Pavol Jozef Šafárik University in Košice

Faculty of Medicine

CLINICAL BIOCHEMISTRY

Eva Ďurovcová
Mária Mareková

Košice 2020

CLINICAL BIOCHEMISTRY

Academic textbook

Author:

MUDr. Eva Ďurovcová, PhD.

prof. Ing. Mária Mareková, CSc.

Faculty of Medicine, Pavol Jozef Šafárik University in Košice

Reviewers:

MUDr. Dušan Dobrota, PhD.

Jessenius Faculty of Medicine in Martin - Comenius University in Bratislava

MVDr. Zuzana Kostecká, PhD.

University of Veterinary Medicine and Pharmacy in Košice

Available at: www.unibook.upjs.sk
Publication date: 25.11.2020

ISBN 978-80-8152-937-5 (e-publication)

Preface to the 2nd edition

After 7 years, we finally reworked the textbook on the subject of clinical biochemistry at UPJŠ FM in Košice. We have innovated information in accordance with recommendations of professional medical societies and added new chapters. As the scope of the subject is limited, the textbook does not cover all issues of biochemical diagnostics. It focuses on disorders of water-ion and acid-base balance disorders, and basic laboratory diagnostics of diseases of selected organs.

The textbook of clinical biochemistry has the ambition to become a practical aid for senior medical students and beginning doctors in the study of laboratory medicine. It should make it easier for them to orientate themselves in routinely used clinical-biochemical examinations and could form a springboard for a more detailed studying of laboratory methods and their use in diagnostic and monitoring algorithms.

Like any medical discipline, clinical biochemistry is evolving rapidly and gradually integrating with other areas of laboratory medicine. New diagnostic methods penetrate deeper into the essence of the biochemical mechanisms of the human body, and "omic" methods are gaining ground. It is almost sure that in a few years, laboratory examination looks quite different from today. If this publication quite hypothetically reaches its next edition, many chapters will need to updated, and new chapters written following the up-to-date status.

Authors thanks to their colleagues and reviewers for their professional comments and constructive criticism of the text - prof. Jaroslav Racek, MD, DrSc. from FM UK in Pilsen.

Authors thanks very kindly too prof. MUDr. Dušan Dobrota, CSc. (JFM UK Martin) and doc. MVDr. Zuzana Kostecká, PhD. (UVMP, Košice), who read and wrote review of this textbook.

Authors, 2020

1

Requesting and interpreting tests

CONTENT

Laboratory diagnostics provides a range of objective data necessary for proper medical decision making. In some diagnoses, it may be up to 70% of all data in a patient 's chart, while it comprises less than 5% of total costs in healthcare. The medical specialty **laboratory medicine** is divided into several subspecialties - **clinical biochemistry**, hematology and blood transfusion, immunology, toxicology, genetics, microbiology, histopathology, cytology. **Clinical biochemistry** is the branch of laboratory medicine which provides such a wide array of tests results, that clinical teams require frequent advice in interpreting results and in choosing appropriate test or test cascade.

1.1 Laboratory organization

The clinical biochemistry laboratory is designed to provide results 24 hours per day. It produces hundreds of tests including **routine chemistry** (e.g. ions, glucose, urea, creatinine, uric acid, bilirubin, proteins, enzymes, lipids, urine analysis, blood gas analysis), **immunochemistry** (hormones, tumor markers, vitamins, cardiac markers, bone markers, markers of inflammation and sepsis, etc.), **toxicology** (e.g. drugs, intoxications, monitoring of drug levels), **special chemistry** (electrophoresis, CSF analysis, amino acid analysis, and others). The set of common tests needed for quick medical decisions is offered by almost all clinical laboratories 24 hours a day in an urgent (or CITO/STATIM) regimen. Many other specialized tests are restricted to larger laboratories or even to regional or national centres. Laboratory professionals are working behind-the-scenes, 24/7, to support the other health care professionals with results of testing.

Large hospital laboratories analyse thousands of samples with a typical profile of 10 tests per sample each day. In dealing with such a huge number of routine test requests the laboratory depends heavily on automated high throughput equipment which is linked to a laboratory information system. That assigns test requests to electronic patient files, maintains cumulative patient records, and generates the printing of test reports. Test requests can be also electronically booked at the ward, clinic, or general practitioner and equally, the test results can be displayed on computer screens at distant locations.

Alongside biochemical analysers in **core laboratory**, small devices/instruments are used to perform certain tests at remote locations, such as at the patient´s bedside in intensive care units, emergency departments, or at home. The regimen of **point of care testing** (POCT) eliminates transport of the sample to the laboratory, tests are minimally invasive (usually need only a spot of whole blood or urine) and results are immediately available (INFO 1.1).

INFO 1.1 Point of care testing

POCT may be performed outside the laboratory by laboratory staff, by nursing or medical staff, or at home by the patient. The frequent reasons for POCT are:

- Tests are of urgent importance, their results affect the immediate management of the patient in situations like following: chest pain (cardiac troponins), acute shortness of breath (NT-proBNP, natriuretic peptide), acute abdomen or gynecological bleeding (hCG, human chorionic gonadotropin), fever (CRP, C-reactive protein), suspicion of pulmonary embolism (D-dimers), suspicion of intoxication (blood gases, electrolytes, ethanol, paracetamol).
- Tests are so common, simple, and relatively cheap that it is more economical to perform them at the point of care - blood glucose, HbA1c (glycated hemoglobin), urinalysis.

Despite the simplicity of POCT tests the training and monitoring of relevant staff performance by laboratory is necessary. POCT disadvantages include higher price per test in comparison to main laboratory test, possibility of access of untrained individuals, less precise calibration and quality control of devices, and non-electronic patient´s records.

1.2 Laboratory testing cycle

All steps between the decision to order a laboratory test and return the result of the testing to the clinician create so-called **brain to brain loop** (Figure 1.1). The whole process can be divided into three phases: preanalytical, analytical, and postanalytical.

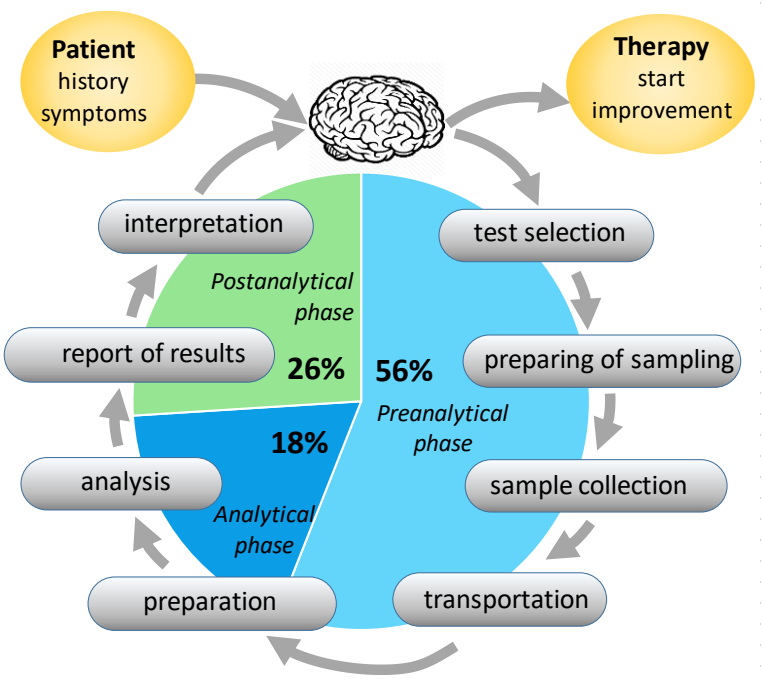


FIGURE 1.1 Brain to brain loop. The incidence of errors (systematic, random) is given in %.

The preanalytical phase begins when a person´s brain, usually that of the physician (patient, other healthcare professional), decides that it would be a good idea to have a lab test and "orders" it. The phase covers test selection, specimen collection, labelling, transportation of sample and request form to the laboratory, the acceptance of specimen by laboratory staff. In the pre-analytic phase, the majority of factors (45 – 60%) occur with a potentially negative effect on the quality and accuracy of laboratory results:

- factors **before sampling** (a rational indication of examination, preparation of a patient before collection;
- sampling-related factors (e.g. inappropriate quantity, type and quality of the sample, confusion of patient or sample;
- **manipulation with biological material** (e.g. storage, transportation, registration of samples in the laboratory).

In the **analytical phase**, specimens are processed and analysed, results are reviewed and verified by medical or technical specialists. During the **postanalytical phase**, results are released to the patient's record and reported to the clinicians in printed or electronic form. At last, clinicians make decisions based on the test results. In the laboratory, the results are stored in the patient databases for a long time.

1.3 Selection and use of laboratory tests

The selection of suitable tests is one of the most important steps in the pre-analytical phase. At present, the physician can choose from a wide range of diagnostic tests, which are increasing as a result of scientific and technological progress in the field of laboratory medicine. The main reasons for biochemical tests requesting are as follows:

- **diagnosis:** to confirm or to reject clinical diagnosis, in the differential diagnosis;
- **monitoring of therapy:** to confirm therapeutic or toxic levels of some drugs (anti-epileptics, some antibiotics, immunosuppressive drugs, etc.), to monitor expected or detect adverse effects of therapy (hepatotoxicity, nephrotoxicity, etc.);
- **monitoring** of **disease:** to follow the course of an illness or to indicate its severity;
- **prognosis** or **risk assessment:** to get information regarding the likely outcome of a disease;
- **screening:** to identify asymptomatic carriers of diseases (in the general population or case-finding screening);
- **other:** for forensic purposes or ethically approved research.

The reasonably ordered test is 'the right test for the right patient at the right time'. Such a test has known and clinically validated diagnostic value. Regardless of the purpose, test results can affect patients' physical and mental health unpredictably, e.g. false positivity of HIV or tumor marker test can cause a cascade of unnecessary and stressful examinations for the patient. Table 1.1 shows examples of test selection for different purposes.

TABLE 1.1 TEST SELECTION FOR THE DIFFERENT PURPOSES

Category	Example
Confirmation or rejection of a clinical diagnosis	TSH and fT4 in suspected hypothyroidism, cardiac troponins in acute myocardial infarction
Differential diagnosis	Bilirubin total and conjugated in different forms of jaundice
Assessment of disease severity	Serum creatinine or GFR in renal disease HbA1c in diabetic patient
Detection of side effect/complications	ALT levels in patient treated with hepatotoxic drugs CK activity in patients treated with statins
Monitoring therapy	Serum drug concentration in patients treated with anti-epileptic drugs, potassium during diuretic therapy
Prognosis	Serial creatinine testing in patients with CKD, NT-proBNP in heart failure
Screening	TSH for congenital hypothyroidism, AFP, βHCG, estriol - prenatal screening of certain birth defects; Plasma glucose or oGTT - risk of DM

When indicating laboratory examinations, the physician is guided by expert recommendations, standard diagnostic procedures, and algorithms based on the principles of Evidence-Based Medicine (EBM). In many countries around the world, the "Choosing wisely" campaign is currently in progress to eliminate unnecessary healthcare for the patient (INFO 1.2).

INFO 1.2 Choosing wisely

The growing evidence has supported that up to 1/3 of healthcare including tests, and treatment procedures are unlikely to be benefit for patients or are not in keeping with evidence-based medicine (EBM). The problem for patients is that all tests and interventions have side-effects and some may even cause harm. CT scan is 200 000 more powerful than an airport scanner; a blood test for may have very few side effects but for an elderly patient it may be distressing and painful.
Choosing Wisely is a global initiative that started in the USA in 2012 aimed at improving conversations between patients and their doctors and nurses in order to make better decisions about healthcare. The initiative encourages them to get the best answers to 4 questions.
What are the benefits? What are the risks? What are the alternatives? What if I do nothing?
Choosing Wisely key aim is to change the culture when it comes to ordering or prescribing. In a field of laboratory medicine, several tests have been identified as of questionable value or their use is not recommended more:

- do not perform a population screening of vitamin D deficiency;
- do not use bleeding time testing, as more suitable coagulation study is available;
- do not use erythrocytes sedimentation rate for diagnostics of acute infection;
- do not use CK-MB and myoglobin in diagnostics of acute myocardial infarction.

1.4 Biological material

Biological materials collected from the patient and used for investigation are called **samples** or specimens. **Analytes** are substances that are tested on the patient´s sample, using appropriate analytical methods. Biochemical tests are most frequently carried out on serum or plasma obtained from venous blood and on urine. In addition, cerebrospinal fluid, feces (stool), kidney stones, pleural, abdominal, amniotic, and other bodily fluids are sometimes required for analysis. **Serum** is the corresponding aqueous phase of blood, which has been drowning into a tube without anticoagulant and allowed spontaneously to clot. For the majority (but not all) biochemical tests it makes little difference whether plasma or serum is used (INFO1.3).

INFO 1.3 Serum and plasma

The majority of laboratory tests are traditionally investigated in serum. In serum clotting factors have been removed naturally by allowing the blood to clot prior to the separation of the liquid component. On the other hand, plasma contains all proteins including clotting factors and fibrinogen. Some biochemical analytes differ in serum and plasma (e.g. phosphate, K, Mg, AST, LDH, NSE), they are mostly increased in about 10% in serum as a consequence of their release from RBC during retraction of a blood clot. Plasma has some advantages and disadvantages when compared to serum.

PROS: Plasma is available for analysis immediately after collection - used for urgent patients.
Plasma is more suitable for critical care patients with clotting disturbance.
Hemolysis of plasma is less frequent in comparison to serum.

CONS: Salts used in additives interfere with mineral analysis (Na, K).
Calcium is falsely decreased in plasma with EDTA, citrate (chelating effect).
Tubes with anticoagulants are always more expensive.

Plasma is obtained by taking whole blood in tubes that are treated with an anticoagulant. It represents the aqueous supernatant of blood after cellular elements have been separated by

centrifugation. In this textbook, the term plasma is used whenever it is intended to describe the concentration of analytes *in vivo*.

1.5 Sampling of specimens

The sample must be collected into a suitable tube or container after proper preparation of a patient. The most often tested material is blood**. Blood specimen types** depend on the collection method and site of collection, the treatment at the time of collection, and the post-treatment processing as described in Table 1.2.

TABLE 1.2 TYPES OF BLOOD SAMPLES USED IN LABORATORY STUDIES

Specimen	Collection	Description	Use
Clotted blood	Venepuncture	No anticoagulant added, activator of clotting	For separation of serum by centrifugation
Serum		Blood is allowed to stand for 20-30 min until clotting is completed and after that centrifuged	Biochemistry testing Serology testing Immunology testing Blood banking
Whole blood		Collection into a tube with anticoagulants which prevent blood from clotting; whole blood includes all cellular and plasma components	Separation of plasma portion by centrifugation, hematology testing in the whole blood
Plasma		Whole blood containing anticoagulants, may be centrifuged immediately after sampling	Coagulation studies Plasma biochemistries
Capillary blood	Skin puncture (finger, heel, earlobe)	A mixture of venous blood, arterial blood and interstitial fluid obtained by a skin puncture	In children, new-borns Neonatal screening, repeated testing in monitoring (e.g. glycemia, ABG)

Venepuncture is a collection method that should be performed exclusively by personnel who have received proper training. As a general rule, the volume of blood drawn should equal 2.5 times the amount of serum plasma required. For example, to obtain 2 mL of serum or plasma, it is necessary to draw at least 5 mL of blood.

Blood samples are collected into color-coded tubes generally depending on the anticoagulant or other additives used (Table 1.3). The color-coding may differ among different manufacturers, however, there are efforts to unify that coding worldwide. Separating gel incorporated into blood collection tubes reacts minimally with analytes in blood and because of its density forms a barrier during centrifugations between supernatant, which is plasma or serum, and the cellular material of the clot. The gel prevents contamination of serum in the primary tube especially during transportation to a remote laboratory and storage.

TABLE 1.3 COLOUR CODING OF LABORATORY SAMPLING TUBES

Tube type	Cap color	Description
Plain tube	Red	Coated with a clot activator. Serum obtained by centrifugation which prevents the exchange of analytes with clot.
Serum separator (gel)	Yellow	Coated with a clot activator, gel barrier allows storage of a primary sample, used in most routine clinical chemistry.
Lithium heparin (plasma)	Green	Available with and without gel barrier. The anticoagulant allows the sample to be centrifuged immediately, suitable for urgent analysis.
Fluoride oxalate (NaF)	Grey	Oxalate binds calcium and NaF inhibits glycolysis, thus maintaining blood glucose for several hours. Used in blood glucose measurements.
Ethylenediamine tetra-acetic acid (EDTA)	Lavender	EDTA chelates calcium preserving cell morphology in: blood counts and films, analytes requiring whole blood (HbA1c and erythrocyte enzyme activities).

The **standardized blood collection** should be performed under the following conditions:

a. in the morning (between 7.00 – 9.00 am),
b. on fasting (no caloric intake for at least 10 h),
c. after overnight rest,
d. usual eating and movement regime 48 h before sampling,
e. exclusion of stress and drugs, which are not vital.

Blood collection tubes must be **drawn in a specific order** to avoid cross-contamination of additives between tubes:

1. tubes for blood cultures,
2. tube containing Na-citrate for routine coagulation tests (PT, APTT),
3. serum tube with or without separating gel or clotting activator,
4. heparin tube (plasma),
5. EDTA tube,
6. tube for glycemia (containing NaF),
7. all other tubes.

The recommended order of draw is also followed if blood cultures are not collected. Important principles in blood collection also include keeping the recommended blood to anticoagulant ratio, thoroughly mixing the tubes by rotating (not shaking), and storing whole blood at room temperature.

Capillary blood obtained from a cutaneous puncture (fingertip, earlobe, heel in very young children) is used when only a small amount of blood is required or possible to collect. The best locations for collecting capillary samples are the 3rd and 4th fingers of the non-dominant hand and the edges of the heel (infants). The sterile disposable retractable lancet for once-time is used most often for safety reasons. Compared to venous blood, this type of sample provides less accurate results, especially for a variable admixture of interstitial fluid, which changes blood composition.

Capillary specimens may be processed for serum, plasma, whole blood, or applied to special filter paper cards and used as dried blood spots. In adults, capillary blood is used frequently for repeated measuring of glycemia, lactate, acid-base parameters.

Urine for analysis is collected either as a spot sample (random, first morning, mid-stream) or a time collection, usually over 24 hours. **Other body fluids** represent cerebrospinal fluid, abdominal, pleural, amniotic fluid, sweet, and rarely saliva. They should be collected into a clean glass or plastic tubes or containers according to local laboratory instructions. A **fecal** examination is currently carried out on small samples taken in special containers with a secure stopper. Other **solid tissue** samples (e.g. stones, nails, hair) are only examined sporadically in the biochemical laboratory, with the exception of specialized centres focused on the analysis of drugs or trace elements.

Each material must be accompanied by the **request form** in written or electronic form, containing at least following:

1. Patient name, date of birth, sex;
2. Ward/clinic/address;
3. Name of requesting doctor (telephone/page number for urgent requests);
4. Clinical diagnosis;
5. Date and time of sampling;
6. Test(s) requested;
7. Type of specimen (other than blood or urine);
8. Special conditions of sampling (function tests);
9. Other relevant information (e.g. drugs, warning about high-risk samples from patients with hepatitis B or C and HIV).

