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Basic biochemical parameters in clinical practice

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Inflammation, Infections

Acute Phase Proteins

- Their levels in serum may be increased (positive APP) or reduced (negative APP) after the onset of a systemic inflammatory reaction
- Produced in response to cytokine production
- **Early acute phase proteins**
  - C-Reactive Protein (CRP), procalcitonin (PCT), serum amyloid A (SAA)
- **Acute phase proteins with a moderate response time**
  - α1-acid glycoprotein, α1-antitrypsin, haptoglobin
- **Slow-reacting acute phase proteins**
  - Complement C3 and C4
- The change in concentration occurs also non-specifically in many other conditions (reaction of the organism to trauma, surgery, cancer etc.)

C-Reactive Protein

- Plasma ß2-globulin that activates complement system
- Produced in the liver in response to tissue damage, infection or other inflammatory stimuli
- Biomarker of inflammation and cardiovascular risk
- CRP is not a specific marker of infectious disease
- The concentration increases in 6–9 h with a maximum in 24–48 h
- In healthy individuals – values <1 mg/L
- Virus infection – values up to 40– 50 mg/L (usually lower – up to 20 mg/L)
- Bacterial infection – values >40–50mg/L (more specific in the presence of fever)
- Increase of CRP levels in postoperative conditions, cancer and inflammations in general

C-Reactive Protein

Indications:

- To distinguish the type of pathogen (bacterial x viral origin) and the degree of immune response
- To monitor the effect of antibiotic therapy
- To monitor the progress of autoimmune diseases
- But in patients with liver failure may be observed low CRP values
- Slower rising of CRP levels in the elderly

Erythrocyte Sedimentation Rate

- Indirect measurement of the degree of inflammation (non-specific marker of inflammation) – measurement of the rate of fall (sedimentation) of erythrocytes
- The rate rises and falls more slowly than do CRP concentrations
- Normal ESR values are specific to age and sex
  - The rate increases steadily with age and is higher in women than in men

Procalcitonin

- New biomarker for early detection of (systemic) bacterial infections
- After immune stimulus, its serum concentration increases within 2–3 hours
- Higher sensitivity and specificity to distinguish systemic and local infection than CRP
- There is no significant increase in its concentration in viral infections, in autoimmune diseases, postoperatively, in cancer
- More suitable (than CRP) in patients with weakened immune system, severe hepatic insufficiency and in corticosteroid therapy

Imunoglobulins

- **IgG**
  - The largest proportion of total immunoglobulins
  - Formed in response to toxins, the products of bacterial lysis, post-vaccination and after viral infections
- **IgA**
  - Are used mainly in the defense of the mucosal surface infections
  - The increase is often found in inflammatory conditions affecting the mucous membranes and toxic hepatopathies
- **IgM**
  - Are formed mainly during the early response to bacterial and viral infections; their synthesis is usually later replaced by IgG synthesis
  - An increase in IgM is observed in some autoimmune diseases

Hepatic dysfunction

- Liver injury of various etiologies (i.e., viral, metabolic, autoimmune, etc.)
- Cirrhosis can result in hepatocyte loss, which affects the liver's ability to **metabolize** and **excrete** drugs
- Determinants in how liver dysfunction affects pharmacokinetics:
  - hepatic intrinsic clearance of the drug (= biotransformation)
  - hepatic extraction ratio of the drug (= efficiency of the liver to remove a drug from the systemic circulation)

Effects of hepatic dysfunction on pharmacokinetic processes

- Cirrhosis reduces the hepatic metabolism and clearance capacities:

  - Cell damage reduces both the quantity and the quality of cells responsible for intrinsic hepatic clearance
  - Fenestrations in the sinusoidal endothelium can occlude, basement membranes can form barriers between the sinusoid and hepatocytes → limitation of drug uptake into hepatocytes
  - ↓ plasma protein synthesis → effect on drug protein binding → ↑ proportion of unbound drug concentration

  → significant alterations in the exposure to many drugs, necessitating dosage adjustment

Child-Pugh score

- Most common system for assessing the prognosis of chronic liver disease, primarily cirrhosis
- Scoring of five clinical biomarkers of liver disease:
  - **total bilirubin**
  - **serum albumin**
  - **prothrombin time or INR**
  - **ascites**
  - **hepatic encephalopathy**

