Methods in pathology
Basic histological techniques

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PATHOLOGY
PATHOLOGY (pathos + logos)

• scientific study of **structural** and **functional** changes of diseased tissues

• studies changes in normal **anatomy**, **histology** and physiology (pathophysiology)
PATHOLOGY (pathos + logos)

- studies **morphologic manifestation** of a disease
Diagnostic methods in pathology

1. **Autopsy**
2. **Biopsy**
3. **Cytology** - classic cytology, cytogenetic testing
4. Diagnostic **molecular pathology** + other diagnostic methods
5. **Experiment**
Autopsy – aims?
Autopsy – aims?

- determination of the **cause of death**
- detection of other / unverified **diagnoses**
- verification of adequacy of **diagnostic** and **therapeutic** procedures
- **education** – doctors (frequently overlooked diagnoses, detection of new diseases, study of disease epidemiology...) and students
AUTOPSY
regulated by

Act no. 581 /2004 Coll.
on health insurance companies, healthcare supervision and on the amendment and supplementing of certain laws, as amended

section 48 – Conduction of an autopsy
AUTOPSY – why?
AUTOPSY – why?

• if the **cause of death is unknown** (confirmation of a disease / diagnostic / therapeutic procedure)
• the **diagnosis is not certain** (unverified malignant tumors, verified malignant tumors of unknown origin – tumor e loco ignoto)
• if the death is related to a **surgery / general anesthesia** (mors in tabula)
• if **iatrogenic damage** is suspected
• if a severe **infectious disease** is suspected
• **sudden death**
• organs collected for **transplantation**
• suspicion of **inadequate health care**
• ...
AUTOPSY - process

- **external** examination □ **internal** examination
- **organs** in complexes
- determination of **cause of death** □ autopsy **protocol**
AUTOPSY - process

- during autopsy: **samples** of tissue for histologic evaluation
- **histologic diagnosis**
- adjustment of diagnoses, conclusion
Biopsy – aims?

- tissue examination during a patient’s lifetime
Biopsy

- **aim:** determination of diagnosis – needed for application of adequate therapy
- any tissues taken from the body for diagnosis of a disease **must be processed** in the histological laboratory to produce **microscopic slides** that are analyzed in the microscope
Biopsy

- standard way of processing the tissue (order of certain steps):

1. labeling
2. fixation
3. macroscopic (gross) description and processing
4. dehydration, cleaning, impregnation
5. embedding
6. sectioning
7. staining
Biological material

0. LABELING

- the material should be adequately labeled
  - name
  - age
  - insurance information
  - clinical diagnosis
  - history
  - symptoms of disease
Biological material

1. FIXATION
Biological material

- **autolysis** (enzymatic decomposition of tissues -> cellular destruction -> great changes in the structure)
- **microbiological spoilage** – by penetration of bacteria and microbiological decomposition (starts with autolysis, these processes are parallel)

1. **FIXATION**

- to kill the bacteria and to stop the enzymatic processes in the cells - stop the autolytic changes
- the purpose of fixation is **to preserve tissues permanently** in as **life-like** state as possible
- should be carried **out as soon as possible** after removal of the tissues
Fixation - types
Fixation - types of fixatives

1. **chemical modification** (formalin)
   - variety of fixatives are available for use (depending on the **type** of tissue and features to be demonstrated)

3. **physical modification** (freezing)
Fixation – chemical types of fixatives

- major groups of fixatives, classified according to mechanism of action:

**Aldehydes** *(formaldehyde, glutaraldehyde)*
- by formation of **cross-linkages** in the proteins (between lysine residues).
- does not harm the structure of proteins greatly -> **antigenicity is not lost**, formaldehyde is good for immunoperoxidase techniques.

**Alcohols** *(methyl alcohol, ethyl alcohol)*
- **protein denaturation**
- very good for cytologic smears because they act quickly and give good nuclear detail

**Oxidizing agents** *(permanganate fixatives, osmium tetroxide).*
- **cross-linkages**, but cause **extensive denaturation**.
- used in specialized applications – electron microscopy

**Mercurials, picrates** fix tissue by an unknown mechanism
Fixation – physical types of fixatives

Frozen Samples

- tissues can be preserved by freezing them directly (snap freezing) at -80°C in a cold environment / by immersing in liquid nitrogen.
- freezing makes tissue solid enough to section with microtome (in a cryostat)
- tissue sections are put on a glass slide and are then ready for staining

- **advantages:**
  1. biological and enzymatic activities of proteins do not change during this process -> suitable for demonstration of enzymes or substances normally washed out (detected by histochemical met.), in techiques for recovery of DNA, mRNA, and proteins
  2. takes only several minutes -> intraoperative diagnostic procedures to guide the surgeon (diagnosis is made quickly)
Gross Examination

- describing the specimen and
- placing all of it / parts of it into a small plastic cassette which holds the tissue while it is being processed to a paraffin bloc

- when a malignant process is suspected - the specimen is often covered with ink with the aim to mark the margins of the specimen
Tissue in fixative solution (formalin) – **FURTHER PROCESSING**

1. **Dehydratation** – removing the water and fixative solution (ethanol, methanol, ...) - In series of concentrations 70%..90%..100%

2. **Clearing** – removing of the dehydrant with a substance that will be mixable with the embedding medium (paraffin). (xylene, toluene, chloroform, ...)