TABLE 1.4 THE MOST FREQUENT CAUSES OF ERRORS DURING BLOOD SAMPLING

Error	Consequence
Confusion of patients during labelling of tubes and request forms	Laboratory results do not correspond to the clinical condition of the patient. In case of pathological findings there is another patient to whom these results belong!
Improper timing of sampling	Important in monitoring drug levels (taking too soon after drug ingestion) or interpreting analytes with pronounced diurnal rhythm (cortisol).
Mistakes during phlebotomy	Too long applying of a tourniquet - haemolysis. Wrong tube, the influence of anticoagulants (Na, K, Ca). Wrong order of tubes - contamination of serum tube with EDTA/citrate results in false hypocalcemia. Wrong blood to anticoagulant ratio distorts coagulation tests.
Mistake after sampling	Improper mechanical manipulation with a sample. Storage of sample at very low or high temperature. Freezing of whole blood (with or without anticoagulant) - hemolysis. Centrifugation of imperfectly clotted blood. Repeated freezing-thawing of a sample.
Blood sampled immediately after or during IV infusion	The visible effect of hemodilution on the serum analytes, except for those contained in the infusion.

Errors in the pre-analytical phase could invalidate the entire subsequent analytical process and lead to incorrect results (Table 1.4). Proper venous blood collection is a key factor in eliminating errors in the preanalytical phase. Professional societies pay close attention to the standardization of blood collection, as evidenced by the new recommendations of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) from 2018. The laboratory, in particular accredited to ISO 15189, is obliged to refuse to perform the tests if the required examinations cannot be clearly assigned to the particular patient or if there is any doubt about the quality of a sample.

1.6 Interpretation of laboratory tests

The results of clinical biochemistry tests are mostly in numerical form. They inform about the concentration or activity of analytes in appropriate units. When interpreting the results, the clinician should answer the following questions, which will be discussed in the next sections:

1. Is it normal or abnormal?
2. Is it different? Has a significant change occurred in results in comparison to those previously reported?
3. Is it consistent with clinical findings? Do any of the results alter my diagnosis of a patient's disease?

Reference ranges

Typically, the result or set of results is compared with 'normal' or reference intervals (RI) or ranges. Those are determined by measuring a set of values in healthy individuals or other well-defined populations (e.g. based on gender, age, ethnicity) and subsequent statistical evaluation of the distribution of the measured parameter in the reference population.

Biochemical laboratories mostly take over their RIs from verified and recognized sources, such as textbooks, monographs, peer-reviewed journals, or diagnostic kit manufacturers' recommendations, as creating their own RIs is time, personnel and financially demanding for them. In certain situations, the laboratory should define and publish its own reference ranges because of existing geographical or ethnic differences.

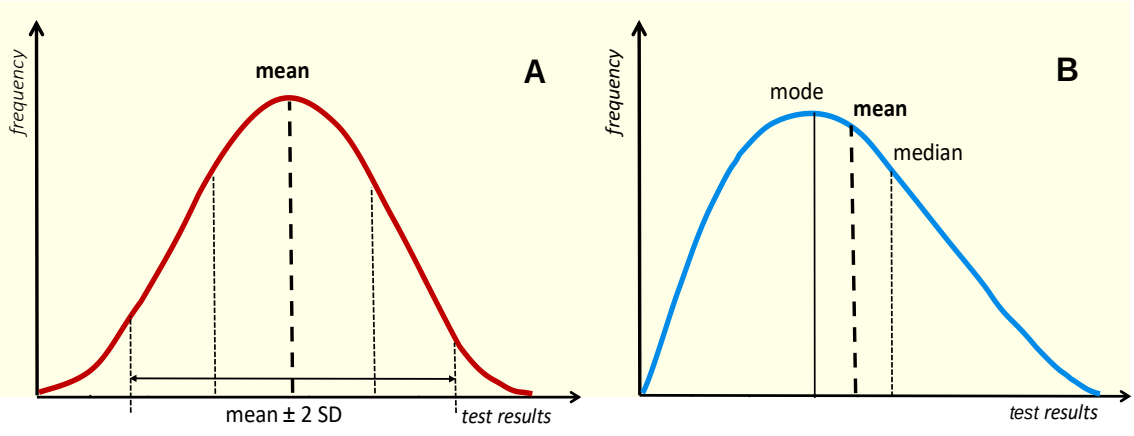


FIGURE 1.2 Gaussian (A) and non-Gaussian (B) distribution of values in reference population. Statistical theory predicts that 95% of the values in population will lie within the range given by mean ± 2 SD.

Most of the biological variables show the symmetric distribution in the form of a typical Gaussian curve (normal distribution). However, many variables have the non-Gaussian asymmetric or skewed distribution (e.g. bilirubin, cholesterol) that is deflected to one side (Figure 1.2). In accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI), RI represents the central 95% interval from the distribution of values obtained from the reference population (statistical mean ± 2 SD). Thus, 5% of healthy individuals (1 from 20!) will not fit in with existing reference ranges. Normal range or value does not mean ideal one nor it is associated with no risk of having/developing the disease. Vice versa, the abnormal result does not necessarily indicate the presence of a pathological process. No absolute demarcation exists between values seen in health and in disease.

If an important decision during patient management is made based on a single test, a proper **cut-off point** or **decision level** may be used instead of reference intervals. These values are recommended by scientific authorities (e.g. medical specialist societies) and are derived from the analysis of dozens of clinical studies, based on the weight of medical evidence. Examples include lipid parameters in cardiovascular risk assessment and in the management of hyperlipidaemias, fasting, and post-load blood glucose in diagnostics of diabetes, NT-proBNP in the exclusion or confirmation of heart failure.

Sources of variation of the test result

Clinicians often compare more results of one test or set of results of the same patient obtained on different occasions. They need to decide, whether a numerical change in sequential testing is clinically significant and reflects a **true change in the patient´s clinical conditions,** e.g. improving or worsening of patient´s disease.

Each laboratory result, however accurate and true, is burdened with some inaccuracy, which is due to the analytical characteristics of the particular laboratory method (INFO 1.4) and the existing biological variability of the measured parameter. **Biological variation** of test results exists in both health and disease. The following influenceable and non-influenceable factors are important sources of **within-individual variability,** thus fluctuations in the level of an analyte during the day or between days in a single individual:

1. *Diet:* Composition of diet affects the level of many analytes, including serum triacylglycerols (TAG), glucose, iron (Fe), phosphorus (P), alkaline phosphatase (ALP), ammonia, etc.
2. *Diurnal rhythm:* Several blood constituents show diurnal variation (cortisol, ACTH, Fe, prolactin, GH) or have seasonal fluctuation (vitamin D, TSH). Within the menstrual cycle, concentrations of pituitary and ovarian hormones naturally fluctuate, but also iron.
3. *Posture:* Protein and all protein-bound constituents show significant differences in concentration between samples collected in an upright and recumbent position. This is due to increased hydrostatic pressure in capillaries, which causes a shift of water from the intravascular space to the interstitium and increases the concentration of large protein molecules (immunoglobulins, albumin, proteohormones) and protein-bound substances (cholesterol - CH, TAG, calcium, Fe). The same changes occur in venostasis, e.g. with a tourniquet used for more than 1 min during blood collection.
4. *Physical activity:* Vigorous or unaccustomed exercise before sampling may increase creatine kinase (CK) activity, myoglobin, or lactate concentration.
5. *Stress:* Physical and mental stress increases ACTH, cortisol, adrenaline, noradrenaline, prolactin, renin, GH, TSH.

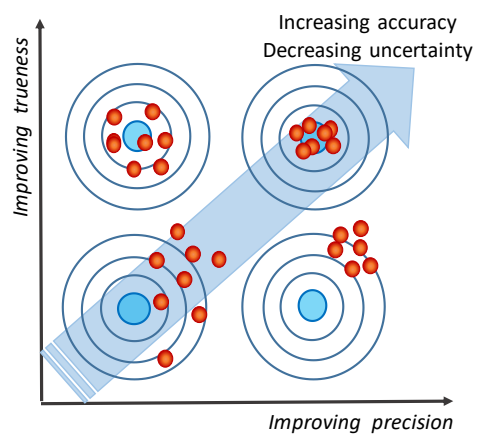
6. *Pregnancy:* An increase in intravascular volume results in dilution of some parameters (e.g. urea, erythrocytes, hematocrit, total protein), GFR increases, glucose reabsorption in renal tubules decreases (renal glycosuria), lipid levels and production of some specific proteins (e.g. ceruloplasmin, transferrin, TBG) increase.
7. *Bodyweight:* Adipose tissue as an endocrine organ markedly influencing metabolism and also laboratory findings. The increase of postprandial blood glucose, insulin, CRP, TAG, cholesterol, uric acid, and cortisol are most common.
8. *Others:* Smoking, alcohol, and plenty of drugs and medicines may affect biochemical results in a different way.

INFO 1.4 Analytical sources of variation

Analytical influence on test results occurs in some systematic degree even in a good laboratory with skilled analysts. It depends on following analytical properties of the test:

- Accuracy
- Precision

The "dartboard" analogy is used to illustrate the meaning and influence of these terms. An accurate method gives results close to the true value. A precise method yields results that are close to one another, but not necessarily to the true value. Blunders (outliers) or grossly inaccurate results have no predictable relationship to the true value and they mostly result from a mislabelling of the specimen at the time of collection or transcription errors during preparing report.



Another group of preanalytical factors causes **between-individual differences** in blood concentration of some analytes represents another source of biological variation. For example:

1. *Age:* Serum alkaline phosphatase activity, phosphate, creatinine, sex hormone, and gonadotropin concentrations are examples for analytes with age-matched reference ranges.
2. *Sex*: influences creatinine, uric acid, CK, GGT activity, and sex hormone concentrations.
3. *Ethnicity:* Asians have lower ADH activity but higher salivary amylase (AMS) activity than the Caucasian population. In Africans and African-Americans, there are lower leukocytes, higher levels of vitamin B12, CK, AMS than in the Caucasian population. Ethnic differences for cholesterol and some plasma proteins have been also reported, as they likely results from environmental and dietary factors;
4. *Genetics:* individuals with blood group 0 have lower von Willebrand factor activity (only 35% compared to other groups), heterozygous for some thalassemia have lifetime lower some hematological parameters (MCV, mean cellular volume).

Interference and sample contamination

Other factors, acting *in vitro,* that contaminate the serum or plasma composition and/or interfere with the laboratory methods, may also bring errors to laboratory results. These include the pathological appearance or consistency of serum - hemolysis, lipemia, hyperbilirubinemia and others, which are listed in Table 1.5.

Hemolysis means the breakdown of red blood cells and the release of their intracellular components into surrounding plasma. Hemolysis of a sample can be detected by eye when the concentration of free hemoglobin reaches more than 0.2 g/L. Causes of **intravascular hemolysis** (*in vivo*) include mostly hemolytic anemias, post-transfusion reaction, immunological, toxic, or mechanical damage of erythrocytes (artificial heart valves, hemodialysis, extracorporeal circulation).

TABLE 1.5 FACTORS CAUSING SAMPLE CONTAMINATION OR ANALYTICAL INTERFERENCE

Pathological serum composition	**Type of interference**
Hemolysis	Interferes with analysis of K, Mg, P, LDH, Fe, ferritin, NSE
Hyperbilirubinemia	Interferes with photometric methods (e.g. creatinine)
Lipemia	Increases glucose, P, BIL, uric acid, TP, HbA1c, TAG, D-dimers, PT, APTT and decreases Na (pseudohyponatremia)
Cold agglutinins	Influences CBC, blood groups, cross match
Anti-animal antibodies (HAAA, HAMA)	Interferes positively or negatively with immunochemical assays
Contaminations	
Anticoagulants	Interfere with K or Na analysis; EDTA and citrate form complexes with Ca, Zn, Cu, Mg; decrease activity of amylase and ALP; EDTA may induce pseudothrombocytopenia
Sodium fluoride (NaF)	Interferes with Na analysis
IV infusions	NaCl: increases S-Cl + dilutes other serum analytes; 5% glucose: increases glycemia + dilutes other analytes
Powder (gloves, catheters)	Contains zinc
Soaps, detergents	Contamination with P and Fe

PT- prothrombin time, APTT- activated partial thromboplastin time

Extravascular hemolysis (*in vitro*) can result from plenty of factors acting during or after blood sampling, particularly following ones:

- too long applying of tourniquet during phlebotomy;
- too high aspiration pressure (thin needle, patients with fragile RBC);
- aspiration of perivascular blood (vein perforation);
- collecting blood from intravenous lines placed distally from cubital veins;
- too intensive mixing and manipulation with non-clotted blood;
- storage at incorrect temperature (too low or high);
- long or too intensive centrifugation of blood;
- freezing of whole blood before separating of serum.

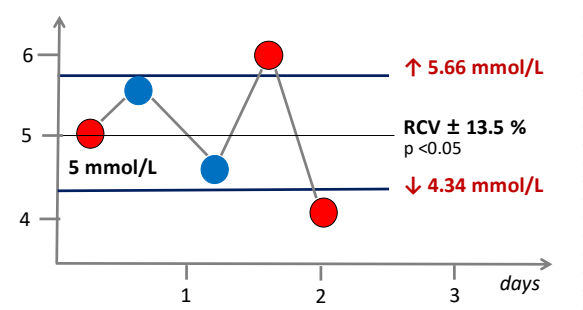
Lipemic sample results from increased content of TAG rich lipoproteins (VLDL, IDL), which change the transparent appearance of serum to turbid or even milky one. The high content of chylomicrons sometimes creates a creamy layer on the top of the serum. Frequent causes of lipemia are intake of food rich in fat and saccharides and/or alcohol, diabetes mellitus, chronic kidney disease, acute pancreatitis, drugs (steroids, estrogens, antiretroviral drugs) and some primary hyperlipidaemias. Lipemia affects laboratory methods that measure reflected light (nephelometry, immunoturbidimetry) but also coagulation tests with optical detection of fibrin.

Hyperbilirubinemia causes pathological coloration of serum (icteric) from pre-hepatic (orange-red colour), hepatic (lemon yellow) or post-hepatic (brown-green reasons. It may interfere with photometric biochemical methods.

Critical change of result

When interpreting repeated laboratory examinations, the physician must decide whether the result is clinically significantly different from the previous result. A useful tool in this decision making is so-called **critical change value** (reference change value RCV), the calculated parameter based on analytical variability (CV_a of a method in a particular laboratory), and biological variability (CV_b available internet databases). If two consecutive laboratory results differ from each other by more than the calculated RCV value, this difference is clinically significant and reflects an improvement or worsening of the patient's disease (INFO 1.5).

INFO 1.5 Reference change value – how does it work?



Value of reference change value is calculated by formula RCV = $2^{½}$ x Z x $[CV_a^2 + CV_b^2]^{½}$, where CV_a is analytical variation, CV_b is biological intra-individual variation, Z = 1.96 for 95% confidence interval. CV_a of most biochemical tests in current laboratories is low (below 5%) due to a high level of automation and standardization of assays.

The database of CV_b is available at http://www.westgard.com/biodatabase1.htm.

Common biochemical test cholesterol shows the analytical coefficient of variation CV_a = 3%, and biological intra-individual variation CV_b = 6%. Calculated reference change value RCV is 13.5 %, which means that interval between 4.34 – 5.66 mmol/L covers the values which could be influenced by analytical or/and biological variation. The result within this interval should not be considered as a clinically significant change.

An integral part of the interpretation of laboratory results is to assess whether they are consistent with the clinical finding in the patient. If so, a laboratory test is a further evidence that supports the clinical or supposed diagnosis. Otherwise, it is necessary to look for an explanation as to why the result does not correspond to expectations (e.g. sampling error, sample swap, interference). If repeated sampling confirms the previous result, the physician should consider the selected test (its sensitivity and specificity) or think about a different diagnosis.

1.7 Clinical assessment of diagnostic tests

When interpreting the results of any test, it is essential to know how the test behaves in health and disease. Sensitivity and specificity of the test are two features informing about the ability of the test to confirm or exclude the particular diagnosis.

Sensitivity (SN) is a measure of the incidence of positive results in persons known to have a disease. It is expressed as a percentage of true positivity (TP) in all individuals with the disease (true positivity and false negativity). Calculation of SN is following:

$$\mathbf{SN = TP/ (TP+FN) \times 100\%}$$

Specificity (SP) is a measure of the incidence of negative results in persons known to be free of disease. It is expressed as a percentage of true negativity (TN) in all individuals without the disease (true negativity and false positivity). Calculation of SP is the following:

$$\mathbf{SP = TN/ (TN+FP) \times 100\%}$$

An ideal diagnostic test with 100% sensitivity and 100% specificity does not exist in reality. There is almost always an overlap between results seen in health and in disease. For example, SP 90% means that 10% of disease-free people would be classified as having the disease on the basis of test results - **false positivity** (FP). Specificity 95% means that 5% of people with the disease would be **false negative** (FN) and 95% would be classified as having the disease. All tests generate false positivity and false negativity. When sensitivity increases, a test tends to decrease its specificity and vice-versa.

It depends on the purpose of the test using, whether it is desirable to maximize specificity or sensitivity (Figure 1.3). For screening the test with high sensitivity is suitable in order 'to catch' all cases of screened disease (comparable with shifting the cut-off value of the test to the right). The test used for confirmation of disease should have high specificity to be able to exclude all individuals without disease (comparable with shifting the cut- off value of the test to the left).

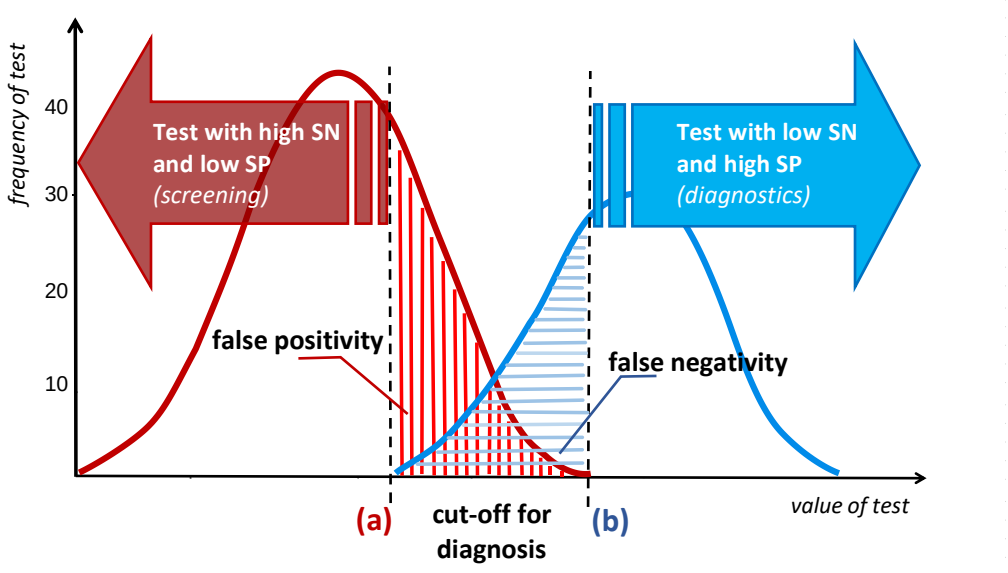


FIGURE 1.3 Different specificity and sensitivity of test depending on the purpose for which test is used. Cut-off for confirmation of disease, (b) cut-off for screening of disease

Another term defining the effectiveness of a test is the **predictive value** of its negative or positive result. The **positive predictive value** (+PV) represents a percentage of individuals with a positive test result who truly have the disease: **+ PV=TP/(TP+FP)**

The **negative predictive value** (-PV) similarly refers to a percentage of individuals with a negative test result who do not have the disease: **- PV=TN/(TN+FN)**

The test with high positive PV has low false positivity and is suitable for example for confirmation of the diagnosis or in a situation where a high number of false-positive results would lead to an extensive following investigation. The test with high negative PV has, by definition, few false-negative results, which is particularly important in the screening program, where it is essential not to miss an individual with the screened disease. Even a good laboratory test can have a poor positive PV and seems to be relatively useless in certain circumstances as for example, low prevalence of the disease in a population. However, if the test is used selectively on the population in which the prevalence of the disease is high, it may have excellent positive PV (Figure 1.4).