Assessment of hepatic damage

ALT = alanine aminotransferase

- Cytoplasmic enzyme – role in the synthesis, degradation and conversion of amino acids, in gluconeogenesis etc.
- Mostly in hepatocytes (intracellular enzyme) – when the cell membrane is damaged → release into the blood
- Determination of serum ALT activity is marker of hepatocyte damage caused by hepatocellular diseases
- Specific indicator of liver diseases – infectious (e.g., acute viral hepatitis), liver cirrhosis, liver tumors, obstructive jaundice, toxic liver damage (drugs ampicillin, clofibrate, statins, contraception, dicumarol, codeine etc.)
- The rate of the increase levels reflects the extent of the damage

Assessment of hepatic diseases

AST = aspartate aminotransferase

- ↑ activity of AST in myocard, liver, skeletal muscles etc. (in general in all tissues with high metabolic activity)

→ ↑ levels of AST is not a specific marker (serum activity is most increased in case of severe hepatocyte damage)

- ↑ levels can be observed in:
  - Liver diseases e.g., hepatitis – acute viral, chronic, other; liver cirrhosis, liver tumors, toxic liver damage (drugs), myocardial lesions, skeletal muscle damage, conditions after heart surgery etc.

Assessment of hepatic diseases

AST / ALT ratio

- The serum AST / ALT activity ratio serves as a prognostic indicator
- Range of 0.5–0.8 – in acute and chronic viral hepatitis
- > 1 – non-alcoholic liver cirrhosis
- The highest values are found in alcoholic liver damage
- Results > 1 may also indicate muscle damage (myocardial infarction, muscular dystrophy)

Drug-induced liver injury (DILI)

- **Intrinsic DILI**
  - dose-related
  - occurs in a large pro-portion of individuals exposed to the drug (predictable)
  - onset is within a short time span (hours to days)
  - E.g., paracetamol, amiodarone, antimetabolites, cyclosporine, valproic acid etc.

- **Idiosyncratic DILI**
  - usually not dose-related, although a dose threshold of 50–100 mg/day is usually required
  - occurs in only a small pro-portion of exposed individuals (unpredictable)
  - exhibits a variable latency to onset of days to weeks
  - E.g., allopurinol, amiodarone, dantrolene, diclofenac, disulfiram, isoniazid, fenofibrate, methyldopa, nitrofurantoin, phenytoin etc.

Drug-induced liver injury (DILI)

- **Acute fatty liver** – clinical syndrome of rapid development of liver and other organ failure associated with extensive microvesicular steatosis (amiodarone, didanosine, stavudine, valproate, zalcitabine)

- **Drug-associated fatty liver disease** – non-alcoholic fatty liver disease attributable to exposure specific medications (methotrexate, 5-fluorouracil, irinotecan, tamoxifen, corticosteroids etc.)

Standard liver biochemistry to assess suspected DILI

- Absence of specific diagnostic biomarker
- ALT, ALP and TBL are the standard analytes to define liver damage and liver dysfunction in DILI
- **Alanine aminotransferase (ALT)** – hepatocellular damage
- **Total bilirubin (TBL)** – cholestasis, impaired uptake, conjugation or excretion, biliary obstruction, haemolysis
- **Alkaline phosphatase (ALP)** – cholestasis, infiltrative disease, biliary obstruction; not specific (bone, salivary glands, intestinal, biliary)
- Etc.

Creatinine

- The product of muscle energy metabolism (product of creatine and creatine phosphate cleavage)
- The main source of creatinine is muscle tissue (98% of creatine is found in the muscles), therefore the plasma concentration of creatinine is largely dependent on the muscle mass of the individual
- Under physiological conditions, creatinine is excreted specifically by glomerular filtration (90%)
- Its values in serum and urine to assess Glomerular Filtration Rate

Glomerular Filtration Rate

- **GFR** (Glomerular Filtration Rate) = a key indicator of renal function
- Rate at which fluid is filtered across the glomerular basement membrane into the renal tubules
- GFR is generally accepted as the best overal index of kidney function
- GFR <60 mL/min/1.73 m2 = decreased GFR
- GFR <15 mL/min/1.73 m2 = kidney failure
- **eGFR** is estimated GFR and is a mathematically derived entity based on a patient's serum creatinine level, age, sex etc.

