diagnosis (presence or absence of specific markers)
- Prognosis (prognostic markers)
- Therapy - presence of markers that are related to the efficacy of drugs, to the resistance to treatment, the suitability of the selected treatment methods

Method:
- Reliable and reproducible
- cheap and low personnel-demanding
- Currently the gold standard of diagnostics

IMMUNOHISTOCHEMISTRY

- 1942
  - Coons, Crewd, Jones, Berliner - by the indirect immunofluorescence method demonstrated the presence of pneumococci in the tissue
IMMUNOHISTOCHEMISTRY

- Diagnostic markers
- Therapeutic markers
- Prognostic markers

FLUORESCENCE METHOD IHC

marked secondary antibody
primary antibody
antigen

Bullous pemphigoid - IgG antibodies against BM

ENZYME METHOD IHC

Soluble chromogen
Insoluble precipitate

marked secondary antibody
primary antibody
antigen

SCC - AE1/3, anti-cytokeratin antibodies

IMMUNOHISTOCHEMISTRY

- Diagnostic markers
- Therapeutic markers
- Prognostic markers

cytokeratins
- Protein filaments present in epithelial cells
  - I. Acidic cytokeratins, 9-20
  - II. Basic cytokeratins, 1-8

Vimentin
- Intermediate filaments (57 kD) present in mesenchymal cells

Neuroendocrine markers: Chromogranin, Synaptophysin
- A group of acid glycoproteins located in neurosecretory granules
- Present in neuroendocrine tumors

Proliferation Markers: Ki67
- Protein expressed by cells in late G1, S, G2 and M phases. Not present in the G0 phase
- The sign of cell growth and division

70 year old female patient


CONCLUSION: Multi-organ failure in sepsis.
Adenocarcinoma of the colon
75 year old patient
Small prominent structure on the skin temporally. Suspected basal cell carcinoma.

60 year old female patient
Metrorrhagia in menopause. Last menstruation: 2014
Endometrioid uterine adenocarcinoma
FIGO Grade 2

ER + 90%
PR + 50%

prognostic and therapeutic markers

ELEKTRON AND RADIOACTIVE MICROSCOPIC METHODS

- scanning electron microscopy
- transmission electron microscopy
- SIMS analysis
- EDAX analysis

GENETIC METHODS
MOLECULAR PATHOLOGY

THE IMPORTANCE OF MOLECULAR MEDICINE IN PATHOLOGY

- Oncology - in the human genome there are described several types of gene changes that lead to oncogenic transformation
  - oncogenes, tumor suppressor genes, "mutator" genes
  - gene, chromosome and genomic mutations
- Current WHO cancer classification often requires genetic testing
- Personalization of medicine / treatment
- The ability to differentiate familial cases from sporadic
- Detection of carriers - genetic consultation, disease prevention, early diagnosis
- Estimation of response to therapy, choice of appropriate therapy (pharmacogenomics)
- Estimation of disease prognosis
- Follow-up of donor cells in transplants
- Detection of minimal residual disease
- Gene therapy - the music of the future