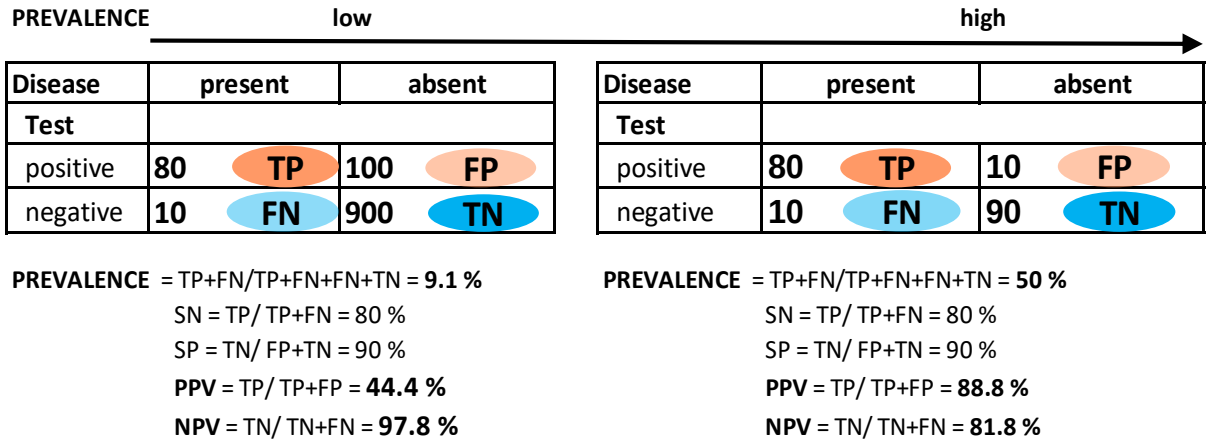


FIGURE 1.4 Influence of prevalence of disease on the usefulness of diagnostic test

Case studies and control questions

Case 1.1

A 35-year-old nurse, healthy, without subjective difficulties, who only takes birth control pills, had her blood taken in the morning after a night's service to check for hypercholesterolemia, which she found during a preventive examination. She put the blood in the refrigerator, later forgot about it, and remembered it at home over lunch when she woke up after a rest. The laboratory results from this blood, which was not tested until around 2:00 pm, were as follows:

Serum	Result	RI
Glucose	2.66	3.3 – 5.59 mmol/L
Urea	4.7	2.0 – 6.7 mmol/L
Creatinine (Crea)	84	44 – 95 μmol/L
Na^+	138	135 – 145 mmol/L
K^+	8.1	3.5 – 5.3 mmol/L
Cl^-	102	98 – 108 mmol/L
Total protein	75	64 – 83 g/L
Albumin (Alb)	48	36 – 50 g/L
Cholesterol (CH)	7.2	<5.0 mmol/L
TAG	2.7	<1.7 mmol/L

Task:
Identify pathological findings and explain their cause.

Case 1.2

The following serum biochemical results belong to a young woman who was hospitalized for a forearm fracture after a fall while skiing. The patient is stable and no remarkable anamnestic data have been reported. After blood collection at the emergency department, the nurse opened an underfilled biochemical tube and supplemented it with blood from the tube for a blood count.

Serum	Result	RI
Urea	6.4	2.0 – 6.7 mmol/L
Na^+	138	135 – 145 mmol/L
K^+	11.1	3.5 – 5.3 mmol/L
BIL	14	3 – 19 μmol/L
ALT	0.51	>0.60 μkat/L
ALP	0.19	0.58 – 1.75 μkat/L
Total protein	74	64 – 83 g/L
Alb	34	36 – 50 g/L
Ca^{2+}	0,6	2.1 – 2.7 mmol/L

Question: How would you explain pathological laboratory results?

Self-assessing questions

1. What type of sample would you send to a biochemical laboratory in a septic patient with coagulation disorders?
2. What is the effect of adding NaF to a blood glucose test tube?
3. Explain why it is important to follow the correct order of the tubes during blood sampling.
4. Explain the causes of false hyperkalemia in samples without visible serum hemolysis.
5. What is a critical difference and what is its significance in the interpretation of laboratory results?
6. Name examples of influenceable and non-influenceable biological factors that are the source of variability of results within an individual.
7. What characteristics should have a test used for screening purposes?

KEY INFORMATION

- ☑ Biochemical laboratory tests are used to support diagnosis, monitoring of treatment and follow-up of disease, assessing its prognosis, and for screening purpose.
- ☑ Any ordered laboratory test should be understood as a question about the patient's condition and each result as an answer.
- ☑ The choice of a laboratory test is the responsibility of the physician, who will interpret the result in the context of the clinical findings of the individual patient.
- ☑ The process of laboratory testing can be divided into three phases: preanalytical, analytical, and postanalytical.
- ☑ Preanalytical phase is a set of all administrative and practical steps during sampling, labelling, transport, and processing of samples preceding laboratory analysis itself.
- ☑ Subjects responsible for adhering to the preanalytical phase conditions are physician, nurse, or phlebotomist, the patient, and laboratory.
- ☑ Errors in the preanalytic phase complicate laboratory work and delay medical decisions about the patient, leading to unwanted or unnecessary testing, delayed reporting of results, and misinterpretation.
- ☑ The most common sample examined in a biochemical laboratory is venous blood collected after proper patient preparation.
- ☑ Reference intervals are used to assess the significance of a particular laboratory result; the individual is compared with the findings in the reference healthy population, generally of the same sex and age.
- ☑ A normal laboratory result does not exclude the presence of a disease and vice versa, a pathological result does not automatically mean the presence of a disease.
- ☑ The laboratory result is influenced by biological variability factors that cause differences in findings between individuals and in one individual. The fluctuation of intra-individual values has an impact on long-term patient monitoring.
- ☑ Critical change/difference of a result is a calculated parameter helping to determine whether the change in consecutive results is clinically significant or whether it is merely the result of random variability.
- ☑ An ideal laboratory test does not exist in reality. Sensitivity, specificity, and predictive values are features of the clinical performance of a test.
- ☑ Laboratory data is never a substitute for good physical examination and patient history. Treat the patient, not the laboratory results.

2

Water and electrolyte disorders

CONTENT

The term electrolyte is used in medical practice especially in relation to two most abundant cations present in biological fluids: sodium (Na^+) and potassium (K^+). Electrolytes solve in water solutions and dissociate into cations (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (e.g. Cl^-, HCO_3^-). Beside an absolute amount of ion in the particular body fluid, volume and distribution of body fluids strongly influence concentrations of plasma ions, particularly of sodium. Therefore, understanding of water balance is essential for interpretation of ion results. Volume of body fluid and concentration of ions are normally maintained within very narrow limits, despite wide variations in dietary intake, metabolic activity, and environmental factors. Disturbances of water and electrolyte balance are common in many clinical settings and they frequently require an urgent management including biochemical investigation.

This chapter informs about:

- distribution of water, sodium and potassium in body compartment and their regulation;
- causes of hypernatremia and hyponatremia;
- causes of hyperkalemia and hypokalemia;
- laboratory testing in diagnostics of water and ion disturbances.

2.1 The basic physiology

The **total body water** (TBW) in adults is about 60% of body weight, representing about 48 L in 80 kg man. Water content in a human body decreases with age and increasing body fat content as well, ranging from about 50% in obese people to 70% in lean people (Figure 2.1). Body water is distributed between two major compartments - **intracellular** fluid (ICF, two thirds of TBW, e.g. 32 L in an average male) and **extracellular** fluid (ECF, one third of TBW, e.g. 16 L), which are in constant equilibrium. About 25% of extracellular fluid is in the **intravascular** compartment, e.g. blood plasma (4 L); the other 75% in the **interstitial** fluid (e.g. 12 L).

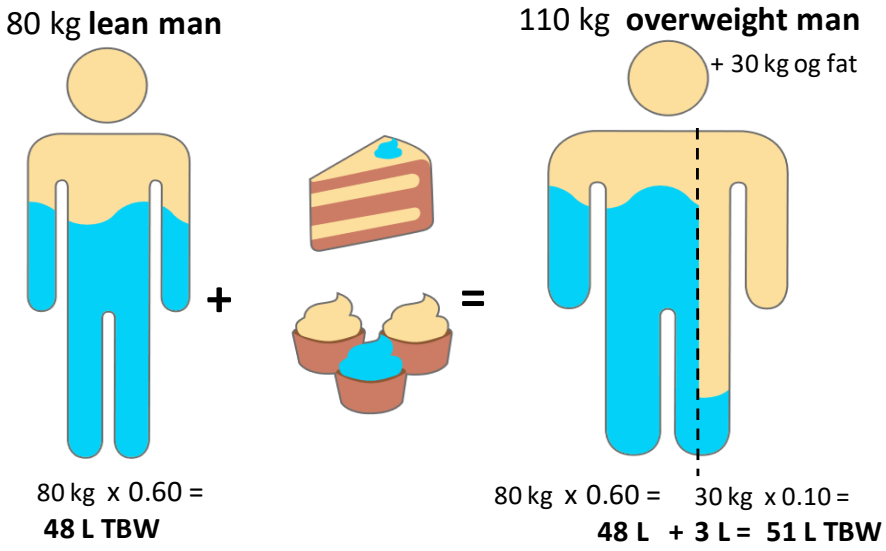


FIGURE 2.1 Influence of body fat on total body water

External water balance is influenced by intake and output of water. Water intake matches water losses in a steady-state situation. Sources of water are fluids in drinks, food and water from cellular metabolism. Obligatory sources and losses of water are summarized in Table 2.1. Water intake is controlled by the sense of thirst, but may be very variable as consequence of social, geographical and individual habits. The minimal daily water intake necessary for maintenance of water balance is approximately 1000 mL/24 h. The minimal volume of urine which is necessary for excretion of waste metabolic products (so called osmotic load) in average adult with normal kidney function is about 500 mL/24 h. The higher volume is needed in individuals with any loss of renal concentrating ability.

TABLE 2.1 AVERAGE DAILY WATER INTAKE AND OUTPUT OF A NORMAL ADULT

Intake	mL	Output	mL
Water in drinks	1 500	Urine	1 500
Water in food	750	Feces	50
Metabolic water	250	Expiration, perspiration	950
Total	2 500	Total	2 500

The distribution of water between compartments, i.e. **internal balance** is influenced by two important factors: effective osmolality and colloid osmotic pressure. Water freely moves across cell membranes between intracellular and extracellular fluid and that movement is determined

by **osmotic content** of both compartments. Under physiological circumstances both spaces have the same content of osmotically active substances, thus the same osmotic concentration.

Any change in the content of solved substances (solutes) within one compartment generates an **osmotic gradient**, causing movement of water between ICF and ECF in order to restore **izotonicity**. Solutes such as urea that freely diffuse across cell membranes have little or no effect on water shifts (little or no osmotic activity), whereas solutes that are restricted primarily to one compartment, such as in ECF Na^+ in ECF or glucose (Glc) in case of insulin deficiency, have the highest osmotic activity. Movement of water molecules from body compartment with low osmolality to a compartment with high osmolality results in increase or decrease of cellular volume. Brain cells are most sensitive to those changes, because they are closed in the limited space of calva. That is the reason why clinical signs of hypoosmolar or hyperosmolar syndromes are mainly neurological.

The movement of water and solutes with small molecules across the capillary wall (between intravascular and interstitial compartments) is a result of net effect of hydrostatic and oncotic pressures. **Colloid osmotic** (=oncotic) **pressure** is a force contributed by plasma proteins, which retains water and small molecules inside capillary walls. Normally, plasma proteins have a small osmotic effect which is counteracted by vascular hydrostatic forces that drive water out of the plasma. When plasma protein concentration is low, e.g. in hypoalbuminemic patients, extravasations of water causes oedemas or increased formation of ascetic fluid.

Osmolality and tonicity of body fluids

Osmolality of body fluid is maintained in the narrow range of 275 – 295 mmol/kg. Serum osmolality informs about situation in ECF and can be assessed by three similar characteristics:

- measured osmolality,
- calculated osmolality,
- effective osmolality or tonicity.

Measured osmolality is examined by laboratory devices - osmometers and reported in units mOsm/kg or mmol/kg. It informs about content of all osmotically active substances; however, it does not distinguish between total osmotic concentration and those solutes, that contribute effective osmolality (= tonicity), a force that causes water diffusion across cell membranes.

In clinical practice is possible to estimate osmolality by calculation based on known substance concentrations of sodium, glucose and urea (in mmol/L), parameters most contributing to the physiological osmolality:

$$\textbf{Osmolality (mmol/kg)} \approx 2 \times [Na^+] + [\text{glucose}] + [\text{urea}]$$

The difference between measured and calculated osmolality, so called **osmolar gap**, is normally less than 10 mmol/kg. The higher osmolar gap signalizes possible presence of osmotically active substance, which has not been included in the calculation and may be of:

- exogenous origin (e.g. ethanol, methanol, ethylene glycol, some drugs or their metabolites) or
- endogenous origin (e.g. ketone bodies, lactic acid, degrading metabolic product of catabolism).

Effective osmolality (= tonicity) is a term describing osmotic activity of substances, which are bound in the certain compartment and cause movement of water across cellular membranes. In general, the tonicity of a solution predicts the effect on cell volume and depends on the relative concentrations of non-penetrating solutes in the cell and the solution. Among physiological components of ECF particularly sodium and its accompanying anions influence the effective osmolality ECF. Pathological hyperglycemia in diabetic patient increases effective osmolality in ECF, as glucose cannot enter cells due to an absolute or relative insulin deficiency.

The situation results in water diffusion from ICF to ECF until osmolality in each compartment equalizes. Normally, urea is a free diffusible solute, thus it does not create difference between osmolality of extracellular and intracellular space. However, a rapid removal of urea during haemodialysis may develop brain oedema in a patient as a consequence of shift of water into cells, where an increased concentration of urea is still persisting.

Unlike osmolality or osmolarity, tonicity has no units. It is a comparative term that predicts changes in cell volume at equilibrium after exposure of the cell to a solution. Tonicity is not measurable; it can be estimated roughly as follows: $2 \times [Na^+] + [Glc]$. For additional information, see also INFO 2.1.

INFO 2.1 Osmolality, osmolarity, tonicity

The terminology associated with calculated and measured osmotic activity is often confusing and is not consistent in the medical literature. Osmolality is a measure of the number of osmotically active particles in a solution. Unit of osmolality is osmole (osmol or Osm) which is defined as one mol of any non-dissociable substance.

In medical practice an osmotic concentration is typically expressed in either milliosmoles/kilogram of solvent (mOsm/kg) - referred to as osmolality, or in milliosmoles/liter of solution (mOsm/L) - referred to as osmolarity. The selection of which term to use (osmolality or osmolarity) depends on how the osmotic concentration was derived. When being measured by osmometers in clinical laboratories, the concentration appropriately referred to as osmolality. Bedside calculation by clinicians using the patient's laboratory data which are measured and expressed in terms of solution (mmol/L), and hence the term osmolarity is appropriate. Because one litre of distilled water weights exactly one kg, both terms - osmolality and osmolarity are used in medicine.

For an ideal solution, osmolarity equals its molarity times a dissociation factor, the number of ions formed from one particle of the solute when placed in water: osmolarity (mosm/L) = molarity (mmol/L) x dissociation factor (n). Because of n=1 for Na^+, Cl^-, K^+, Ca^{2+}, urea, glucose, units mOsm/kg and mmol/L may be used interchangeably in medical settings.

The tonicity of a solution predicts the effect of the solution on cell volume at equilibrium and depends on the relative concentrations of non-penetrating solutes in the cell and the solution. Under physiological conditions the most important is the osmotic pressure of sodium that causes the movement of water out or into cell. Tonicity is not measurable. ECF and ICF both have equal osmolality, they are isotonic.

Hormonal regulation of ECF volume and osmolality

Osmolality and volume in ECF are under control of following hormonal systems: antidiuretic hormone (ADH or vasopressin), renin-angiotensin-aldosterone system and natriuretic peptides.

Antidiuretic hormone takes part in maintaining of ECF osmolality by the mechanism of excretion or retaining of water (=solute free) in collecting ducts of kidney. Water deprivation leads to an increase in osmolality of ECF including plasma. Even a small (e.g. 1 – 2%) increase of plasma osmolality is detectable by **osmoreceptor** in the hypothalamus and results in two physiological responses; stimulation of thirst and releasing of antidiuretic hormone (ADH, or vasopressin) from the posterior pituitary gland. Secretion of ADH increases steadily and its level ensures maximum antidiuresis at plasma osmolality ~290 mmol/kg. ADH activates specific V2 receptors in cells of collecting ducts and increases their water permeability by transfer of water channels (aquaporin 2) to the apical surface of tubular cells. Renal water retention and formation of concentrated urine increases blood volume and together with increased intake of water restores osmolality at normal value (Figure 2.2). Urinary osmolality raises above 600 mmol/kg.

However, if ECF osmolality rises due to increased concentration of freely diffusing solutes (urea, glucose), ICF osmolality also increases and osmoreceptors are not stimulated. The secondary effect of ADH is constriction of arterioles which increases arterial pressure. Decrease in osmolality of ECF supresses ADH secretion, kidneys produce diluted urine with low osmolality and after water excess has been excreted, plasma osmolality normalizes.

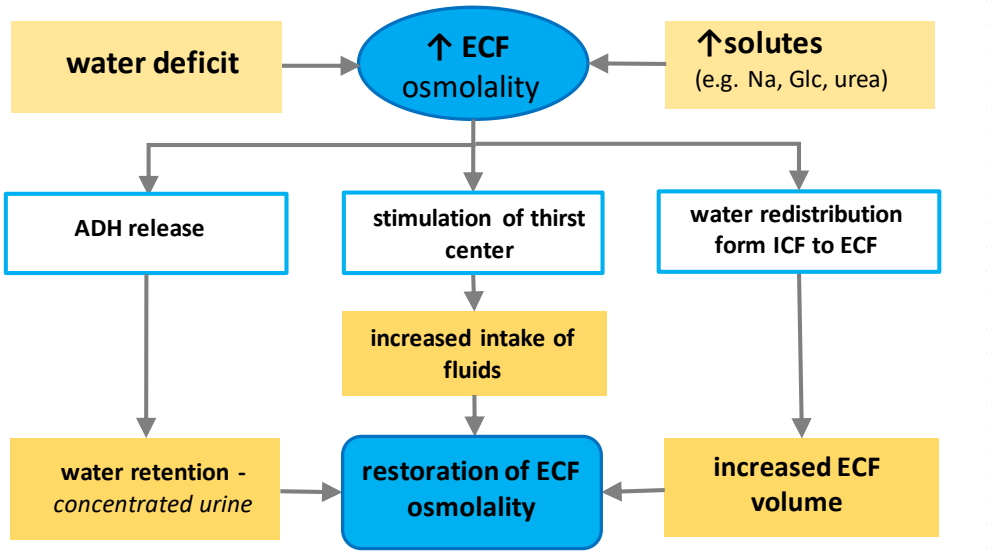


FIGURE 2.2 Regulation of ECF osmolality - major physiological responses following a rise of osmolality

There are also **non-osmotic stimuli** increasing ADH secretion, particularly hypovolemia, pain, stress, nausea and some drugs (Table 2.2). Mechanism controlling osmolality changes also volume of water in body compartments including circulating volume and consequently blood pressure. Osmotic regulation and mechanism controlling water volume cooperate together. If the circulating volume decreases by more than in 10%, hypovolemia becomes a more powerful stimulus than osmolality and ADH is secreted regardless of the existing hypotonicity in the ECF.