3. **Impregnation** – embedding medium (paraffin)

Tissue impregnated in embedding medium can be embedded
Dehydratation and clearing

10% formaline
70% ethanol
95% ethanol
100% ethanol
100% ethanol
100% ethanol
100% ethanol
+Xylen
Xylen
Xylen
Paraffin
Paraffin
Embedding

**Aim:** processing into a form from which the thin microscopic sections can be prepared.

• tissue can’t be cut right away -> it has to be embedded in a suitable medium
• the medium should be **solid**, but also **cut-able**
• embedding media must **fill all spaces within the tissue** to support cellular components adequately during microtomy
• must be **elastic** enough to recover sectioning deformation

...advantages of PARAFFIN (similar in density to tissue, adequate viscosity and melting point, can be sectioned at anywhere 3-10 um)
Embedding

• impregnated tissue is put in a metal container -> embedded in liquid warm paraffin
• cools down -> paraffin block with embedded tissue
Sectioning

- samples are cut into sections that can be placed on a slide
- microtome and ultramicrotomes
Processing of a sectioned material
Drying

Storage

Deparaffinisation
(paraffin is only used as a medium needed for sectioning, has to be removed before staining)
Staining

- **Acidofilic stains**
  - Eosin, Azokarmine, Anilin blue
  **Substrates**: Cytoplasm, intercellular substances,...

- **Bazofilic stains**
  - Metyl blue, Toluidine, Hematoxylin
  **Substrates**: Chromatin, Ribosomes,...

- **Impregnation methods**
  - Salt of silver or gold
  - neurons and glial cells
Staining
Methods of staining:

???
Methods of staining:

1. **STANDARD STAINING** – hematoxylin and eosin

2. special techniques (used for demonstration of various substances / antigens) – **HISTOCHEMISTRY, IMMUNOHISTOCHEMISTRY...**
STANDARD STAINING: hematoxylin a eosin

- Nucleus: blue
- Cytoplasm: light red
- Collagen: red
Special methods - HISTOCHEMISTRY
Special methods - **HISTOCHEMISTRY**

- if we want to demonstrate certain **specific substances / components of cells** -> various subst. stain in different colour (depends on characteristics of stained subst. and stain itself)

- amyloid, sacharides, lipids, proteins, NA, connective tissue, nervous tissue...

- congo red, PAS, van Gieson, PWH, Gram, Z-N...
Green trichrome

- Nucleus: blue
- Cytoplasm: lightred
- Collagen: green
Staining for lipids (oil red)
Periodic Acid Schiff (mucin in goblet cells)
Alcian blue – mucin in goblet cells
Gram

Diagram:
- **GRAM +**
  - Fixation
  - Crystal Violet
  - Iodine treatment
  - Decolorization
  - Counter stain (safranin)

- **GRAM -**
PWH – fibrous connective tissue
Special methods - IMMUNOHISTOCHEMISTRY

- used for demonstration of **presence and location of a certain antigen** in / on a cell (membrane, cytoplasm, nucleus)
- with the use of specific **antibodies** and **chromogens**
- colour is always the same (brown)

*Diagram 1: Illustration of Indirect Immunohistochemistry and Immunofluorescence methods.*
Immunohistochemistry

- Protein
- Glykoprotein
- Ag (Antigen)
- Primary antibody
- Secondary antibody
- Peroxidase
- Precipitate
- DAB (Diaminobenzidine)
- Microscopic evaluation
Ki67
poorly dif. squamous cell carcinoma
cytokeratins
epidermis
IMMUNOHISTOCHEMISTRY – use
IMMUNOHISTOCHEMISTRY – use

• tumors of unknown primary site
• prognostic markers of tumors
  (expression of HER-2/neu in breast Ca, Ki67)
  – modification of therapy!!!
• prediction of response to therapy
  (estrogen receptors in breast Ca)
• infectious diseases – viruses (Ab against RNA / DNA, HPV, herpesviruses), bacteria, parasites
IMMUNOHISTOCHEMISTRY – use
CYTOLOGY - material

• **EXFOLIATIVE**
  • material: spontaneous detachment of cells from epithelial surfaces, scraping, brushing, lavage of mucosal surfaces

• **INTERVENTION**
  • material: obtained by aspiration, curettage,... / during surgery (FNAC – fine needle aspiration cyt.)
CYTOLOGY - use
CYTOLOGY - use

- dg and management of **tumors**
- dif.dg. of **benign and malignant** tumors (breast)
- dg during surgery
- dg of specific **infectious diseases** (TBC from LN)
- dg non-tumorous, **inflammatory lesions** (Hashimoto disease)
- **cytogenetic** testing
FNAC – fine needle aspiration cytology

- **use**: palpable lesions (breast, LN, thyroid, soft tissue, salivary glands, intraabdominal lesions, testicles)

- **advantages**: no need for hospitalisation and anesthesia, method is quick, safe, repeatable, painless and cheap