Relation between pathobiochemistry and clinical biochemistry. Clinical-biochemical analytics and its specific features. Terminology of clinical biochemistry. Material analyzed. Material collection.

Relation between pathobiochemistry and clinical biochemistry

- **Pathobiochemistry** = a field dealing with biochemical processes under pathological conditions/biochemical mechanisms that lead to the development of a certain disease.
- **Clinical biochemistry** = interdisciplinary field focused on research of basic biochemical processes in human organism from the point of view of human health resp. from the point of view of the treatment of diseases, eventually to prevent the onset of the disease.

Clinical-biochemical analytics

- **Biochemical analyte** = investigated biochemical substance.
- The analytes may be of **endogenous** or **exogenous** origin.
  - **Analytes of endogenous origin**: ***substrates and products of cellular metabolism***: ions, minerals, enzymes, vitamins, trace elements and other substances that are physiologically present in the human body.
  - **Analytes of exogenous origin: *substances of natural origin*** (toxins of animals, fungi, plants, moulds, bacteria, allergens), ***drugs*** and their metabolites, **a*lcohols***, ***addictive substances***, **substances abused for doping**, ***industrial products***, ***industrial waste***.
- **Laboratory analysis** of the analyte is carried out by a well-defined examination method and consists of **3 parts**: ***pre-analytical, analytical*** and ***post-analytical phases.***

Preanalytical phase

- **Preparation of patient** for biological sample collection.
- Biological sample collection.
- **Pre-analysis adjustment of the sample** after collection (addition of an anticoagulant - obtaining non-clotting blood, NaF for glucose testing in plasma, conservants…).
- Fill in **the request form**.
- **Sample transport to clinical laboratory**.
- **Receiving of the sample**.
- **Separation of serum/plasma** from the cellular component of blood.
- **Storage of samples/aliquots** (if the sample is not immediately examined).
- Temporary archiving of sample for possible repeat analysis.
- **Preparation of analytical samples** by physical and chemical adjustment **from biological samples** (pH adjustment, concentration, dilution, dialysis, sedimentation, filtration, extraction into organic phase, protein denaturation, mineralization, amplification - molecular biological examination, drying, homogenization, lyophilization…).
- **Non-standard conditions of the preanalytical phase** (incorrect collection of sample, unacceptable transport time, unclear sample identification, insufficiently filled request form, spilled tube contents…) → **clinical laboratories are obliged to reject the material.**

Analytical phase

- **Preparation of the analytical system:** start-up of laboratory instruments, tempering water baths, performing of the optical tests and calibration, internal quality control).
- Analysis of the biological sample by an **appropriate examination method.**
- **Conversion of specific physicochemical quantities** (absorbance, fluorescence, chemiluminescence, etc.) to **analyte concentrations expressed in usual units.**

Postanalytic phase

- Expression of the obtained result, rounded to the appropriate number of decimal places, and insertion of the obtained values into the formulas (e.g. for the calculation of renal clearance).
- **Interpretation of the result** with respect to the physiological range of values, diagnosis, stage of the disease, stage of its treatment and also analyte values measured at the previous collection.
- **Confirmation of the result** by **medical laboratory technician** and **clinical biochemist.**
- **Sending the result** (printed/electronic) **to the doctor.**
- In some cases, the result may be delivered directly to the patient or persons legally authorized to take it over.

Preanalytical influences on the laboratory tests

- The results of the laboratory examination can be influenced in all three phases.
- **The most important** from the point of view of **influencing the results is the preanalytical phase.**
- In the **preanalytical phase** the result may be influenced by:
  - ***Person of patient***
  - ***Collection of a sample***
  - ***Transport of sample***
  - ***By storing the sample before analysis***
  - ***Sample preparation for processing***

Person of patient-controllable factors

- **Gender** (most laboratory tests do not depend on gender, but we also find methods where the reference range differs for women and men, e.g. red blood cell parameters).
- **Race, ethnic or social groups of population** (e.g. negroid races have significantly less granulocytes than europoid race, they also differ in reference values of other tests such as amylase, alkaline phosphatase, creatine kinase). Members of ethnic groups may differ, for example, in the frequency of certain genes and thus in the occurrence of hereditary metabolic disorders. The reference values may also be influenced by typical eating habits of some ethnic or social group.
- **Age:** most tests have a lower upper limit of the reference range in childhood. E.g. higher activity of acid alkaline phosphatase and inorganic phosphorus concentration due to bone formation. The results of some laboratory tests (e.g. creatinine clearance) in children should be corrected according body weight or body surface area.
- **Simultaneous other disease** (e.g. in a patient with rheumatoid arthritis, we find an elevated c-reactive protein).
- **The half-life of the analyte** (e.g. diagnosis of myocardial infarction according to enzyme activity and levels of muscle protein in serum). Knowledge of the rate of elimination of a substance from the body is of great importance in the evaluation of drug levels and toxic substances **= half-life of xenobiotics**.
- **Method of setting reference values.**

Person of patient-controllable factors

- **Pregnancy:** proteins and other substances produced by trophoblast or fetal organs (hCG, SP-1, α1-fetoprotein, placental alkaline phosphatase, estrogens…) appear in the mother's blood and urine. Hormonal effects are also applied to some common metabolites: cholesterolemia increases in the last trimester, hemoglobin concentration decreases due to hemodilution, increased rate of glomerular filtration → decreased creatinine and urea in serum…
- **Cyclic changes** (e.g. diural rhythm of cortisol, monthly rhythm of female sex hormone secretion) The concentration of some other analytes is influenced by circadian rhythm, too.