Glomerular Filtration Rate

- The reference values for GFR (creatinine) according age:
  20-40: 78 to 150 mL/min
  40-50: 75 to 132 mL/min
  50-60: 69 to 120 mL/min
  60-99: 66 to 114 mL/min

- Relationship of GFR and creatinine concentration is not linear: the value of GFR can be estimated from about 20 different equations involving various corrections, or using other parameter, particularly cystatin C

Albuminuria

- Assessment of albumin levels in the urine
- Earliest marker of glomerular diseases, it generally appears before the reduction in GF

- Normative values for albuminuria and proteinuria are in general expressed as the **urinary loss rate**
- Urinary loss rate of albumin = **albumin excretion rate (AER)**
- The normal value – cca 10 mg/24 hours

Cystatin C

- Physiological significance: cysteine protease inhibitor, a protein with MW 14 000
- Freely filtered through the glomerular membrane with subsequent reabsorption, a small amount is also excreted in urine of healthy individuals

- In clinical diagnosis:
  reduction in glomerular filtration = ↑ CC in serum
  renal tubular dysfunction = ↑ CC in urine

Chronic Kidney Disease (CKD)

= abnormalities of kidney structure or function present for more than 3 months

- Criteria (present for > 3 months):
  - Decreased GFR – **GFR < 60 mL/min/1.73 m2**
  - Albuminuria – ≥ 30 mg/24 hours
  - Urine sediment abnormalities
  - Electrolyte and other abnormalities due to tubular disorders

  etc.

Complications connected with CDK
– Drug toxicity

= complication, which is of relevance to all patients with CKD and reduced GFR

- Altered pharmacokinetics of drugs excreted by the kidney
- Increased risk of drug-interactions

→ Risk of errors in dosing, toxicity to the kidney (→ AKI) or systemic toxicity

→ Requirement of an adjustment in the dosage of many drugs

- At lower GFR – also changes in pharmacokinetics and pharmacodynamics of drugs not excreted by the kidney

Cautionary notes for drug administration in people with CKD

Examples:

- NSAIDs – Avoid in people with GFR <30 mL/min/1.73 m^2; not recommended in people with GFR <60 mL/min/1.73 m^2
- Aminoglycosides – Reduce dose and/or increase dosage interval when GFR <60 mL/min/1.73 m^2, monitor serum levels (trough and peak), avoid concomitant ototoxic agents (furosemide)
- Macrolides – Reduce dose by 50% when GFR <30 mL/min/1.73 m^2
- Fluoroquinolones – Reduce dose by 50% when GFR <15 mL/min/1.73 m^2
- Sulfonylureas – Avoid agents that are mainly renally excreted
- Cisplatin – Reduce dose when GFR <60 mL/min/1.73 m^2, avoid when GFR <30 mL/min/1.73 m^2
- Methotrexate – Reduce dose when GFR <60 mL/min/1.73 m^2, avoid, if possible, when GFR <15 mL/min/1.73 m^2

Acute Kidney Injury (AKI)

- Abrupt decrease in kidney function
- Various etioliogies – specific kidney diseases (e.g., acute interstitial nephritis, acute glomerular and vasculitic renal diseases); non-specific conditions (e.g, ischemia, toxic injury) etc.
- AKI is defined as any of the following (SCr = Serum creatinine) :
  - Increase in SCr by ≥0.3 mg/dL (≥26.5 μmol/l) within 48 hours; or
  - Increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or
  - Urine volume <0.5 ml/kg/h for 6 hours

Drug-induced acute kidney injury

- Acute tubular necrosis – aminoglycosides
- Osmotic nephrosis – e.g. hypertonic solutions
- Interstitial nephritis – acute allergic (penicillins), chronic (calcineurin inhibitors), papillary necrosis + decreased intrarenal blood flown (inhibition of prostaglandin-induced vasodilation) (NSAIDs), glomerulonephritis

Lipids, lipoproteins

- Major lipids in plasma: fatty acids, triglycerides, cholesterol and phospholipids
- **Triglycerides** (triacylglycerols)
  - From dietary fat, synthesis in liver and other tissues
  - Source of stored energy (lipolysis hydrolysis catalyzed by lipases)
- **Cholesterol**
  - Component of the cellular membranes, precursor of steroid hormones, bile acids, vitamin D_3
- In plasma, lipids are transported in association with proteins:
  - Free fatty acids with albumin
  - In complexes as **lipoproteins**
    - **Core – hydropfobic;** triacylglycerols, cholesteryl ester
    - **Outer layer** from phospohlipids (outer hydrophilic part), cholesterol, apolipoproteins