Renin-angiotensin-aldosterone system (RAAS) is the hormonal mechanism regulating the extracellular volume (e.g. blood plasma, lymph and interstitial fluid), as well as blood pressure by constriction of the arteries and blood vessels. **Renin**, also called angiotensinogenase, is an enzyme (called angiotensinogenase) secreted by the kidneys from the specialized cells in the juxtaglomerular apparatus. The secretion of renin is stimulated by the following factors:

- When a fall in arterial blood pressure is detected by baroreceptors in the arterial vessels.
- When a decrease in sodium chloride is detected in the distal tubules by the macula dense in the juxtaglomerular apparatus.
- When sympathetic nervous system activity is detected through beta1-adrenergic receptors.

TABLE 2.2 FACTORS INFLUENCING ADH SECRETION

Stimulating factors	Inhibiting factors
↑ effective osmolality in ECF	↓ effective osmolality in ECF
Hypovolemia, hypotension (decrease in >10%)	Hypervolemia
Stress, pain, nausea	
Drugs: e.g. opiates, carbamazepine, nicotine, antidepressants, chloropropamide	Alcohol, β-adrenergic agonists

Aldosterone, the end part of RAAS, is a mineralocorticoid produced within adrenal cortex and its release is influenced by angiotensin II or directly by hyperkalemia. The main effect of aldosterone is on the distal renal tubule where it enhances the reabsorption of sodium in exchange for potassium and hydrogen ions. The raising plasma concentration of Na^+ increases osmolality and ADH release. Extracellular retention of sodium together with isosmotic water retention increases or normalizes volume of ECF. Aldosterone secretion is also influenced (but not regulated) by ACTH and directly and indirectly, by atrial natriuretic factor (ANF).

The **natriuretic peptide** family consists of three main peptides, each with unique tissue expression and regulation: atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). The stimulus for natriuretic peptide release is an increased myocyte stretch. ANP is secreted primarily from the cardiac atria in response to increased atrial wall tension, reflecting volume and pressure loads. BNP is secreted primarily from the cardiac ventricles in response to increased left and/or right ventricular volume and pressure loads. Natriuretic peptides produce opposite effects to those of RAAS. They reduce renal Na^+ reabsorption (cause natriuresis), inhibit production of renin and aldosterone, and act as vasodilators. Natriuretic peptides have received attention as cardiac markers, especially of congestive heart failure.

Sodium is the major osmotically active ion in ECF. Changes in sodium reabsorption and excretion regulate plasma volume, but not plasma osmolality. Deficiency or excess of total body content of Na^+ causes depletion or overload of ECF volume. Despite different physiological mechanisms controlling water and sodium balance, they need to be considered together during assessment and understanding of a patient's natremia and volume status. Nevertheless, from didactic reason, disorders of fluid volume and sodium will be discussed separately.

2.2 Disorders of fluid volume

Lack or excess of body fluids results in **dehydration** and **hyperhydration,** respectively. The main causes of depletion and excess of water involve inadequate intake, excretion and renal or extra-renal losses. Losses or gains of **pure water** are distributed across all compartments due

to free movement of water between ECF and ICF. On the contrary, losses or gains of **isotonic fluid** (water + Na^+) are limited to ECF (Figure 2.3). For that reason, it is more urgent to replace losses of isotonic fluids than losses of pure water. Similarly, circulatory overload is more likely present after excessive administration of isotonic Na^+ solution than after infusion of isotonic glucose, which after its metabolism is pure water. Some disorders of water balance may be associated with pathological serum [Na^+], but it is not the rule. For example, acute loss of isotonic fluid (e.g. plasma, blood, interstitial fluid) may cause severe hypovolemia and Na^+ depletion with shock, but patient's serum [Na^+] may be normal or even raised.

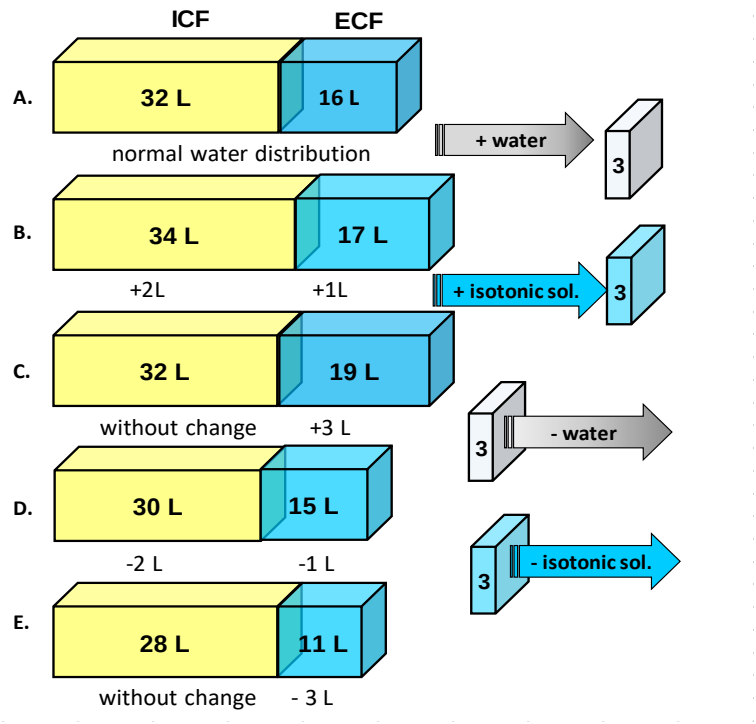


FIGURE 2.3 Different effects of fluid changes on the body´s fluid compartments:
A. normal water distribution
B. gain of 3 L water,
C. gain of 3 L of isotonic saline
D. loss of 3 L of water
E. loss of 3 L of isotonic saline
The volumes shown relate to a 80 kg adult.

Dehydration generally means decrease in ECF volume, particularly low **effective circulating volume,** which in turn causes decreased organ perfusion and further clinical sequelae. Common causes of volume depletion are listed in the Table 2.3. For most frequent clinical signs read INFO 2.2.

TABLE 2.3 CAUSES OF VOLUME DEPLETION

Extrarenal loss	Example	Renal loss	Example
Bleeding	GI bleeding, trauma, surgery	Acute renal failure	Diuretic phase of recovery
Dialysis	Hemodialysis, peritoneal dialysis	Adrenal insufficiency	Hypocorticism, Hypoaldosteronism
GI losses	Vomiting, diarrhoea, nasogastric suction	ADH deficiency	Diabetes insipidus (trauma, tumour, infection)
Skin losses	Burns, excessive sweating, exfoliation	Osmotic diuresis, Diuretics	Diabetes mellitus, Diuretics: loop and thiazide
3rd space losses	Intestinal lumen, intra-, retroperitoneal	Salt-wasting renal disease	Interstitial nephritis Medullary cystic disease

Hyperhydration generally refers to expansion of ECF in all cases of fluid gains and leads to **volume overload** and **edema**. The disturbance is sometimes more complex, as excess of water (and ions) may

affect all body compartments as a whole, e.g. total body water, or only one of them, e.g. plasma or interstitial fluid. ECF volume expansion typically occurs in heart failure, cirrhosis, renal failure, syndrome of inappropriate ADH secretion and in pregnancy. An increase of total body sodium is the key pathophysiological event, which increases osmolality in ECF and triggers compensatory mechanisms that cause water retention. Serum sodium concentration can be high, normal or low in volume overloaded patients despite the increased total body Na content.

INFO 2.2 Symptoms of hypovolemia

Mild volume depletion (<5%) is accompanied with only diminished skin turgor (tested at the upper torso), dry armpits, sense of thirst in patient and oliguria. Dry mucosa membranes do not always correlate in elderly and in mouth-breathers.
Volume depletion by 5 – 10% has typically signs orthostatic tachycardia and/or hypotension, except low skin turgor. When ECF volume falls by 10%, signs of shock can occur: tachypnea, tachycardia, hypotension, poor capillary refill, and anuria.
Laboratory signs: low urinary concentration of Na^+, ratio of urinary Na^+/K^+ excretion below 1.0, increase in serum urea>creatinine, increase in haematocrit.

Laboratory diagnostics of water balance disorders is only a supplement to the detailed clinical investigation of a patient. Dehydration and hyperhydration usually manifest as hyperosmolar or hypoosomolar syndromes and the laboratory tests which may be helpful in their differential diagnostics are listed in the Table 2.4.

TABLE 2.4 LABORATORY FINDINGS IN WATER BALANCE DISORDERS

Dehydration	Hyperhydration
↑Na^+ connected with ↑osmolality ↓U-Na^+ < 30 mmol/L - extrarenal losses U-Na^+/K^+ <1.0 - secondary hyperaldosteronism ↑urea >↑creatinine - sign of prerenal kidney injury ↑ TP, albumin, HTK, Hb - hemoconcentration	Serum [Na^+] - low informational value ↓ Na^+ connected with ↓osmolality ↓ TP, albumin, HTK, Hb - hemodilution

2.3 Disorders of sodium balance

Basic physiology

The total body sodium pool in average adult man is about 3 500 mmol. Approximately 75% is found in ECF, the remaining 25% is found in bone and soft tissues. The daily intake of sodium in Western diet (100 – 200 mmol or 2.3 – 4.6 g NaCl) is greater than recommended daily dose. An excess of Na^+ is excreted by kidney; obligatory losses into faces and sweat are lower than 10 mmol/day.

Concentration of sodium [Na^+] in serum representing ECF is **135 – 145 mmol/L,** while in ICF is only 4 – 10 mmol/L. The concentration gradient between both compartments is maintained by active transporting system of Na^+/K^+-ATP-ase. Serum [Na^+] does not necessarily reflect its total body content. In general, the concentration of any solute (including sodium) may change because of change of either amount of solute, amount of solvent, or both. Thus, serum [Na^+]

is more often abnormal as a result of changes of body water rather than of sodium losses or gains. Sodium has an essential role in maintaining the volume of ECF and accounts for the majority (>90%) of the osmotic activity in plasma. Serum [Na^+] is maintained by feedback loops involving kidney, adrenal gland and hypothalamus. The ability of kidney to conserve or excrete Na^+ is substantial in regulation of sodium homeostasis.

Hypernatremia and hyponatremia are **disorders of water balance,** rather than sodium balance, e^- losses or gains of sodium. Elderly people are more susceptible to sodium imbalance due to age-related decrease or decline in: total body water, thirst mechanism and renal function (maximal urinary concentrating ability, ability to excrete water load). Elderly people have also multiple comorbidities and take more drugs affecting renal function and serum [Na^+].

2.3.1 Hyponatremia

Hyponatremia is defined as serum [Na^+] below 135 mmol/L. This most common electrolyte abnormality occurs with frequency up to 30% in patients in hospitals and 5 – 10% in healthy elderly population, respectively. Severe hyponatremia (<125 mmol/L) is associated with increased mortality, morbidity and length of hospital stay. Hyponatremia is mostly a result of excess of body water as a consequence of non-osmotic stimulation of ADH secretion (dilutional hyponatremia) or may be caused by depletion of Na^+.

TABLE 2.5 CLASSIFICATION OF HYPONATREMIA BASED ON ECF VOLUME

Type	Causes	Comments
Hypervolemic hyponatremia	Congestive heart failure Liver failure (cirrhosis) Renal failure (acute, chronic) Nephrotic syndrome	TBW and sodium pool are increased but the increase of water is greater than that of sodium
Euvolemic hyponatremia	SIADH Adrenal and thyroid deficiency Primary polydipsia Low intake of solutes in food	TBW increases while total body sodium is normal/near normal U-Na^+ >30 mmol/L U-osmolality >100 mmol/kg
Hypovolemic hyponatremia	GI losses - vomiting or diarrhoea Skin losses- burns, sweating Renal losses - diuretics, osmotic diuresis (diabetic ketoacidosis), aldosterone deficiency 3rd space losses - pancreatitis, peritonitis, rhabdomyolysis	Deficit of total body Na^+ and water but with relatively greater loss of sodium Cause hyponatremia, if replaced fluids are hypotonic compared with losses

Because sodium is the main contributor of serum osmolality, the majority of the patients with hyponatremia have also low osmolality - so called **hypotonic hyponatremia.** Patients with hyponatremia can be divided into three categories according to their ECF volume: with **hypovolemia, euvolemia, hypervolemia** (Table 2.5). The serum [Na^+] does not reflect total body sodium, which may be low, normal or increased, respectively.

The most frequent cause of euvolemic hyponatremia is syndrome of **inappropriate antidiuresis** (inappropriate antidiuretic hormone secretion, SIADH, Schwartz-Barter

syndrome). ADH secretion is inappropriately increased regardless effective serum osmolality or circulating volume. Hyponatremia in SIADH is due to renal water retention frequently combined with secondary sodium loss. Despite clinically undetectable expansion of ECF volume (patient has no edema), moderate increase in ECF volume is present and stimulates natriuretic peptide secretion resulting in natriuresis ((U-Na >30 mmol/L). Because of ADH activity, urine osmolality will be inappropriately high (usually >100 mOsm/L) which is one of the criteria required for a diagnosis of SIADH (INFO 2.3).

INFO 2.3 SIADH

DIAGNOSTIC CRITERIA:
1. Serum [Na^+] <134 mmol/L and serum osmolality <280 mmol/kg;
2. U-osmolality >100 mmol/kg or U-Na >30 mmol/L;
3. Euvolemic patient - no clinical signs of dehydration or hyperhydration;
4. Normal cardiac, renal, hepatic function, adrenal, thyroid and, no diuretic use.

ETHIOLOGY:
1. Malignancies: lung (especially small cell lung carcinoma), pancreas, duodenum, head and neck;
2. CNS disturbances: tumour, stroke, infection, trauma, surgery;
3. Pulmonary disorders: pneumonia, tbc, mechanical ventilation, chest surgery;
4. Drugs: SSRI, other antidepressants, desmopressin, oxytocin, nicotine, opiates, NSAID, ACEI, ect;
5. Major surgery: anaesthesia, pain and stress - non-specific but potent stimuli for ADH secretion in hospitalized patients;
6. Others: porphyria, AIDS, sclerosis multiplex.

Serum osmolality is decreased in all above mentioned cases. However, hyponatremia may also occur with normal or even high osmolality if serum contains any additional osmotically active substances, such as glucose in diabetic patient, urea, ethanol, mannitol or some other toxins. All mentioned molecules increase osmolality in ECF and cause shift of water from the cells to EC compartment, resulting in a dilution of serum [Na^+]. Those conditions are referred as **non-hypotonic or false hyponatremia**.

Pseudohyponatremia is serum [Na^+] below the reference range due to artificial cause. It is associated with extremely **high concentration of lipids or proteins** in the blood specimen, which cause decrease of water content of plasma (normally approximately 93%). Despite normal [Na^+] in water phase of serum, some methods measure concentration of ions in whole plasma/serum volume and yield false low result. Another cause of pseudohyponatremia is *in vitro* **hemolysis**. When red blood cells lyse, their intracellular content of water and ions is released into plasma. Lower concentration of Na^+ in RBCs results in decrease of serum [Na^+].

Clinical consequences of hyponatremia

Hyponatremia represents a **low effective osmolality (= hypotonicity)** in ECF, which causes movement of water into cells with higher osmolality compared to ECF and followed by increase of cellular volume. Predominantly neurological symptoms of hyponatremia are caused by brain edema and increased intracranial pressure. Clinical symptoms of hyponatremia depend on rapidity, and intensity of decrease in natremia. For example, slow fall of serum [Na^+] from 140 to 120 mmol/L can be asymptomatic. In contrary, the rapid fall of serum [Na^+] from 135 to 125 mmol/L may have dramatic clinical symptoms. Lack of clinical symptoms in some patient with hyponatremia is a consequence of adaptive changes occurring in brain after onset of

hyponatremia. Brain cells actively reduce the number of their osmotically active particles (mostly potassium and organic ions) in an attempt to reduce water shift and volume of cells. This process takes up to 48 h, hence the reason for using that threshold to distinguish acute (< 48 h) from chronic hyponatremia (> 48 h or unknown duration). Distinguishing between acute and chronic hyponatremia influences the treatment mode, particularly its rapidity and intensity (INFO 2.4). Because of poor correlation of symptoms with hyponatremia is necessary to monitor frequently serum and urine electrolyte during treatment.

INFO 2.4 Basic principles of therapy in hyponatremia

Treatment of hyponatremia depends on its severity (mild-moderate-severe), duration (acute-chronic) and on extracellular volume. The therapy is aimed to decrease the brain oedema and prevention of brain stem herniation. Frequency of serum [Na^+] monitoring should be higher in the early phase after starting therapy (every 3 – 6 h during the first day), later at least every 12 – 24 h. Too rapid correction of hyponatremia may lead to osmotic dehydration and demyelination of brain. The basic principles used during therapy are:

- In patients with severe acute hyponatremia and significant neurological symptomatology an increase of natremia in 5 mmol/L by rapid hypertonic NaCl infusion usually ameliorates clinical symptoms. Increase in serum [Na^+] should be higher than 10 mmol/L during the first 24 h, 8 mmol/L during next days.
- Therapy of a mild or asymptomatic hyponatremia is based mainly on fluid restriction (in case of hypervolemia) and elimination of all possible/potential factors of existing hyponatremia (hypotonic solutions, medicines). In SIADH per oral administration of urea or NaCl is optional.
- In hypovolemic patients is necessary to normalize ECF volume with isotonic NaCl solution.
- Vaptans - nonselective inhibitors of V2 receptors (e.g. tolvaptan, conivaptan) are newer promising drugs in treatment of hypervolemic and euvolemic hyponatremia.

Laboratory diagnostics of hyponatremia

S-osmolality distinguishes between hypotonic and non-hypotonic hyponatremia.

U-osmolality <100 mmol/kg coexisting with serum hypoosmolality indicates, that kidneys excrete solute-free water (maximal dilution of urine) in attempt to normalize ECF hypoosmolality. ADH secretion is supressed.

U-osmolality >100 mmol/kg informs about an inappropriately high concentration of urine when the current serum osmolality is low. It is marker of the inappropriate ADH activity.

Urinary sodium concentration (U-Na^+) helps to distinguish between renal and extrarenal sodium losses. **U-Na <30 mmol/L** means an adequate response of kidney to hyponatremia which is caused by extrarenal sodium loss. It is also sign of secondary hyperaldosteronism in response to hypovolemia. The value **>30 mmo/L** indicates renal sodium losses, caused either by mineralocorticoid deficiency (including Addison´s disease), diuretic therapy, renal disorders (salt wasting tubulopathy) or prerenal kidney injury due to hypovolemia and hypoperfusion. In the last case patients have oliguria and increased nitrogen compounds (urea, creatinine, uric acid).

S-Cl and **HCO_3^-** concentrations inform about presence of expected decrease of anions accompanying fall in S-Na in order to maintain electroneutrality in ECF.

TSH, liver function tests, cortisol are test necessary for exclusion of other causes of hyponatremia in diagnostics of SIADH.