Person of patient-uncontrollable factors

- **Physical activity** (e.g. Increased concentration of total protein and substances bound to it, pH changes (under anaerobic load), lactate concentration increases, muscle proteins are released into the blood circulation (increased activity of CK, AST, LD, myoglobin concentration)… changes depend on a number of factors such as the duration of exercise, exercise intensity (aerobic, anaerobic), individual training.
- **Psychological stress** leads to release of adrenal hormones and their metabolic effects, e.g. hyperglycemia, increase of free fatty acid concentration…
- **Food, alcohol, fluid intake.**
- **Smoking** (e.g. may affect the rate of metabolism of the drug theophylline).
- **Drugs:** the drug can affect the metabolism of an analyte (it changes its rate e.g. by inducing synthesis or inhibiting enzymes, affects binding to transport proteins…) or interferes with its own chemical reaction (e.g. ascorbic acid may mask the presence of glucose/blood in urine.
- **Surgery**: narcotics (hepatotoxicity), contusion and damage of tissue (increased CK, AST, LD, myoglobin concentration), hormonal response to stress.

Basic samples of biological material

- Whole blood
- Serum
- Plasma
- Urine (single, collected)
- Sweat
- Gastric content
- Cerebrospinal fluid
- Exudation and other body fluids
- Stool
- Bone marrow

Collection of sample

- It depends on the **patient's position** for some time before and during collection and the **type of collected blood**. **Additives** to the collected blood and the **type of container** may also influence the result.
- **Collection of venous blood:** from the **elbow vein**.
  - **Patient position: lying down** is recommended. Standing up there is a transfer of fluid from the intravasal space to the interstitium and the concentration of high-molecular substances (and substances bound to them) in the blood, including hematocrit, can increase by 10 - 15%.
  - **Skin disinfection:** use of alcoholic disinfectant solution before blood collection to determine alcohol concentration → false positive result. Skin disinfection with surfactant (Ajatin) → haemolysis.
  - **Arm tightening + exercise** should be as **short** as possible, otherwise the above fluid movement, anaerobic metabolism (local acidosis) may occur.
  - **Beware of vacuum during blood collection.** Vacuum too **high** → mechanical **hemodialysis**.
  - **Collection of other types of blood:** capillary blood (e.g. from the fingertip for blood glucose measurements), arterial blood (blood gas testing), arterialized capillary blood (acid-base balance).

Containers/tubes

- Fill in etiquette of conatainers (tubes) – **name of patient** → **identification** of patient. It is also possible to identify the patient **using a code**, e.g. Bar code.
- Drain blood with the syringe slowly, never through the needle (to avoid hemolysis).
- Most examinations are performed from **serum** → blood must be clotted.
- **One-use only containers** are **the most convenient.** They are mostly made of **plastic** (blood clots slowly and insufficiently, haemolysis occurs more often) → the tubes with a **layer of kaolin or glass wool** → **acceleration of precipitation**. A special **internal gel** allows **separation of serum** (gel forms the interface between the blood cells and the serum layer).
- **Glass tubes** → **better blood clotting**, **BUT:** possibiltiy of a **rupture of tubes** in centrifugation (specimen degradation, centrifuge contamination). **Before reuse**, the container must be **decontaminated** and **washed** → **reagent residue** in the test tube → **devalued assay results.**

Examination from the whole blood and plasma

- It is important to maintain the prescribed r**atio between the volume of anticoagulant solution and the added blood.**
- **Blood is always added to the tube with the anticoagulant**, never vice versa.
- The **anticoagulant influences the composition of the collected blood**. E.g. all anticoagulants, including heparin, bind Ca^{2+} and Mg^{2+} and decrease their concentration. To determine the Ca^{2+} concentration we must use heparin titrated by these ions. Various enzymes may be inhibited.
- All anticoagulants are anions where the accompanying cation is Na^+ (K^+, NH_4^+, Li^+). E.g. for the determination of ions from collected blood in a tube designed for the determination of blood glucose (contains NaF and Na_2EDTA), we get the increased value of Na^+ concentration.
- **Examples of analytes:** levels of glucose, lactate, glutathion, acid-base balance, glycated hemoglobin (**whole blood**), fibrinogen, acid phosphatase (**plasma**).

Sample transport

- The blood is transported in **closed containers.**
- **Protect samples of blood** during transport from **extreme temperatures** (inactivation of enzymes in the heat, glucose concentration decreases faster, frost can cause hemolysis).
- Exposure of blood to light may cause degradation of bilirubin.
- Transport must be **fast enough** (1-2 hours from collection depending on the analyte).
- We always **prefer to send serum** over longer distances than whole blood (because mechanical haemolysis).