Lipoproteins

Classification od lipoproteins				
Lipoprotein	**Source**	**Density (g/ml)**	**Function**	**Risk of atherosclerosis**
Chylomicrons (CM)	Intestine	< 0.95	Transport of exogenous triglycerides	---- (CL remnants ↑↑)
VLDL	Liver	0.96 – 1.006	Transport of endogenous triglycerides	↑
IDL	Catabolism of VLDL	1.007 – 1.019	Precursor of LDL	↑↑↑
LDL	Catabolism of VLDL Via IDL	1.02 – 1.063	Cholesterol transport	↑↑↑
HDL	Liver, intestine, catabolism of CM and VLDL	1.064 – 1.21	Reverse cholesterol transport	↓↓↓

Lipids, lipoproteins

- Reference values (adults):

Total CH: up to 5.2 mmol/L (up to 5.8 mmol/L for individuals over 40 years old)

HDL-CH: male: over 1.1 mmol/L female: over 1.3 mmol/L

LDL-CH: 1.2 - 4.5 mmol/L

TG: 0 - 25 years old: up to 1.4 mmol/L

25 - 50 years old: up to 1.55 mmol/L

over 50 years old: up to 1.7 mmol/L

Dyslipidemia

- Medical condition of an abnormal level of blood lipids
- Results from increased synthesis or decreased catabolism of lipoprotein particles
  - which ensure the transport of fatty substances (cholesterol, triglycerides, phospholipids and fatty acids)
- The clinical consequence of DLP is **atherosclerosis**
- Part of a condition called metabolic syndrom

→ co-occurence of cardiovascular risk factors as:

atherogenic dyslipidemia (hypertriglyceridemia + reduced high-density lipoprotein cholesterol (HDL)), elevated fasting glucose, obesity and hypertension

Glycemia

Reference value: adults 3.3–5.6 mmol/L

Diagnosis of diabetes mellitus (DM):

1) fasting blood glucose level exceeds 7 mmol/L in 2 separate visits

2) individual testing exceeds 11.1 mmol/L

3) after 2 hours in "oral glucose tolerance test" exceeds glycemia exceeds 11.1 mmol/L

If fasting glycemia < 7 mmol/L, and simultaneously in "oral glucose tolerance test" after 2 hours glucose still exceeds 7.8 mmol/L (but less than 11.1 mmol/L) = impaired glucose tolerance

→ self-monitoring of blood glucose in DM

Glycated proteins

- modified proteins that are formed by the addition of glucose molecules to amino acid chains

- **Glycated hemoglobin (HbA1C)** is an important glycated protein assayed to diagnose and monitor diabetes

→ reflects long-term glycemic status

- HbA1C is used in routine as the best parameter to assess compensation of patients with DM (efficacy of the treatment)

→ an indicator of so-called "long-term blood glucose" because it provides information on blood glucose for a period of 2-3 months

Acute Coronary Syndrom (ACS)

= AMI with or without ST elevation (EKG), unstable angina pectoris

hypoxia - necrosis

Biochemical investigation

enzymatic markers: creatinkinase, lactate dehydrogenase

non-enzymatic markers : troponin, myoglobin

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Creatinkinase (CK)

3 cytosolic isoenzymes : CK-1 (CK-BB), CK-2 (CK-MB), CK-3 (CK-MM) a 1 mitochondrial (CK-Mt), 3 genes coding subunits CK-M, CK-B a CK-Mt

The most significant: **total CK, CK-2 (CK-MB)**

CK-MM is the most abundant isoform of CK in both heart and skeletal muscle, CK-MB is more specific for heart

- heart: total CK consists of about 20% CK-MB
- skeletal muscle: total CK consists of 2% CK-MB (reflects status in healthy individuals)

AMI: increased enzymatic activity both total CK and CK-2 as well, but CK-2 is raised relatively much higher than total CK

Skeletal muscle damage: increased enzymatic activity both total CK and CK-2 as well, and the ratio between total CK and CK-2 is identical to healthy individuals

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High-sensitivity Troponin (hs-Trop)

- detection of myocardial infarction

- detects much lower concentrations of the troponin protein → shortening the time interval required to identify myocardial injury

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