2.3.2 Hypernatremia

Hypernatremia is serum [Na^+] >145 mmol/L, severe hypernatremia is defined as a serum [Na^+] >155 mmol/L. Hypernatremia is most frequently caused by deficit of water in relation to sodium stores in the body (dehydration, negative water balance). Another cause is excessive sodium intake in form of hypertonic intravenous solutions and drugs, or retention of sodium due to mineralocorticoid excess (positive sodium balance). In most situations, the cause of hypernatremia will be apparent from clinical settings. The highest risk of hypernatremia occurs among:

- infants and elderly people who cannot maintain adequate fluid intake without assistance;
- people with impaired mental status who are unable to ask for water;
- people with uncontrolled diabetes;
- people with impaired thirst mechanism;
- hospitalized patients receiving hypertonic infusions, tube feedings, osmotic diuretics, lactulose or those on the mechanical ventilation.

Clinical diagnostic evaluation of patients with hypernatremia should be focused on:

- **detailed personal history** (e.g. peroral or parenteral sodium intake, polyuria, increased or in opposite, missing sense of thirst, hypertension, muscle weakness) and
- assessing **circulating volume** status which direct clinicians to the common causes of hypernatremia with hypovolemia, euvolemia or hypervolemia (Table 2.6).

TABLE 2.6 CLASSIFICATION OF HYPERNATREMIA BASED ON ECF VOLUME

Type	Causes	Findings
Hypovolemic hypernatremia	Renal losses: loop diuretics, osmotic diuresis (mannitol, hyperglycemia, hypercalcemia), renal disease with impaired concentrating ability (diabetes insipidus central and nephrogenic), GI losses (substituted with salt solutions): vomiting, diarrhoea Skin losses: excessive sweating, heat stroke, burns Decreases intake of water	U-Osmolality 300 – 600 mmol/kg U-Na^+ >30 mmol/L U-Osmolality >600 mmol/kg U-Na^+ <30 mmol/L
Euvolemic hypernatremia	Renal losses: central and nephrogenic diabetes insipidus, drugs (e.g. lithium, aminoglycosides, amphotericin B) Extra-renal losses: respiratory tract-tachypnea, skin-fever Decreased water intake: disorders of thirst perception, inability to access water (comatose pts, disabled pts, children, lack of water)	Initial stage of hypervolemic hypernatremia ↑ECF-osmolality in 2% normally increases sense of thirst)
Hypervolemic hypernatremia	Iatrogenic: Hypertonic fluid administration, enteral feeding, hypertonic haemodialysis, salt laxatives) Mineralocorticoids excess (Conn´s disease) Glucocorticoids excess (Cushing´s sy, ectopic ACTH) Acute salt poisoning (salt tablets, sea water)	Personal history ↓ S-K^+, hypertension ↑ aldosterone/renin ratio ↑ U-cortisol/24 h

Laboratory diagnostics of hypernatremia

Following laboratory tests are helpful in differential diagnostics of hypernatremia:

S-osmolality: In hypernatremia it is always high, however, comparison of measured and calculated osmolality (osmolar gap) may reveal presence of other osmotically active substances in some patients with signs of dehydration or hyperhydration (e.g. urea, glucose, ethanol).

U-sodium: In patient with hypovolemia helps in distinguishing of renal and extrarenal losses of fluids. U-Na^+<30 mmol/L indicates possible extra-renal losses or increased renal reabsorption due to secondary hyperaldosteronism. U-Na^+>30 mmol/L informs about possible renal losses (diuretics, osmotic diuresis, intrinsic renal disease).

U-osmolality is an indirect marker of ADH activity.
Value >800 (~500) mmol/kg is a sign of maximal concentrating ability in case extra-renal fluid loss. ADH secretion is also activated by existing hypovolemia.
Value <800 (~500) mmol/kg informs about submaximal concentrating ability of renal tubules and about possible renal fluid losses resulting in hypernatremia (diabetes insipidus, water diuresis).

U-Na^+/K^+ ratio is above 1 under physiological settings. Value U-Na^+/K^+<1 is a marker of hyperaldosteronism (primary and more frequently secondary).

Urea, creatinine, glycemia, K, Ca are additional tests, which are capable to reveal a cause of dehydration and hypernatremia.

Hemoglobin, hematocrit, total proteins: Usually increase or decrease as indirect markers of hemoconcentration (dehydration) and hemodilution (hyperhydration), respectively.

Diuresis: Specific condition associated with hypernatremia is **diabetes insipidus**, which is characterized by production of high volume of dilute urine. Patients present with **polyuria,** an excessive urine output, and secondary **polydipsia** (excessive thirst). **Hypernatremia** develops when urine losses exceed water intake. Two types of diabetes insipidus are distinguished: central and nephrogenic. Central type arises from failure of ADH secretion which occurs following a cranial injury or tumour. Nephrogenic diabetes insipidus is due to failure of renal collecting ducts to respond appropriately to ADH, mostly due to mutation of V2 receptors or aquaporin 2. Specific diagnostic tests for both types of diabetes insipidus are described in chapter 5 (Tubular function tests).

INFO 2.5 Clinical symptoms of hypo- and hypernatremia

Hyponatremia: Mild chronic hyponatremia (Na^+ >120 mmol/L, duration >48 h) is accompanied by mild symptoms: adynamia, attention and memory disorders, headache, depression, anorexia. Moderate symptoms are accompanied by mild acute (Na^+ >120 mmol/L, duration <48 h) or severe chronic hyponatremia (Na^+ < 120 mmol/L, duration >48 h): lethargy, unsteady gait, falls, confusion, anorexia, nausea, vomiting. Severe, acute hyponatremia (Na^+ <120 mmol/L, duration <48 h) is manifested by convulsions, delirium, stupor to coma, respiratory arrest, and death in brainstem hernia.
Hypernatremia: Thirst is the main symptom of hypernatremia (and hyperosmolality). Lack of thirst in conscious patient signalizes damaged thirst mechanism. Manifestations of acute hypernatremia are caused by dysfunction of brain cells during their dehydration (transfer of water from ICF to ECF): confusion, neuromuscular irritability, hyperreflexia, convulsions, and even coma. Cerebrovascular complications, e.g. bleeding or venous thrombosis, osmotic demyelinating syndrome are potential causes of mortality in patients with severe hypernatremia.

2.4 Disorders of potassium balance

Basic physiology

Physiology of potassium balance is easier and logical compared to sodium. The body of an average 70 kg adult person contains about 3600 mmol of potassium predominantly located in the ICF (98%). Potassium is the most abundant intracellular cation and contributes significantly to intracellular osmolality. The difference between ICF and ECF concentrations of K^+ cations [K^+] maintained by transporting activity of Na^+/K^+-ATP-ase creates an electrochemical gradient. The gradient strongly influences cell membrane polarization, which in turn modulates important cell processes, such as conduction of nerve impulses and muscle (including myocardial) cell contraction. Consequently, relatively small alterations in serum [K^+] can result in significant clinical manifestations. The tight control over both the intracellular and extracellular potassium pools is necessary because the movement of only 1% of the intracellular potassium to the extracellular compartment can stop the heart.

The physiological concentration of potassium in serum (S-K^+ or [K^+]) is maintained in the range 3.5 – 5.3 mmol/L. Potassium enters extracellular compartment only by 3 ways):

1. resorption from food in small intestine,
2. reabsorption in renal tubules,
3. redistribution/ shift from cellular pool (Figure 2.4).

The external potassium balance depends on its intake and intestinal resorption and renal handling which is under hormonal control of aldosterone. Potassium reabsorption in proximal tubules normally exceeds excretion in distal tubules and collecting ducts, so normal urinary excretion takes maximally 20% of the filtrated amount.

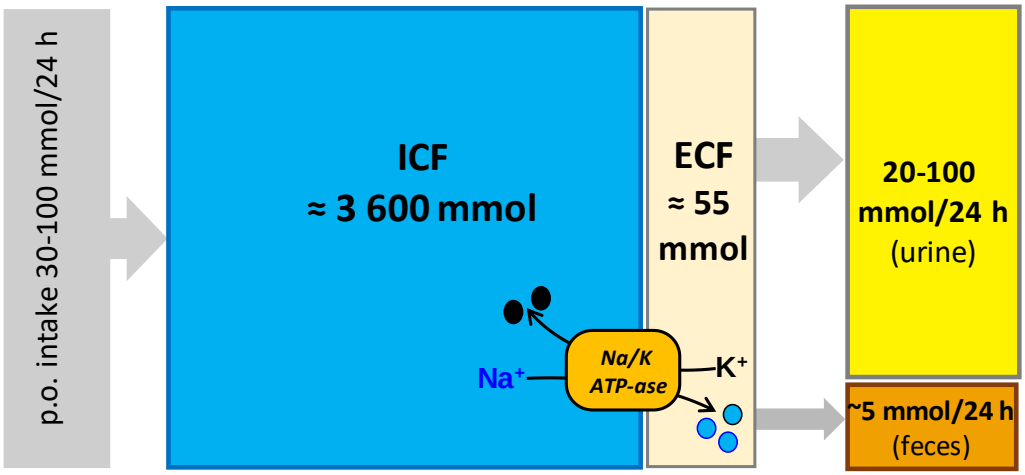


FIGURE 2.4 The intake, distribution, and renal excretion of potassium

An actual potassium ECF level is controlled by its intracellular and extracellular exchange, depending mostly on function of Na^+/K^+ pump. Factors that influence activity the enzyme pump include insulin concentrations, drugs with β-adrenergic activity and acid-base status (for details see also INFO 2.6).

INFO 2.6 Shift of potassium between ICF and ECF

INSULIN "pushes" potassium into cells; high concentrations of insulin thus lower serum [K^+] concentration, as within refeeding syndrome. Low concentrations of insulin, as in diabetic ketoacidosis, cause potassium to move out of cells, thus raising serum [K^+], sometimes even in the presence of total body potassium deficiency.

B-ADRENERGIC AGONISTS, especially selective β_2-agonists stimulate activity of Na^+/K^+ pump, whereas β-blockers and α-agonists promote movement of potassium out of cells.

ACID-BASE STATUS: Acute acidosis causes potassium to move out of cells, whereas acute alkalosis causes potassium to move into cells. For example, serum [K^+] increases by app. 0.5 mmol/L for every pH decrease in 0.1. Correction of acidosis/alkalosis will produce a decrease/increase in serum [K^+]. Metabolic acid-base disorders influence serum [K^+] more than respiratory. Nonetheless, serum potassium concentration should always be interpreted in the context of the serum pH (and HCO_3^- concentration).

NECROSIS/PROLIFERATION OF CELLS: Breakdown of cells (e.g. rhabdomyolysis, tumor lysis syndrome) results in release of potassium and increase of serum [K^+]. Rapidly growing tumour or therapy of severe megaloblastic anemia with vitamin B12 and folate may cause shift of potassium into cells and hypokalemia.

Potassium is one of the most frequent laboratory tests used by clinicians. However, there are many preanalytical influences affecting of serum [K^+]. Hemolysis causing an artificial hyperkalemia (pseudohyperkalemia) is the commonest observation when reviewing pathological appearance of serum (Table 2.7).

TABLE 2.7 CAUSES OF POSSIBLE ARTIFICIAL CHANGE OF POTASSIUM IN SERUM

Procedure	Causes	Comments
Blood sampling	Long use of tourniquet Too thin needle Inappropriate specimen tube Incorrect ratio of blood to anticoagulant	Mechanical or chemical hemolysis
Storage	High or low temperature	Activity of Na^+/K^+-ATP-ase is insufficient to hold K^+ in ICF
Transport	Careless handling with tube (vigorous mixing) Delayed transport (>1 hour after sampling)	Mechanical hemolysis
Centrifugation	Delayed centrifugation Centrifugation of unclothed blood specimen Repeated centrifugation + mixing of clotted sample	Mechanical hemolysis
Patient´s conditions	Intravascular hemolysis Chronic severe illnesses Platelet count >1000 × 10^9/L Leucocytosis >100 × 10^9/L	Increased fragility of RBC due to illness, lack of energy - ATP release of K^+ from platelets or WBC during clotting

The cause of hemolysis is usually fragility or breakdown of erythrocytes, which contain much higher concentration of potassium compare to surrounding plasma. An increased permeability of blood cells membrane (e.g. leukemia, polycytemia, severe leucocytosis and thrombocytosis, deficit of energy) may cause shift of intracellular substances including potassium into plasma even without visible hemolysis.

2.4.1 Hypokalemia

Hypokalemia is defined as the concentration of plasma/serum potassium below 3.5 mmol/L, value bellow 2.5 mmol/L represents severe hypokalemia. In hospitalized patients it occurs with frequency up to 20%, however clinically significant form of hypokalemia is seen in about 5% of patients. Based on fact that only 2% of potassium exist in ECF, serum [K^+] is not a good predictor of total potassium stores. Decrease in serum [K^+] of about 1 mmol/L indicates a total K^+ deficit of about 200 to 400 mmol. Patients with [K^+] <3 mmol/L typically have significant potassium deficit.

Hypokalemia is usually caused by excessive losses of K^+ in the urine or from the gastrointestinal tract. Less common causes include low intake or abnormal redistribution of potassium from ECF into cells (Table 2.8). Gastrointestinal K^+ losses during prolonged vomiting frequently combined with renal K^+ losses due to metabolic alkalosis and stimulation of aldosterone due to volume depletion.

TABLE 2.8 CAUSES OF HYPOKALEMIA

Causes	Examples
GI losses	Vomiting, diarrhoea, laxative abuse, gastric suction, intestinal tumour (villous adenoma of colon), malabsorption
Renal losses	Thiazide and loop diuretics, diuretic phase of acute RF, Mineralocorticoids excess (primary and secondary hyperaldosteronism, Cushing´s syndrome); Renal tubular acidosis, Bartter´s and Gitelman's syndrome
Transcellular shift	Alkalosis, insulin overdose, treatment of diabetic ketoacidosis, β-adrenergic agonists, familial periodic paralysis; Drugs: β_2-adrenergnic agonists (theofyllin), risperidone
Decreased intake	Anorexia nervosa, alcoholism

Renal handling of potassium is primary influenced by aldosterone, thus hypokalemia can arise when this hormone is present in excess. Primary hyperaldosteronism (Conn´s syndrome) results from overproduction of aldosterone, most frequently due to adrenal adenoma. Secondary hyperaldosteronism develops as a consequence of renin synthesis stimulated by hypovolemia and hypoperfusion of kidney. Bartter´s and Gitelman's syndromes, uncommon genetic disorders affecting renal tubules, are characterized by hypokalemia due to renal potassium wasting (they imitate therapy with high doses of loop or thiazide diuretics).

Clinical signs and symptoms

Mild hypokalemia (3.0 – 3.5 mmol/L) rarely causes symptoms. Serum [K^+] < 3 mmol/L generally causes muscle weakness and other mostly neuromuscular symptoms listed in the Table 2.9. Persistent hypokalemia usually impairs renal concentrating ability, causing polyuria with secondary polydipsia. Severe hypokalemia < 2.5 mmol/L deserves urgent treatment and careful monitoring because of the dangerous effect of rapid change in the serum [K^+]. Even severe hypokalemia may be asymptomatic!

TABLE 2.9 CLINICAL SIGNS OF HYPOKALEMIA

Disorder	Feature
Neuromuscular	weakness, constipation, confusion, hypotonia, paralytic ileus, leg cramps, respiratory failure, rhabdomyolysis
Cardiac	arrhythmias, potentiation of digoxin toxicity, ECG: ST depression, prolonged P-R interval, biphasic T wave
Renal	Impaired concentrating ability – polyuria, polydipsia
Metabolic	alkalosis

Laboratory testing of hypokalemia

U-K^+ informs about renal loss as a possible cause of hypokalemia, if concentration in random urine sample is >20 mmol/L. In contrary, concentration U-K^+ <20 mmol/L signalizes possible extra-renal losses or inadequate intake of potassium.

U-Na^+/U-K^+ <1 suggests hyperaldosteronism.

Acid-base parameters inform about possible transcellular shift in alkalosis; alkalosis is present also in hyperaldosteronism due to increased renal reabsorption of HCO_3^-.

S-Mg: Hypomagnesemia can cause an unexplained hypokalemia, as it affects tubular reabsorption of potassium.

U-Cl^-: Increased renal excretion together with potassium loss are regular indices of diuretic therapy.

Aldosteron/renin ratio measured in plasma distinguishes primary and secondary hyperaldosteronisms.

2.4.2 Hyperkalemia

Hyperkalemia is serum [K^+] >5.5 mmol/L resulting from a positive potassium balance (i.e. excretion lower than intake) or from an abnormal potassium distribution from cells to ECF. The kidneys normally excrete excess of K^+ in case of potassium overload, fractional excretion of potassium (FE-K^+) increases from normal 20% to 100%. Therefore, only transitory hyperkalemia develops after increased per oral or parenteral potassium intake in individual with normal renal functions. Sustained hyperkalemia usually implies diminished renal K^+ excretion.

Hyperkalemia due to total body K^+ excess is particularly common in oliguric states (especially in acute kidney injury) connected with rhabdomyolysis, burns, bleeding into soft tissue or gastrointestinal tract and adrenal insufficiency. In chronic renal failure, hyperkalemia is uncommon until the GFR falls to <0.25 mL/s unless dietary or IV intake of potassium is excessive (Table 2.10).

TABLE 2.10 CAUSES OF HYPERKALEMIA

Causes	Examples
Decreased excretion	Acute and chronic renal failure - mostly decreased GFR, oliguria; Renal tubular disorders - defects in tubular K^+ secretion; Drugs: K^+ sparing diuretics, ACE inhibitors, angiotensin receptor blockers, antagonists of aldosterone receptors, NSAIDs; Aldosterone deficiency: Addison´s disease, adrenalectomy; Ureter jejunostomy
Excessive intake	Parenteral infusion; Transfusion of long stored blood; Oral K^+ supplements - rare
Transcellular shift	Cell necrosis: crush and non-crush trauma, tumor lysis syndrome; Bleeding into soft tissue or gut; Catabolic states, burns (low ATP causes K^+ leakage from ICF); Systemic acidosis: DKA, lactic acidosis; Hyperosmolar syndrome (dehydration, extreme exercise)

Clinical symptoms and signs

The most significant clinical effect of hyperkalemia is decreased neuromuscular excitability, which negatively affects cardiac function; it disturbs cardiac conduction, causing cardiac arrhythmias or event cardiac arrest. Electrocardiographic changes become apparent at lower concentrations of potassium and are more prominent if another ion and acid-base disturbance is associated with hyperkalemia, e.g. hypocalcemia, hyponatremia, hypermagnesemia or acidosis (Table 2.11).

TABLE 2.11 CLINICAL SIGNS OF HYPERKALEMIA

Disorder	Findings	Serum [K^+]
Cardiac	ECG changes peaking of T-waves	>6 mmol/L
	loss of P waves, broad QRS	>7 – 8 mmol/L
	abnormal QRS, deep S waves	
	Arrythmia, ventricular fibrillation, cardiac arrest	>9 mmol/L
Neuromuscular	Weakness, muscle aches, Paralysis (begins on inferior extremities)	>7 – 8 mmol/L

Laboratory evaluation of hyperkalemia

In any case of laboratory confirmed hyperkalemia it is important to confirm that the serum [K^+] is a true reflection of *in vivo* concentration. Storage of whole blood in refrigerator at 4°C is widely practiced by physicians in the belief that it will aid the preservation of a specimen. This practice, however, can increase serum [K^+] without any evidence of hemolysis. The reason is inhibition of activity Na/K-ATP-ase by low temperature; potassium leaks from cells and increases serum [K^+] without visible hemolysis. Hyperkalemia in patient with extremely high WBC or platelets counts should be confirmed in freshly separated plasma, rather than serum. Having confirmed true hyperkalemia further laboratory tests usually follow:

Acid-base parameters exclude acidosis as a possible cause of distributional hyperkalemia;

S-Na, S-Cl are parameters used for calculation of anion gap;

U-K is indicator of hypoaldosteronism (low U-K), that is necessary to confirm by additional tests (cortisol, aldosterone, renin);

FE-K is an alternative parameter for assessing of renal potassium excretion. Value <0.04 is suggestive of hypoaldosteronism, value >0.20 reflects adaptational changes in tubules after decrease of GFR;

Creatinine, estimated GFR: Hyperkalemia is likely of renal origin, if GFR is less than 0.25 mL/s;

Myoglobin: If increased, it serves as evidence of muscle cells necrosis, resulting in shift of intracellular potassium.

