Pathology - division of the field, methods of examination and technical sample processing

Pathology

The name is composed of the Greek pathos (=disease) and logos (=doctrine). Pathology is thus the study of disease processes and changes in the human organism. It is concerned with the etiology of disease and the mechanism (how disease occurs), with morphological changes in cells and organs and with the significance of these changes for their function.

Division of the field

The knowledge of pathology is applied in all clinical disciplines, but its contribution to the diagnosis of patients coming to clinical departments is also significant.


Microtome

General pathology

- deals with basic pathological processes in cells and tissues
- alteration of development, complications in general terms (necrosis, inflammation, infection)

Special pathology

- uses terms from general pathology without re-explanation
- describes damage and disease conditions in specific organs or organ systems (circulatory system, GIT,...)

Methods of examination

1. **Immunohistological methods**
   - based on the principle of the reaction of the antigen in the tissues with an antibody that is labelled with fluorochlorine - can be observed in a fluorescence microscope; also used for formalin
2. **Indirect immunohistological method**
   - similar to the indirect immunofluorescence method
   - 2. the antibody is labelled with root peroxidase
   - its localization is determined by several types of chromogen (the resulting product is solid, dark brown or red)
3. **Indirect immunofluorescence method**
   - used for formalin-fixed tissues
   - the primary antibody is not labelled, only the secondary antibody is labelled with fluorochlorine and reacts with the primary substance
   - Disadvantage of fluorescence methods - gradual degradation of fluorochrome - after a certain time it turns off
4. **Direct immunofluorescence method**
   - used for native (non-formalin-fixed) preparations
   - the fluorochrome-labelled antibody is not pipetted onto the cut slide and the result of the colour reaction is observed in a fluorescence microscope
5. **Avidin-biotin method**
   - the indirect avidin-biotin method is used
   - an avidin-biotin complex labelled with horseradish peroxidase is bound to the secondary biotin antibody
6. **In situ hybridisation method**
   - allows the identification of specific NK sequences in situ (in the genes of histological or cytological cell preparations) - e.g.: position of genes on the chromosome, genes of viruses, proteins, oncogenes
   - labelled probes are used which hybridise to complementary stretches of DNA or RNA
7. **Fluorescence in situ hybridization (FISH)**
   - Probes are labelled with fluorochrome and observations are made using a fluorescence microscope
8. **Polymerase chain reaction (PCR)**
   - amplification (= propagation, takes place in thermocyclers) of DNA fragments and their identification; e.g.: electrophoresis, Southern hybridisation and DNA sequence determination

Technical processing

Biopsy

- histological examination from living tissue

- Tissue is obtained in various ways:
  1. surgery - larger tissue sections, parts of organs or whole organs (measure, weigh)
  2. spontaneous excretion - exclusion of tissue from the body's hollow organs by natural means (cough, runny nose, stool, urine, genital bleeding)
  3. probatorial excision - removal of part of the lesion and histological evaluation
  4. probatorial puncture (attempted puncture) - the puncture is performed with a wider needle → the tissue under examination is punctured → it remains in the needle and is evaluated microscopically (e.g. kidney, liver)
  5. curettage (scraping) - a curette is used to scrape mucous membranes and pieces of tissue from some cavities; e.g.: uterine mucosa, ear canal, nasopharynx, bone deposits
  6. endoscopic excision - fibroscopes are used → the altered part of the mucosa is removed in a targeted manner; e.g.: GIT (intestine - ulcer), airways, urinary tract

Methods of sending and processing tissues

Tissue fixation

- molecular biological and cytogenetic tests - unfixed tissue is taken histological examination - a representative part of the removed tissue is used
- the tissue is immediately (to prevent drying and autolysis) immersed in a container (labelled!:name, surname, department and clinic sending the tissue) with fixing fluid (to prevent decomposition of cells, tissues)
- common method of fixation - 10% formalin (= 4% formaldehyde, dilution of 40% HCHO from the pharmacy 1:9, in a fume hood) sufficient amount of formalin:
  - 10x-20x greater volume than the fixed tissue
  - pH of the fixation solution is neutral
- fixation solidifies the tissue in the state in which it is left in the container, which is why the container has a wide neck smaller flat particles that might curl up (when loose) are placed on a piece of stiffer paper before fixation
- for electron microscopic examination, glutaraldehyde fixation is used; to fix the tissue well, it must be divided into small pieces of about 1 mm3
- peroperative examination:
  - in situations where the doctor is not sure what the pathological process is
  - using a cryostat, the tissue is frozen (liquid nitrogen) to allow a thin section to be made through the microtome, the pathologist can view the slides in a few minutes and communicate the resulting diagnosis to the surgeon

After tissue fixation

- usually 15-24 hours (shorter fixation time is sufficient for smaller samples)
- after fixation with formalin, the tissues are blocked (cut)
- larger parts of tissues or organs are weighed and measured during blocking, pathologically described → several smaller pieces (blocks, size: 1.5 cm. 1 cm. 0.5 cm) are taken → into a special device → where the tissues are drained → saturated with molten paraffin
- the paraffin blocks are cut into thin slices (5-7 µm) with a microtome → they are stretched on slides → the tissue is dewaxed on the slides → they are usually stained with haematoxylin-eosin → the slides are then sealed (mounted) in Canadian balsam and covered with a cover slip → the slides are numbered and can be viewed under a microscope

Links

Used literature

- CHADIMOVÁ, M. *Patologie*, Přednáška, Praha: 2.LF UK, 22.2.2011.
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