Storage of samples

- **Storage conditions** affect the **stability of the analytes**.
- **Prolonged standing** of the collected blood leads to depletion of energy sources of erythrocytes (glucose) → impaired membrane function (ensures transport of Na^+ out and K^+ to the cell) → K^+ leakage from erythrocytes → hyperkalaemia.
- **Heat storage** → leukocytes consume oxygen + CO2 production + anaerobic glycolysis in erythrocytes → **decreased blood pH**. Therefore, blood for examination of acid-base balance and blood gases is kept on ice.
- **Addition of NaF in the blood for glucose/lactate assay** → **inhibition of glycolysis**. Without the addition of NaF, blood glucose decreases by erythrocyte activity.
- **Most examinations** are performed from **serum**. Store in a fridge at **+ 4 °C** in a **well-closed tube** to avoid evaporation of the sample (analytes and enzymes are stable for several days). **Prolonged storage** → storage of **frozen serum** (at **- 20 °C/- 80°C**).
- **Chemical conversants** are rarely used to stabilize serum (they should not interfere with the analysis). They are used for conversation of urine samples → we always choose them according to the type of analysis. Examples of chemical conversants: thymol in isopropanol, HCl, formalin (cell fixation).

Processing and sample preparation for analysis

- **Centrifugation** of blood. **Too strong** → **haemolysis** of blood!
- **Separation of serum/plasma.**
- **Deproteinization** of the sample (usually using chemical reagent → select according to subsequent analysis).
- **Thickening of the sample** (e.g. concentration of proteins contained in urine/cerebrospinal fluid prior to their separation by electrophoresis, choosing the right pore size).
- More rarely other procedures (e.g. erythrocyte washing).

Hemolysis

- = **disintegration of erythrocytes + spillage of their contents, including red blood agent into plasma (serum**).
- Haemolysis usually occurs *in vitro*, i.e. during blood collection, transport and processing.
- **According to the cause we divide hemolysis into:**
  - **Mechanical** (too strong shaking, centrifugation at high speed, transporting whole blood over a long distance, etc.)
  - **Osmotic** (wet tube)
  - **Thermal** (blood exposed to frost/high temperature).
  - **Chemical** (disinfectant that disrupts the erythrocyte membrane).
- **Influence of results by hemolysis:**
  - Intracellular erythrocyte content is released from the erythrocytes into the plasma → **increased concentration of intracellular components** (e.g. LD, AST, ACP, potassium).
  - The **red color of hemoglobin** interferes with the photometric determination of analytes.
  - **Hemoglobin acts as a buffer and changes the pH of the reagent** → it affects the reaction (e.g. determination of albumin, hemolysis decreases the result).

Examination methods in clinical biochemistry

- **Laboratory observations:**
  - analyte examined by the **human senses** (sight, smell).
  - Often a **microscope** is used, such as the microscopic determination of urinary tract stones.
  - **Laboratory observation** = **qualitative analysis** → we evaluate what is **present** in the sample, not expressing the amount, content or concentration of the analyte.
  - Qualitative biochemical methods belong to the **Identification methods** → we perform the **detection of the analyte** = **we determine the presence/absence of the analyte in the sample.**

Examination methods in clinical biochemistry

- **Laboratory measurements:**
- The analysis is performed **in biochemical analyzers**.
- Examination based **on scanning physical or chemical quantities** using detectors → laboratory measurements.
- **Instrumental examination methods**: **optical methods** (spectrophotometry in UV-VIS, reflex photometry, atomic absorption spectrometry, luminescence spectrometry, turbidimetry, nephelometry), **separation** (chromatography, electrophoresis, mass spectrometry, ultracentrifugation), **electrochemical** (potentiometry, amperometry) , **physical** (osmometry, flow cytometry) and **microscopic** (diascopic and episcopic microscopy).
- **Laboratory measurement of a qualitative character**: the measurement result corresponds to the positive negative response of the detector. E.g. Detection of gene mutation by real-time PCR, in laboratory finding we evaluate presence absence of mutation.
- **Laboratory measurements of quantitative character:** the detector response is proportional to the intensity of the measured physico-chemical quantity (**Lambert-Beer's law**) in a certain measuring interval. If we have a sufficiently large number of **calibration standards**, we can determine by their **analysis the dependence of the measured quantity on the analyte concentration** → get the **calibration equation** → determination of the **analyte concentration**. The result is expressed numerically and is provided with units (mmol/l, µmol/l, g/l, mg/l, µkat/l). We determine the concentration/amount of the analyte of interest in the sample.
- **Laboratory measurements of semiquantitative character:** we do not have a reference sample (calibrator) for the analyte evaluation → we are **not able to define the equation of the calibration curve**. We can **only estimate the concentration**, whether it is pathological, physiological or low, moderate, high (based on experience, reliability of instruments and reference samples containing a known amount of a substance with similar physico-chemical and biological properties as the analyte of interest). E.g. immunochemical analysis of benzodiazepines (the device is calibrated for one drug from the benzodiazepine family and other drugs in this group are tested with less reliability).

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