If hyperkalemia is a result of increased body stores of potassium, then it is possible to roughly predict potassium excess from serum [K^+]. Although the relationship of plasma potassium to excess body potassium is highly variable, elevation of serum [K^+] by 1 mmol/L above normal will equate with a 200 mmol total excess.

Case studies and control questions

Case 2.1

71-year old retired man, formerly a miner, smoker (40 years 20 cigarettes/day), has been suffering from a persistent pulmonary infection with cough and copious sputum for the past 3 months. Finding on physical examination included clubbed fingers, dry and wet rales on both sides and signs of small pleural effusion, cardiac finding is without pathology. No oedemas or signs of dehydration are present. Biochemical results in serum and random urine sample summarizes the following table.

Serum	Result	RI
Urea	2.2	2.3 – 8.0 mmol/L
Creatinine	79	60 – 106mmol/L
eGFR	1.45	>1.5 mL/s
Na^+	118	135 – 145 mmol/L
K^+	4.3	3.6 – 5.3 mmol/L
Cl^-	87	95 – 105 mmol/L
Osmolality	260	275 – 295 mmol/kg
Urine (random sample)		
Na^+	54	60 – 220 mmol/24 h
Osmolality	370	50 – 1200 mmol/kg

Questions:

a. What is the probable cause of patient´s hypernatremia and hyperosmolality?
b. Name further tests necessary for confirmation of a provisional diagnosis.

Case 2.2

6-year old girl was admitted to a hospital after 3 days of diarrhoea and vomiting. On physical examination somewhat dry mouth mucosa, decreased skin turgor, hypotension 74/50 mm Hg, tachycardia 120/min. Laboratory finding on admission are in the following table.

Serum	**Result**	**RI**
Urea	19	1.8 – 6.7 mmol/L
Creatinine	107	32 – 88 mmol/L
Glucose	3.3	3.3 – 5.5 mmol/L
Na^+	168	135 – 145 mmol/L
K^+	3.3	3.6 – 5.3 mmol/L
Cl^-	91	95 –1 05 mmol/L
Urine (random sample)		
Na^+	<10	50 – 120 mmol/24 h
K^+	45	20 – 60 mmol/24 h
Osmolality	590	50 – 1 200 mmol/kg

Questions:

a. Calculate serum osmolality.
b. What is the probable cause of hypernatremia and hyperosmolality?

Self-assessment questions

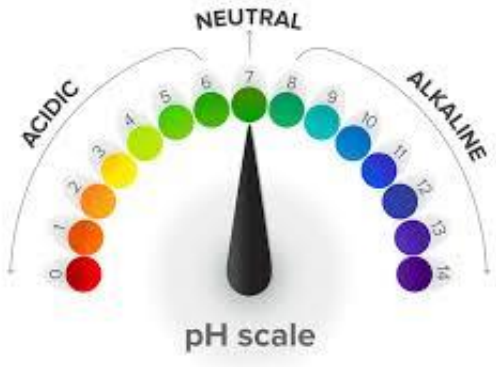
1. Explain difference between measured and calculated osmolality?
2. Name non-osmotic stimuli of ADH secretion.
3. What is the most frequent cause of hyponatremia?
4. Which biochemical test may be helpful in distinguishing of renal and extra-renal sodium losses?
5. Name common causes of pseudohyperkalemia.
6. Which biochemical test would you order in differential diagnostics of hyperkalemia in a patient with normal GFR?

KEY INFORMATION

- ☑ Losses and gains of water are distributed through both the ECF and ICF. Intake and output are controlled by thirst and vasopressin, respectively.
- ☑ In general, the ECF volume reflects total body volume and is normally controlled mainly by aldosterone.
- ☑ Osmolality of body fluids depends on the number of osmotically active particles (ions and molecules) dissolved in a kg of body water. In body compartments, water will move from a region with low osmolality to a region with high osmolality.

- ☑ Sodium content is the principal determinant of volume status and must be rigorously guarded. Sodium retention will expand ECF volume, and vice versa.
- ☑ Serum sodium concentration does not reflect sodium content in the body. It depends on sodium to water ratio in ECF.
- ☑ Hypernatremia and hyponatremia are more disorders of water balance than sodium balance.
- ☑ Acute losses of isotonic fluid usually cause hypovolemia, with clinical symptoms of hypotension, etc., but not hyponatremia.
- ☑ Inadequate intake of water or loss of hypotonic solution (excessive sweating) results in hypernatremia.
- ☑ Water retention in due to increased ADH activity or decreased renal excretion causes hyponatremia.
- ☑ Increased ADH secretion is a part of the metabolic response to trauma or surgery (non-osmotic stimuli). Hypotonic fluids should be used with caution during or after surgery, since there is a risk of acute water intoxication.
- ☑ Changes in plasma [K^+] are usually caused by changes in internal balance (acute shift of potassium between compartments) and/or changes in its external balance (intake and renal excretion).
- ☑ Hyperkalemia due to hemolysis of blood samples *in vitro* is caused by incorrect sampling procedure, manipulation with tubes after sampling and during transportation and storage.
- ☑ Hyperkalemia, especially acute one, can cause potentially lethal neuromuscular abnormalities, and should be treated urgently with appropriate biochemical and ECG monitoring.
- ☑ Persistent electrolyte disturbances require additional laboratory testing in blood and urine to identify specific abnormalities of renal and endocrine functions.
- ☑ Severe disorders of electrolytes could be life threatening and rapid communication of abnormal test results by laboratory staff to clinical teams is essential.

3



Acid-base balance disorders

CONTENT

The maintenance of hydrogen ion concentration [H^+] within narrow limits is one of the vital functions of living organisms. Several homeostatic mechanisms responsible for the maintenance of stable acidity (pH) of body fluids have evolved, including buffer systems, respiratory and renal activity. Laboratory investigation is a necessary part in assessment of acid-base disorders (ABD). This chapter focuses on:

- Physiological principles of acid-base balance regulation;
- Investigation of acid-base parameters including preanalytical requirements;
- Classification of ABD;
- Interpretation of laboratory results in ABD;
- Oxygen transport and disorders.

3.1 Basic physiology

Normal concentration of H^+ (or protons) $[H^+]$ in extracellular fluid is kept within narrow limits 36 – 44 nmol/L which is by five orders lower than concentration of other ions found in ECF. Because of extremely varying $[H^+]$ in nature it was impractical to deal with such a large range of concentration ($10^1 – 10^{14}$). For that reason, Danish chemist Sörensen invented the pH scale in 1909 (INFO 3.1). The physiological reference range for pH is **7.36 – 7.44** in ECF, pH of intracellular fluid is lower, but also strictly controlled. Values of pH in arterial blood lower than 6.8 and higher than 7.8 are considered incompatible with life.

Changes in pH influence more protein features, especially **ionization of amino-groups** and stability of hydrogen bounds which alter tertiary structure and consequently their biological functions, e.g. structural, transporting, enzymatic, hormonal, signalling, or receptor. An extreme change of pH can cause denaturation of proteins with entire loss of their biological activity. Despite the strict homeostatic control of pH in ECT, some exceptions exist for individual enzymes requiring specific pH for their optimal activity, for example pH 1.5 – 3 for pepsin or pH 10 for alkaline phosphatase.

Changes in pH influence **distribution and biological availability** of some cations, mainly calcium and magnesium, which are partially bound to proteins in biologically inactive state. Increase in $[H^+]$ causes release of those cations from proteins and concentration of their biologically active form increases. The decrease in $[H^+]$, thus alkalosis, results in opposite changes.

In addition, change in $[H^+]$ can affect state of **ionization** of large number both **small and large molecules** and alter their ability to pass across cell membranes. Pathological distribution those molecules within cells and between compartments may have adverse consequences.

INFO 3.1 Relationship between H^+ concentration and pH

pH is calculated by taking the negative logarithm to base 10 of the H^+ concentration (pH = - log $[H^+]$). Understanding of pH can be problematic for most of us probably because it reflects concentration of $[H^+]$ in logarithmic manner. What does the negative logarithm to base 10 actually mean?

1. The log to power 10 is the power to which 10 must be raised to produce that number. For example, log 100 = 10^2 = 2, log 1000 = 10^3 = 3, log 10000 = 10^4= 4, etc.
2. The pH scale is logarithmic; a change in pH of 1 is equivalent to a 10-fold change in $[H^+]$.
3. The pH scale is the inverse to the $[H^+]$. Thus, the lower the H^+ concentration, the higher the pH value. For example, if $[H^+] = 10^{-7}$ mol/L, pH = -log $[10^{-7}]$ =7.

Physiological pH = 7.7 represents $[H^+]$ 40 nmol/L. Change of pH in 0.3 means 100% change $[H^+]$; thus, decrease of pH to 7.1 means $[H^+]$ 80 mmol/L, at pH 6.8 is $[H^+]$ 160 mmol/L. On contrary, pH 7.7 represents $[H^+]$ only 20 mmol/L.

Sources of hydrogen ions

The normal metabolism produces daily in plenty of **aerobic or anaerobic** reactions an **excess of H^+**, which must be neutralized or eliminated in order to maintain acid-base balance. The relatively small amount of acids are ingested by food, however, the principal source of H^+

is **cellular metabolism** producing two main types of acids: **metabolic** („fixed, non-volatile") and **respiratory** ('volatile') acids.

Metabolic acids are derived mainly from:

- catabolism of proteins and oxidation of sulphur-containing amino acid (cysteine and methionine) or cationic amino acids (arginine, lysine);
- anaerobic glycolysis (pyruvate and lactate);
- anaerobic metabolism of fatty acids;
- catabolism of phospholipids and nucleic acids (phosphoric and uric acid).

During mentioned processes cells release into ECF 50 – 100 mmol [H^+] per day (~1 mmol/kg/day). Organic acids are reutilized in metabolism, while inorganic acids (sulphate, phosphate) need to be excreted by kidney. If those metabolic acids were not neutralized by buffers or excreted by kidney, then the [H^+] of blood would dramatically raise resulting to pH of less than 3.

Respiratory component of acid-base balance represents carbon dioxide - CO_2, produced by cells in end-stage reactions of saccharide and lipid degradation in the daily amount of 15 000 – 20 000 mmol (depending on physical activity). A small part of CO_2 reacts with water to form carbonic acid - H_2CO_3, which dissociate to HCO_3^- and H^+. The wasting majority of CO_2 (volatile acids) is eliminated from the body by lungs.

3.2 Acid-base homeostasis

The organism responds to deviations of pH, e.g. changes of [H^+] by three interrelated mechanisms: **buffering systems, respiratory and renal system.** The characteristic features of these complex processes are following:

1. Buffering systems in ECF and ICF response to pH change **immediately** (in minutes), but their efficiency is limited.
2. The respiratory system controls partial pressure of CO_2 (pCO_2) by changing in alveolar ventilation. That response begins almost immediately (in tens of minutes).
3. Response of the renal system is the slowest one (in days), but its biological significance is extraordinary, because as the only system enables elimination of buffered H^+ into urine and simultaneously regulates plasma [HCO_3^-] in ECF.
4. In terms of time, the response of the body to acid-base disturbance can be divided into three steps:
   - **buffering** (chemical buffers);
   - **compensation** (kidneys, lungs, other organs) - failure of one system is compensated by another system;
   - **correction** (kidney, lungs) - affected system corrects its own failure.

The buffer systems

Buffers provide the first line defence which minimizes pH changes by binding or releasing H^+. From the chemical point of view, the buffer is pair of weak acid and its conjugate base/salt -

HA/A^- (INFO 3.2). All buffering systems in a body coexist in the mutual equilibrium. Four important buffers in ECF are:

- bicarbonate,
- hemoglobin,
- protein (mostly albumin),
- phosphate.

The major buffer in ECF is **carbonic acid/bicarbonate** system (**H_2CO_3 /HCO_3^-**), accounting more than 70% of total buffering capacity in plasma and also presents at lower concentration in erythrocytes (Table 3.1). The Henderson-Hasselbalch equation for that buffer system is following: $pH = 6.1 + \log [HCO_3^-]/[H_2CO_3]$, where 6.1 means pK_a of carbonic acid.

TABLE 3.1 BUFFERS AND THEIR DISTRIBUTION

Composition	Distribution	pK	% in blood
H_2CO_3 /HCO_3^-	ECF and ICF (RBC), urine	6.1	53%
Hprotein/protein$^-$	ICF, plasma	6.4 – 7.0	7%
$H_2PO_4^-$/HPO_4^{2-}	Urine, ICF, RBC, bone,	6.8	5%
HHb/Hb^-	RBC (part of EC compartment)	7.9	9%
NH_4^+/NH_3	urine	9.2	
HCO_3^-/CO_3^{2-}	bone		

Measuring of H_2CO_3 concentration in blood is not available in all routine clinical laboratories, therefore it has been replaced by pCO_2 value that is in equilibrium with H_2CO_3 and is easy to measure on acid-base analysers.

The bicarbonate to carbonic acid ratio in blood at physiological pH 7.4 is 20:1 and its pK_a is 6.1. Both values are significantly different from the values desirable in an ideal buffer (1:1 and 7.4 respectively). Despite that there are two factors helping the bicarbonate-carbonic acid pair to be effective at maintaining of physiological pH:

1. Remarkable high concentration of **HCO_3^-** in blood/plasma;
2. The system is opened and both its components (CO_2 and H_2CO_3) are actively regulated according the actual body needs by two mechanisms affecting the production or elimination of H_2CO_3. The lungs eliminate CO_2, utilized H_2CO_3 is substituted from reaction of CO_2 with water and from renal reabsorption of HCO_3^-. The next formula shows relationships among all three members of buffer system:

$$\mathbf{CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+}$$

Non-bicarbonate buffering systems - proteins and phosphates, despite having some buffering capacity in ECF, represent the key **intracellular buffers**. **Proteins**, especially albumin have feature of weak acid due to its high concentration of negatively charged amino acids. On the other hand, histidine residues of albumin (pK_a=6.8) react with H^+ and are the most important buffer groups of proteins at physiological pH. Similarly, haemoglobin buffer system within erythrocytes also contains histidine groups. **Hemoglobin** (Hb) buffer system in erythrocytes is considered as extra-cellular buffer, because it contributes significantly to control

pH in ECF by buffering and transporting of CO_2 (Figure 3.1). Considering its buffering capacity, it is the second most efficient buffer in extracellular compartment.

Phosphate buffer system consists from the pair of $H_2PO_4^-$ and HPO_4^{2-}. At physiological pH most of phosphate in plasma exists in form of monohydrogen phosphate (HPO_4^{2-}), which can accept H^+ to form dihydrogen phosphate ($H_2PO_4^-$). Due to pK_a of this buffer pair (6.8), which is close to the pH of glomerular filtrate, the buffer is very effective in urine. Low concentration of phosphates in ECF results in their insignificant buffering capacity within that body compartment. High concentrations of phosphate are found in the ICF and in bone.

Urinary buffers (bicarbonate, ammonia, and phosphate) play a special role in maintaining of acid-base homeostasis, as they provide the major mechanism for excretion of H^+ from the body and are important for the generation of HCO_3^-. Urinary pH may fall to 4.5 which represents in comparison with plasma 1 000-times greater $[H^+]$.

INFO 3.2 Acids, bases, and buffers

The traditional approach defines acid as any compound that is proton donors in the solution. Bases are compound able to accept protons. An acid dissociates in water to form H^+ and a conjugate base: $HA \leftrightarrow H^+ + A^-$. Strong acids dissociate entirely with no recombination, while weak acids dissociate only partially and may recombine with their bases until a chemical equilibrium is established.

Buffering capacity means the ability of buffer system to resist change in pH and depends on 2 factors:

1. concentration of buffer in body fluids;
2. the position of its equilibrium, being the most effective at the $[H^+]$ at which acid and base are present in equal concentrations. The most efficient buffer has pK_a value close to the pH of solution (for ECF pH 7.4 would be suitable pK_a 6.4 – 8.4).

In body fluids effectiveness of buffer is also influenced by its availability within different cells, tissues, organs. Applying the Henderson - Hasselbalch equation, pH of buffer depends on the logarithm of base/acid ratio: $pH = pK_a + \log [A^-]/[HA]$.

An "ideal" buffer should have a base/acid ratio of 1:1 and pK_a close to 7.4.

Respiratory system in maintaining of acid-base balance

Aerobic cell metabolism produces daily up to **20 000 mmol** of **CO_2**, that must be transported via blood into pulmonary capillaries and eliminated by lungs. **In tissues** CO_2 diffuses freely down the concentration gradient across cell membranes into ECF and erythrocytes where no CO_2 is produced due to anaerobic metabolism (Figure 3.1).

The total CO_2 content in body fluids is expressed as its **partial pressure pCO_2** with the normal value of **5.33 ± 0.5 kPa** in arterial blood. Reaction between CO_2 and water producing carbonic acid goes quicker in erythrocytes, due to presence of enzyme carbonate dehydratase (or carbonic anhydrase) catalysing the following reaction: $CO_2 + H_2O \leftrightarrow H_2CO_3$. The next dissociation of carbonic acid occurs rapidly and spontaneously: $H_2CO_3 \leftrightarrow H^+ + HCO_3^-$. Then, H^+ ions are neutralized inside RBC by haemoglobin buffer, which is more effective in deoxygenated state, thus after releasing of oxygen in tissues. Bicarbonate ions pass from RBC down their concentration gradient into plasma, in exchange for chloride ions to maintain electrical neutrality. The net effect is transformation CO_2 to HCO_3^-.

In the lung alveoli low pCO_2 is maintained by ventilation, pCO_2 in blood of pulmonary capillaries is higher than pCO_2 in the alveoli, thus the gradient is reversed. CO_2 diffuses therefore from blood into alveoli and is eliminated by the lungs. The following buffering reaction runs in opposite direction compared to the peripheral tissues:

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$$

HCO_3^- is supplied to that reaction from plasma in exchange for chloride. Decreased ventilation causes elevation of pCO_2 called **hypercapnia**, which results in high concentration of non-buffered H^+ and thus in acidosis (low pH). Increased ventilation lowers pCO_2 (**hypocapnia**) and causes low concentration of H^+ and thus alkalosis (high pH).

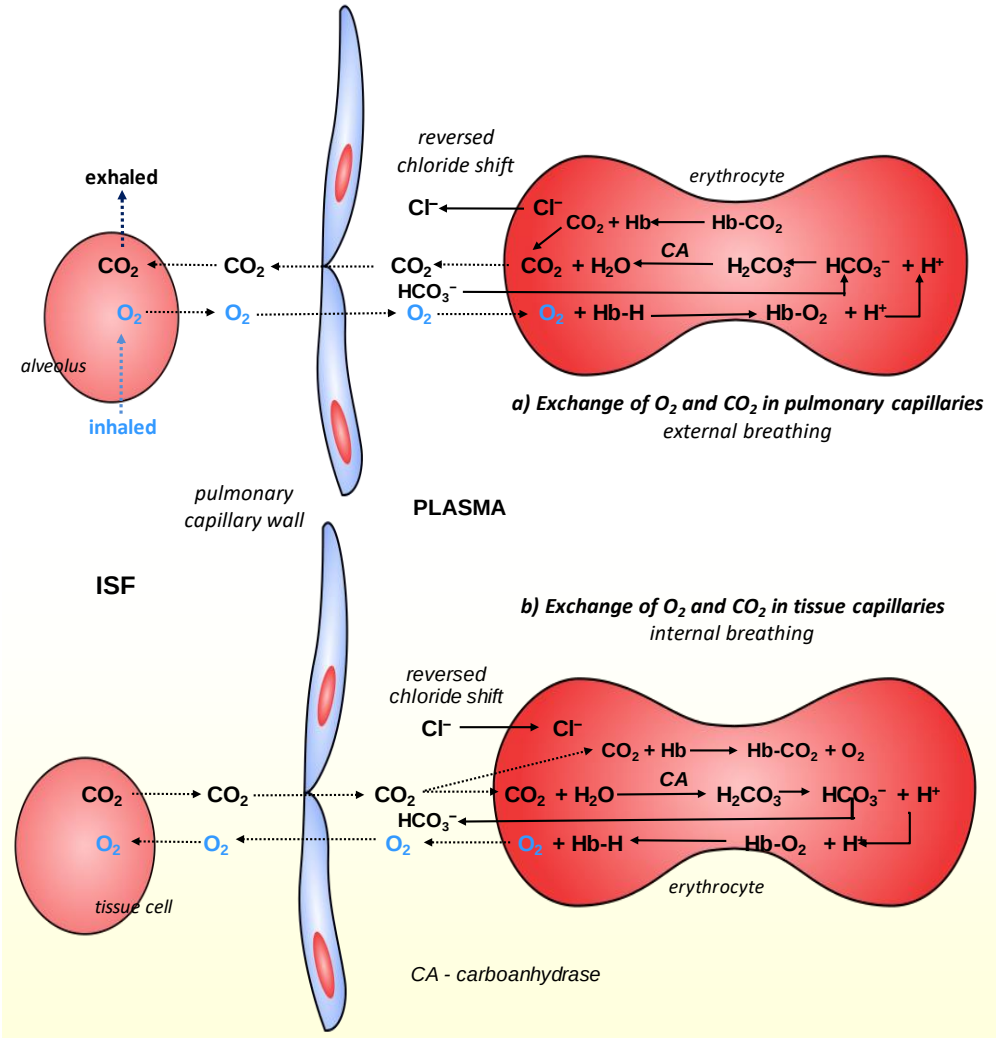


FIGURE 3.1 Transport of blood gases into RBC and buffering activity of Hb in tissues and lungs

Renal mechanisms for acid-base maintenance

The kidney has two principal tasks in acid-base regulation:

1. reabsorption of filtered HCO_3^- and
2. excretion of daily produced H^+ originated from non-volatile acids.

The glomerular filtrate contains the same concentration of HCO_3^- as plasma - 24 mmol/L. Renal tubular mechanisms are responsible for reabsorption of virtually all filtered amount of HCO_3^-

(180 L × 24 mmol = 4 300 mmol/day). If they failed, a large amount of HCO_3^- would be lost into urine, resulting in a decrease of total buffering capacity and acidosis. The mechanism of HCO_3^- reabsorption is indirect, as bicarbonate anions are not able to cross the luminal membrane. The end result of tubular processing of glomerular filtrate is reabsorption of filtered HCO_3^- with no net excretion of H^+ (Figure 3.2).

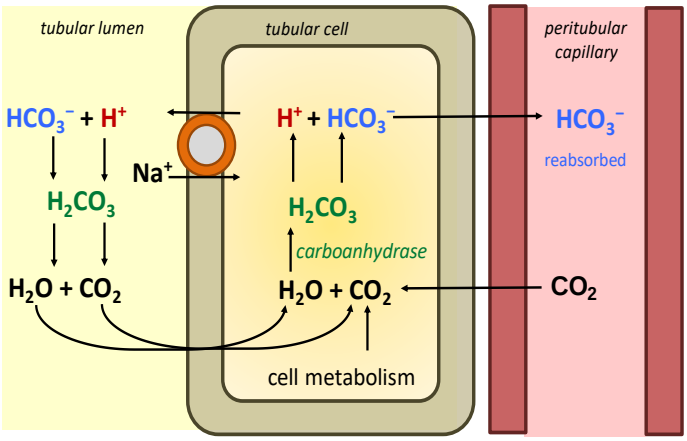


FIGURE 3. 2 Reabsorption of filtered bicarbonate in renal tubule

This process needs two steps: secretion of H^+ on luminal membrane into tubular lumen and HCO_3^- reabsorption on basal membrane into interstitial fluid

The renal tubules are also responsible for excretion of additional 50 – 100 mmol/L H^+ originated from "non-volatile" metabolic acids. As only 1/1000 of that amount can be eliminated in a free form, excretion of H^+ depends on the presence of suitable buffers in urine. The main urinary buffer is **phosphate** ($HPO_4^{2-}/H_2PO_4^-$), which is present mainly as HPO_4^{2-} at physiological pH of glomerular filtrate and can combine with H^+ to form $H_2PO_4^-$.

Ammonia ions represent another important 'carrier' for H^+ excretion into urine. Ammonia is formed by the deamination of glutamine in renal tubular cells in reaction catalysed by enzyme glutaminase and diffuses into the tubular lumen where it combines with H^+ to form NH_4^+ (Figure 3.3). This molecule does not pass across the cell membrane, thus must be eliminated into urine together with an additional anion (phosphate, chloride). In chronic acidosis activity of glutaminase increases, resulting into high ammonia production and therefore increased urinary H^+ excretion.

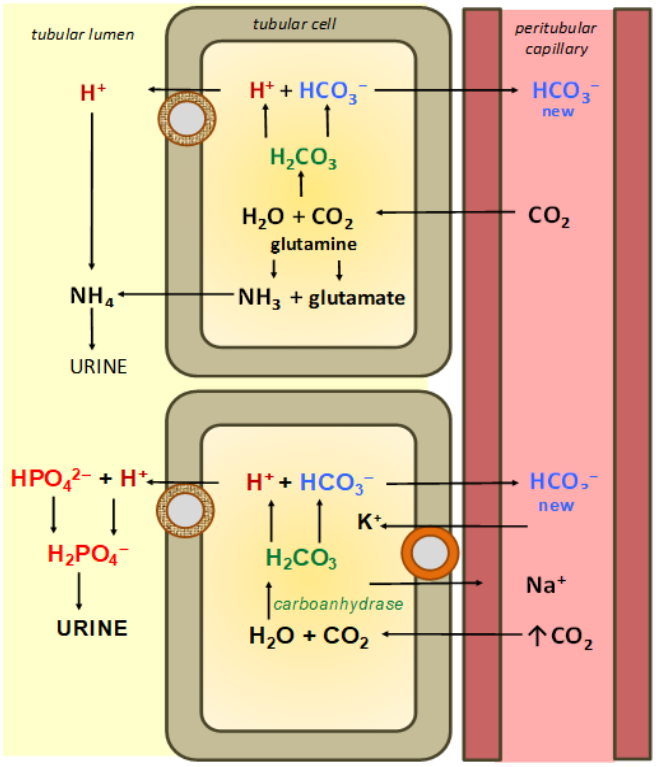


FIGURE 3.3 The regeneration of bicarbonates by renal tubular cells

In this process H^+ is excreted into lumen of tubule with simultaneous generation of 'new' HCO_3^-; the H^+ is buffered either by HPO_4^{2-} or NH_3

In addition to all mentioned mechanisms further organs also contribute to regulation of acid-base balance. The **liver** has a large capacity to metabolize many organic acids, e.g. utilize

lactate for gluconeogenesis. Based on actual acid-base situation the liver metabolizes ammonia from protein degradation in two ways. In alkalosis it prefers utilization of ammonia for synthesis of urea (the cycle produces also H^+), in acidosis glutamine is synthesized instead, which serves in renal tubules as source of ammonia, the important acceptor of H^+ in the urine. The heart is able to metabolize ketone bodies and lactate as a source of energy.

3.3 Laboratory investigation of acid-base disorders

Preanalytical conditions

The most appropriate specimen for assessing of acid-base status is **arterial blood,** collected from the radial artery. Its disadvantage is a limited number of repeated samplings in the monitoring of patients whose clinical conditions are changing rapidly. An alternative is **arterialized capillary blood** sample collected from skin puncture (finger, earlobe, lateral part of heel in new-borns). For correct specimen collection it is to ensure following preanalytical conditions:

- An adequate **preparation of patient**, who must be relaxed and without undesirable hyperventilation which may decrease pCO_2 (e.g. due to insertion of an arterial cannula, apprehension or fear, crying children).
- The **sampling site** in case of capillary blood should have good blood supply and blood should flow freely. In patients with peripheral vasoconstriction or venostasis the capillary sample does not inform only about acid-base state in the local peripheral tissue.
- Use of heparinised syringe or capillary tubes with **sufficient content of heparin** as anticoagulant. Excess of heparin, which is acidic, can change pH value and even cause hemolysis. If ionised calcium is to be measured simultaneously, calcium-balance heparin must be used.
- The specimen must be well mixed, free of air bubbles, since they cause a rise in pO_2 and fall of pCO_2.
- Analysis of acid-base sample should be performed immediately after sampling (using POCT devices) or transported to a laboratory under permanent chilling of a sample (e.g. in ice water). Maximal accepted delay of analysis after sampling is up to 1 hour. Otherwise, the acid-base composition of blood sample alters rapidly due to production of lactic acid during glycolysis in blood cells.

Laboratory parameters

Laboratory testing of acid-base status is based on traditional measured (**pH, pCO_2**) and calculated (**HCO_3^-** by Henderson-Hasselbalch equation) acid-base parameters in arterial or arterialised capillary blood (Table 3.2). These three parameters are necessary for an understanding of acid-base disturbances. The modern acid-base analysers allow to measure on very small sample volume (app. 100 µL) a set of analytes, which influence acid-base balance or they change significantly in ABD:

1. anions: Na^+, K^+, Ca^{2+};
2. anions: Cl^-, lactate, albumin;
3. metabolites: glucose, urea, creatinine, ketone bodies.

TABLE 3.2 OVERVIEW OF LABORATORY ACID-BASE PARAMETERS

Abbrev	Name	RI	Comments
pH	pH $[H^+]$	7.36 – 7.44 36 – 44 nmol/L	pH inversely correlates with concentration of H^+
pCO_2	Partial pressure of carbon dioxide	4.8 – 5.8 kPa 35 – 45 mmHg	Respiratory component of AB balance
HCO_3^-	Bicarbonates	22 – 26 mmol/L	calculated from actual pCO_2 and pH Metabolic component of AB balance
BE	Base excess	0 ± 2.5 mmol/L	express the deviation (excess /deficit) from normal $[HCO_3^-]$
pO_2	Partial pressure of oxygen	10 – 13 kPa 80 – 100 mmHg	Marker of oxygen composition of the blood and indicator of oxygen availability
$SatO_2$	Oxygen saturation of hemoglobin	95 – 97.5%	At normal atmospheric pressure

Note: measured parameters /calculated parameters

The **anion gap** (AG) is useful calculated biochemical mean helping in differential diagnostics of metabolic acidosis. AG represents the difference between the total concentrations of the measured cations minus the measured anions. Sodium and potassium make up more than 90% of plasma cations (remaining are Ca^{2+}, Mg^{2+}, and some positively charged proteins, mainly γ-globulins). Chloride and bicarbonate make up 80% plasma anions, while the remaining come from negatively charged proteins (albumin), sulphate, phosphate, urate, lactate, and some other organic anions.

The AG reference range is typically cited as 16±4 mmol/L, when the following formula is used: **$AG = [Na^+] + [K^+] - [Cl^-] - [HCO_3^-]$**. The simpler formula for AG calculation is also used in medical practice based on the concentration of the most abundant ions in plasma: **$AG = [Na^+] - [Cl^-] - [HCO_3^-]$**. Reference range is logically lower because of potassium is excluded (12±4 mmol/L).

Decreased values of anion gap are frequent in patients with hypoalbuminemia, when missing negative charge of albumin has been compensated by an increase of other anions, mostly Cl^- and HCO_3^-. If albumin decreases in 10 g/L, AG decreases approximately in 3 mmol/L.

3.4 Classification of acid-base disorders

Terms and definition

Acidemia is acid-base disturbance with high $[H^+]$ in blood, when resulting pH is less than 7.34.

Alkalemia is acid-base disturbance with low $[H^+]$ in blood, when resulting pH is higher than 7.46.

Acidosis and **alkalosis** describe pathophysiological processes acidemia or alkalemia. Both disturbances may co-exist simultaneously, resulting in **mixed acid-base** disturbances, which may present with an increased, normal or decreased pH value.

Respiratory acid-base disorders (acidosis or alkalosis) are caused by respiratory disorders with the primary change in pCO_2 (hyperkapnia-hypokapnia) which is related to changes in ventilation or ability of gases to diffuse across the alveolar membrane.

Metabolic acid base disorders (acidosis or alkalosis) are caused by metabolic or non-respiratory disorders, which initially result in changes in $[HCO_3^-]$ in ECF. This usually occurs due to production or loss of H^+ and HCO_3^-, respectively.

The **acute** acid-base disorder is recently developed disturbance characterized by changes of pH and pCO_2 in case of respiratory disorders; or pH and HCO_3^- in metabolic disorders. Compensatory response of opposite organ is not present.

Compensation is the physiological response to any acid-base disturbance, which tends to eliminate the change in pH caused by the primary process. Respiratory disorders (change in pCO_2) are compensated by change in renal HCO_3^- reabsorption. This results in a change of blood $[HCO_3^-]$ in the same direction as pCO_2 was changed. Similarly, metabolic acid-base disorder causing in change in blood HCO_3^- concentration is followed by respiratory compensation, which changes pCO_2 concurrently with the changes in $[HCO_3^-]$.

Metabolic acidosis

Metabolic acidosis (MAC) is an acid-base disturbance characterised by low pH and low concentration of HCO_3^- followed by compensatory fall in pCO_2. It is caused by increased endogenous production or intake of H^+, decreased excretion of H^+ or loss HCO_3^- (Table 3.3).

TABLE 3.3 CAUSES OF METABOLIC ACIDOSIS

Mechanism	Examples	Source of disturbance
Increased H^+ production in excess of body excretory capacity	Ketoacidosis - diabetic, alcoholic, starvation	Acetoacetic acid, β-OH butyric acid
	Lactic acidosis - hypoxic, shock, drugs, metabolic disease	Lactic acid
	Intoxications	Methanol, ethylene glycol, salicylate
Failure to excrete H^+ at the normal rate	Acute or chronic renal failure Distal renal tubular acidosis	Accumulation of H^+
Loss of HCO_3^-	GI: diarrhoea, pancreatic fistula	Loss of alkaline gut fluid
	Renal: proximal RTA, inhibitors of carbonic anhydrase, ureteroenterostomy	Decreased reabsorption and regeneration of HCO_3^-
Administration of chloride or acids	NaCl, ammonium chloride	Gain of H^+
Expansion of ECF	Dilutional MAC	Dilution of $[HCO_3^-]$ in ECF + ↓ renal HCO_3^- reabsorption

All conditions result in accumulation of H^+ in ECF, which immediately triggers neutralizing buffer activities. H^+ combines with HCO_3^- to form H_2CO_3, followed by splitting into CO_2 and water. Because an excess of produced CO_2 is immediately eliminated by the lungs, a new equilibrium is being established with a bit higher pH (still acidic), but at the expense of fall in [HCO_3^-], which has been consumed in this **buffering process**. If MAC is caused by renal or extra-renal loss of HCO_3^-, the fall in [HCO_3^-] is less dramatic, rarely being bellow 15 mmol/L.

After immediate but limited buffer reaction the compensatory **respiratory response** occurs in tens of minutes after onset of MAC. Acidosis stimulates respiratory centre and causes compensatory hyperventilation in form of Kussmaul´s breathing, which reduces pCO_2 and helps to normalize pH. However, the normal pH value is never achieved by hyperventilation. The limiting factor of compensation is [H^+] that decreases due to hyperventilation and its ability to drive an efficient hyperventilation decreases consequently.

In addition, if renal function is normal, an increased **renal** excretion of H^+, i.e. production of acidic urine (U-pH <5.5) will complete the **correction** of metabolic acidosis. Urinary [H^+] rises to its maximal possible value if two natural acceptors H^+ are available in urine: HPO_4^{2-} and ammonia produced from glutamine (MAC stimulates activity of glutaminase).

The metabolic acidosis occurs in two subtypes according anion gap value (Table 3.4):

1. **MAC with high AG (HAGMAC)** is caused by excessive production of H^+ mostly from organic acids of endogenous (ketones, lactic acid) or exogenous origin (degrading products of alcohols, toxins, etc.). Those acids, accumulate in ECF and HCO_3^-, together with anions of other buffers, are consumed in reactions neutralizing H^+ and they are replaced by anions of all mentioned acids (e.g. lactate), which are called as **unmeasured anions**.

   Hypoalbuminemia, frequently found in critically ill patients, decreases value of AG. It is possible to calculate AG corrected for normal albumin concentration according to the following formula: $\mathbf{AG_{corr} = AG + 2.5 \times (40 - S\text{-}Alb)}$

TABLE 3.4 EXAMPLES OF MAC WITH HIGH AND NORMAL ANION GAP

HAGMAG	NAGMAG
Ketoacidosis - diabetes, alcohol, starvation	Diarrhea
Lactic acidosis	Loss of intestinal and pancreatic fluids
Toxins - ethanol, methanol, ethylene glycol	Renal tubular acidosis (type 1+2)
Uremia - advanced stage CKD	Addison´s disease
Salicylates (aspirin) overdosing	Extra chloride/HCl ingestion
Propylene glycol	Carbonic anhydrase inhibitors

2. **MAC with normal AG** (**NAGMAG):** If MAC is caused by loss of HCO_3^- (e.g. severe diarrhea); there is a compensatory increase in chloride concentration (due to restore electroneutrality in ECF) and AG remains unchanged. This type of MAC is also called **hyperchloremic metabolic acidosis**.

Metabolic alkalosis

Metabolic alkalosis (MAL) is characterised by pH higher than 7.44 and increased [HCO_3^-]. MAL is a frequent acid-base disorder generated by at least one of the following mechanisms (Table 3.5):

- loss of H^+ from ECF (renal, extra-renal),
- shift of hydrogen ions into ICF (during hypokalemia),
- increases renal reabsorption of HCO_3^- (volume depletion),
- alkali administration (oral, parenteral).

TABLE 3.5 CAUSES OF METABOLIC ALKALOSIS

Primary cause	Example	Mechanism
A. Chloride-responsive (with Cl^- depletion)		U-Cl^- <20 mmol/L
Gastrointestinal loss of H^+	gastric fluid (vomiting, NG suction) colonic fluid (laxative abuse, congenital chloride diarrhea)	Loss of H^+ without HCO_3^-
Renal loss of chloride and H^+	Loop diuretics, thiazide Post hypercapnic MAL	↑HCO_3^-reabsorption, Persisting renal response after restoration of normal pCO_2
Intake of alkali	Infusion of HCO_3^-, massive blood transfusion (citrate) Milk-alkali syndrome,	Citrate metabolizes to HCO_3^- Large doses of carbonates
B. Chloride-resistant (without Cl^- depletion)		U-Cl^- >20 mmol/L
Renal loss of H^+	primary hyperaldosteronism, Cushing´s syndrome Bartter, Liddle, Gitelman syndrome	Increased tubular Na^+ reabsorption in exchange for K^+ and H^+
C. Other		
Loss of water	Dehydration, volume depletion	Contraction alkalosis
Hypoalbuminemia	Critical care patients, malnutrition	Loss of negative charge on albumin compensated by HCO_3^-

In MAL the equilibrium in buffering reaction is shifted to the right, toward dissociation of H_2CO_3 to H^+ (which is being lost and needs to be replaced) and HCO_3^- concentration raises. **Respiratory compensation** of MAL manifests as hypoventilation, which is limited by simultaneous fall in pO_2, that in turn, represents a potent stimulus of ventilation. The limiting value of pCO_2 during respiratory compensation is around 8 kPa, higher values of pCO_2 are usually associated with hypoxia.

Normally, plasma [HCO_3^-] is maintained at a stable level by two processes: tubular reabsorption of all daily filtered load of HCO_3^- and excretion of the net daily production. Whenever plasma [HCO_3^-] raises above 24 mmol/L, healthy **kidney** is able to excrete that excess rapidly with consequent formation of alkaline urine. Therefore, MAL is usually of short duration. Persistent MAL develops if there is any additional process which impairs renal HCO_3^- excretion:

a. decrease in ECF volume (frequently also with decrease in GFR),
b. chloride depletion,
c. potassium deficiency.

For clinical management of therapy is practical to divide MAL into:

1. **MAL with chloride deficit** (=**chloride-responsive MAL)** in hypovolemic patients with low effective circulating volume, which responds well to ECF volume substitution with solutions containing chlorides. The principal cause of chloride-responsive MAL is **loss of gastric fluid** due to vomiting or suction or losses of chloride and potassium into urine during **diuretic therapy** (loop and thiazide). Urinary Cl^- concentration <20 mmol/L is a marker of chloride depletion in the body.
2. **MAL without Cl^-** deficiency (=**chloride-resistant MAL)** is usually caused by renal H^+ loss due to excess of mineralocorticoids. Patients are mostly euvolemic, with hypertension (primary hyperaldosteronism) or without hypertension (inherited or acquired tubular defects). Serum [Cl^-] decreases in order to compensate for an increase in [HCO_3^-]; it does not reflect decreases body stores (depletion). Administration of chloride therefore does not solve MAL, more important is normalization of hypokalemia and treatment of hyperaldosteronism.

Respiratory acidosis

Respiratory acidosis (RAC) is caused by decreased pulmonary elimination of CO_2 due to hypoventilation from pulmonary, neurological, or mechanical (muscle, bones) reasons. Other possible causes are impaired distribution of gases during ventilation, decreased alveolar gas exchange, an excess CO_2 in the inspired air or rarely increased production of CO_2 in the body (Table 3.6).

TABLE 3.6 RESPIRATORY ACIDOSIS - OVERVIEW OF POSSIBLE CAUSES

Normal function of lungs/airways	Abnormal function of lungs/airways
CNS depression: anaesthetics, drug overdose (sedatives, hypnotics, opiates), brain injuries, tumours and infections, stroke	Extra-pulmonary airways obstruction: aspiration, tumour, laryngospasm, angioedema, strangulation, stenosis
Neuropathy/myopathy: spinal cord trauma, status epilepticus, myasthenia gravis, multiple sclerosis, poliomyelitis, toxins (curare, succinylcholine, organophosphates)	Intra-pulmonary airways obstruction: asthma, bronchospasm, bronchiolitis, CHOPD, emphysema
Failure of ventilation: chest trauma, pneumothorax, kyphoscoliosis, obesity	Low alveolar diffusion: ARDS, pulmonary oedema, fibrosis
Iatrogenic: wrong placed endotracheal cannula, bronchoscopy, artificial ventilation	Low pulmonary perfusion: cardiac arrest, circulatory failure, thromboembolism

An elevation of pCO_2 in acute RAC shifts the equilibrium in buffering reaction to the right, with a resulting increase of both [H^+] and [HCO_3^-] in blood. Although, because of the large difference in their basal concentration (e.g. nmol/L and mmol/L), the change in concentration will be relatively small or even undetectable:

$$\uparrow\mathbf{CO_2} + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + \uparrow\mathbf{H^+}$$

In the first line, intracellular and extracellular **buffer systems** answer to change in pH and in minutes equilibrate [H^+] until a new steady state is achieved. If RAC persists longer (its cause has not been quickly resolved or treated), **renal compensatory response** causes restoration almost the normal value of pH in 3 – 5 days by following mechanisms:

1. increased reabsorption of HCO_3^- in proximal tubules, with parallel losses of chloride.
2. excretion of H^+ (acidic urine) - synthesis and excretion of ammonia increases and combines with production of 'new' HCO_3^- in collecting ducts.

Patients with **chronic compensated RAC** have typically pH slight lower or close to normal, increased pCO_2 and compensatory elevated [HCO_3^-], which may cross concentration above 45 mmol/L at maximal compensation. After a new steady state has been achieved, renal processes (e.g. HCO_3^- retention and H^+ excretion) return to normal. Final correction of the primary cause of RAC often needs restoration of is well tolerated until it is not combined with hypoxia. Post-hypercapnic metabolic alkalosis sometimes persists longer despite resolution of chronic RAC. The reason is chloride deficiency worsened frequently by diuretic treatment and salt restriction in diet.

Respiratory alkalosis

Respiratory alkalosis (RAL) is acid-base disturbance caused by increased pulmonary elimination of CO_2 (hyperventilation). List of most frequent cause of RAL is in Table 3.7. The reduced pCO_2 shifts the equilibrium in buffering reaction to the left, with a resulting decrease of both plasma [H^+] and [HCO_3^-], although the relative change in HCO_3^- is small:

$$\downarrow\mathbf{CO_2} + H_2O \leftarrow H_2CO_3 \leftarrow HCO_3^- + \downarrow\mathbf{H^+}$$

TABLE 3.7 RESPIRATORY ALKALOSIS - OVERVIEW OF POSSIBLE CAUSES

Central (direct action via respiratory center)	**Pulmonary** (act via intrapulmonary receptors)
– Head injury – Stroke – Anxiety, pain - hyperventilation syndrome – Drugs: analeptics, salicylate intoxication – Endogenous compounds (progesterone in pregnancy, cytokine in sepsis, toxins in liver failure)	– Hypoxemia (via peripheral chemoreceptors) – Pulmonary embolism – Pneumonia – Asthma – Pulmonary edema

An early response to RAL is provide intracellular non-bicarbonate buffers, which yield H^+ needed for restoration the pH ($HBuf \rightarrow H^+ + Buf^-$). If condition lowering the pCO_2 persists longer **renal compensatory response** follows in form of excretion of HCO_3^- and retention of H^+ and new steady state with near normal pH and low [HCO_3^-] will be achieved in few days. However, the renal response in RAL is not so powerful than in RAC, thus it is unusual to find [HCO_3^-] below 12 – 14 mmol/L even in severe RAL.

Mixed acid-base disorders

In medical practice we often meet patients with comorbidities, which logically cause different types of acid-base disorders. This mixed acid-base disorders are even more frequent than

simple ones. They can combine any two or more single disturbances, except for the combination of RAC and RAL that cannot exist simultaneously. In addition, one single disturbance may have more causes. If the primary conditions cause the opposite effects on pH (e.g. MAC + RAL), the resulting pH value may be in the normal range. Alternatively, if the primary conditions are additive (for example, MAC + RAC), their effect on pH summarizes and pH changes more in comparison to a single disturbance.

3.5 Interpretation of acid-base data

Assessing of acid-base status of a patient is a part of complex diagnostic procedure involving detailed history, physical examination, laboratory data and results of other available diagnostic methods. This should identify pathological condition, which has caused an acid-base disturbance. Severe acidosis and alkalosis have severe clinical consequences which are predictable especially in critically ill patient (INFO 3.3).

INFO 3.3 Systemic effects of acid-base disorders

Acidosis affects function of following systems:

1. Cardiovascular (CV): negative inotropic (low cardiac output), arteriolar vasodilatation (CNS), venoconstriction (increased pulmonary vascular resistance)
2. Oxygen delivery to tissues: shift of the oxyhemoglobin dissociation curve to the right, decreased liver and kidney perfusion (stress reaction)
3. Potassium homeostasis: transcellular movement from ICF into ECF
4. Bone: significant buffering H^+ by bone in chronic MAC leads to demineralisation
5. Immune effects: activation of WBC, increased blood viscosity (due to RBC swelling) - regional hypoperfusion
6. Water and salt retention and hypervolemia due to stimulation of RAAS, cortisol and ADH

Systemic effects of alkalosis are difficult to distinguish from the effects of associated issues - hypovolemia, Cl and K depletion, decrease in ionized calcium:

1. Cardiovascular: decreased myocardial contractility, arrhythmias, decreased cerebral blood flow
2. Consciousness: confusion, mental obtundation, lethargy
3. Neuromuscular excitability: tremor, paraesthesia, seizures
4. Impaired peripheral O_2 unloading: shift of oxygen dissociation curve to the left.

The traditional approach to interpretation of acid-base disturbance is based on laboratory parameters **pH, blood gases, HCO_3^-**. Analysis of ions (Na^+, K^+, Cl^-) measured simultaneously with blood gases provides helpful information. In clinical setting charts, calculators and software applications are often used for acid-base data interpretation. However, they are not helpful for patients with rapidly changing status and usually lose precision at extreme values.

Modified Boston´s approach to evaluation of acid-base status

There are many recommended guidelines to understanding the interpretation of acid-base data. The simple approach is based only on result of blood gas analysis (pH, pCO_2, HCO_3^-), the more complex one recommends to consider more laboratory and clinical data (ions, lactate, urine analysis, albumin, etc.). The standard procedure is following:

1. **Initial clinical assessment:** The essential first step is to assess the available clinical information (history, physical examination) and use them to make a clinical decision about the possible or most likely acid-base diagnosis. The more we know about a patient, the closer we are to the correct diagnosis. The knowledge of pathophysiology of conditions which usually cause acid-base disorders is extremely useful in making these initial assessments (Table 3.8).

TABLE 3.8 EXAMPLES OF CLINICAL CONDITIONS WHICH CAUSE ABD

Clinical cause	Acid-base disorder
Hypoventilation	RAC from CO_2 retention MAC from hypoxia (lactic MAC)
Hyperventilation	RAL out-breathing of CO_2
Vomiting, NG suction	MAL from HCl loss
Diarrhoea, laxative abuse	MAC from loss of alkaline intestinal fluid
Starvation, malnutrition	MAC (ketoacidosis)
Renal failure	MAC from retention of organic/inorg. acids, renal loss of HCO_3^-
Hyperhydration	Dilutional MAC ($\downarrow HCO_3^-$)
Dehydration	Contraction MAL ($\uparrow HCO_3^-$) - also due to secondary hyperaldosteronism
Diuretics (loop, thiazides)	MAL- loss of Cl^- and K, activation of RAAS
Ethanol	MAC - acetic + lactic + 3-OH-butyric acids
Methanol	MAC - formic + lactic + 3-OH-butyric acid
Ethylene glycol	MAC - glycolic acid, oxalic acid + lactic acid
Salicylates overdose	MAC - different organic acids RAL from stimulation of respiratory centre
Severe hypoproteinemia	MAL (drop of $Prot^-$ and $\uparrow HCO_3^-$)

2. **Compare pH to the normal range:** The pH determinates whether the primary disorder is an acidosis or an alkalosis. A normal pH indicates either none acid-base imbalance, completely compensated (rare) acid-base disorders or mixed disorders.
3. **Identify the primary process that has led to the change in pH:** Look for the suggestive **pattern in pCO_2 and $[HCO_3^-]$** (Figure 3.4):
   - **a.** Low both pH and $[HCO_3^-]$ indicates a possible metabolic acidosis. Low pCO_2 informs about respiratory compensatory response (hyperventilation), which tends to occur relatively quickly. Occasionally, a patient with a very acute MAC presents with no change in pCO_2.
   - **b.** High both pH and $[HCO_3^-]$ indicate a possible metabolic alkalosis. MAL is acute, if pCO_2 is normal, higher pCO_2 is a signal of compensatory response (hypoventilation).
   - **c.** The combination of low pH and increased pCO_2 indicates respiratory acidosis. $[HCO_3^-]$ is mostly normal in the acute phase of RAC, while in chronic conditions $[HCO_3^-]$ elevates recognizably with an increase in pH close to normal value (compensated RAC).
   - **d.** An increase in pH combined with decreased pCO_2 indicates respiratory alkalosis. In that condition $[HCO_3^-]$ is expected to fall due to compensatory renal response (HCO_3^- excretion).
   - **e.** Any other combinations of acid-base data with high probability suggest presence of mixed acid-base disorder.

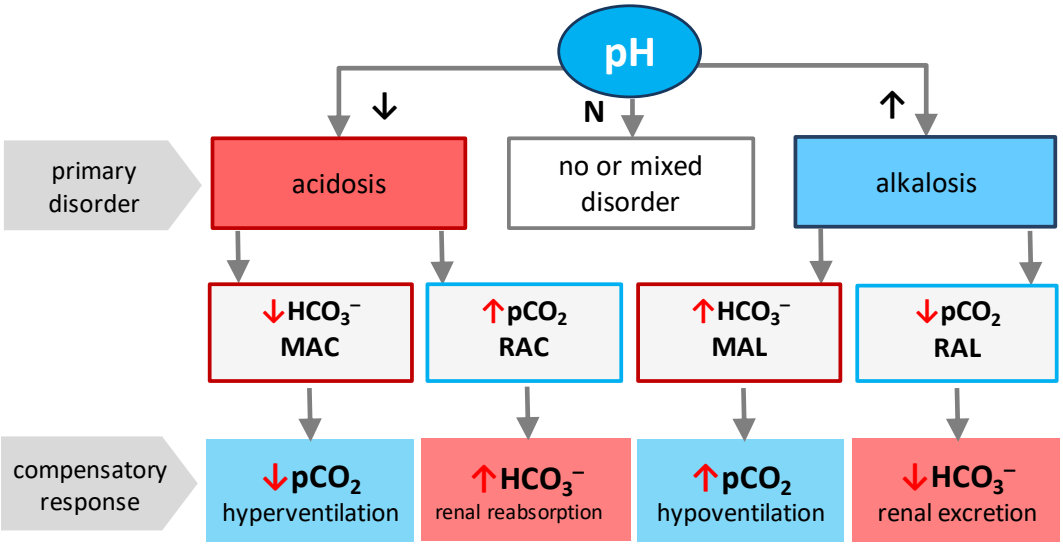


FIGURE 3.4 An algorithm for assessing acid-base disorders

4. **Assess compensatory process:** The target of compensatory response is the normalization of pH and its basic principle is to maintain the $pCO_2/[HCO_3^-]$ ratio. Therefore, the direction of the compensatory response is always the same as that of the initial change. Compensatory response in respiratory disorders has two steps; a fast response of buffering systems and a significantly slower (in 3 – 5 days) renal response. The compensatory response to metabolic disorders involves only an alteration in alveolar ventilation. Metabolic disorders are usually not defined as acute or chronic in terms of respiratory compensation because the extent of compensation is the same in each case.

   To assess degree of compensation of acid-base abnormalities is also possible by using formulas calculating an expected value of plasma $[HCO_3^-]$ in respiratory disorders or expected pCO_2 in metabolic disorders. If calculated value for maximal compensation matches the real measured value, a single fully compensated acid-base disorder is confirmed. In opposite, if the calculated value is different from the measured one, a mixed acid-base disorder should be considered.

5. **Check for additional clues in other investigations:**
   a. **Anion gap:** The parameter is useful in differentiating of metabolic acidosis. Value of AG in the range 20 – 30 mmol/L suggests HAGMAC with 70% probability; AG > 30 mmol/L confirms presence of HAGMAC definitively.

   b. **Delta ratio** ($dAG/dHCO_3^-$): is another auxiliary parameter in differential diagnostics of mixed acid-base disorders. In uncomplicated HAGMAC, the decrease (**d**ifference) in plasma bicarbonate should be roughly equal to increase (**d**ifference) in the anion gap. That statement is based on simplified stoichiometry, where one millimole of any acid neutralizes one millimole of bicarbonate. Delta ratio ($dAG/dHCO_3^-$) in typical HAGMAC is usually close to 1.0. Whenever the AG changes disproportionally to that of bicarbonate, this is suspected of a co-existence of a mixed acid-base disorder. Thus, the $dAG/dHCO_3^-$ ratio outside the range 0.5 – 1.5 suggests presence of another metabolic acid-base disorder. Hyperchloremic MAC causes a more intensive decrease in $[HCO_3^-]$, delta ratio falls below 0.5. On the other hand, simultaneous MAL ameliorates decrease in $[HCO_3^-]$, corresponding to increase in AG, value of delta ratio is >1.5.

c. **Clues in the other laboratory results**: some typical laboratory findings suggest a possible cause of acid-base disorder, for example hyperglycemia with ketones (DKA), hyperglycemia without ketones (hyperosmolar hyperglycemic state), hypokalemia and/or hyperchloridemia (MAL), increased creatinine and urea (uremic MAC).

6. **Identify a possible mixed ABD** Laboratory results (AB parameters, ions, AG) may be the first indicators of mixed acid-base disorders. For example, if pH is relatively normal compared to the pathological values of the pCO_2 and/or $[HCO_3^-]$, high AG is at normal or elevated pH, signs of extreme compensatory response in pCO_2 or $[HCO_3^-]$, hypochloridemia combined with normal or higher pH are present, one can consider existence of a mixed abnormality. The detailed knowledge of clinical findings in a patient is essential in differentiating and interpreting of mixed acid-base disorders. Typical clinical conditions resulting in mixed acid-base disturbances are listed in Table 3.9.

TABLE 3.9 EXAMPLES OF MIXED ACID-BASE DISORDERS

Combination	Cause of mixed acid-base disorder
MAC + RAC	Heart arrest, respiratory failure (ARDS) + septic shock
MAL + RAL	Vomiting + liver failure, consequence of treatment of DKA (alkaline solution + insulin)
MAC + RAL	Septic shock, hepatorenal syndrome, salicylate overdosing,
MAC+ MAL	Vomiting combined with ethanol intoxication, uremia, diabetic ketoacidosis or lactic acidosis
MAC + MAL + RAC	All situations mention above combined with respiratory failure
MAC + MAC	Diarrhoea in patient with renal tubular acidosis or adrenal failure (Addison´s disease)

Approach to acid/base disorders interpretation according to Stewart

Interpretation of respiratory A-B disorders is the same as in the traditional approach, thus based on pCO_2. Interpretation of metabolic component of acid-base balance utilized the principle of electroneutrality, i.e. the equal sum positive and negative charge on cations and anions, respectively (INFO 3.4 and Figure 3.5).

Stewart's model of acid base physiology assumes that pH and $[HCO_3^-]$ are **dependent variables** and do not change without change in any of three **independent controlling variables**:

- partial pressure of carbon dioxide,
- strong ion difference, and
- total weak acid (weak anions) concentration.

All plasma **cations are strong** (= almost completely dissociated and chemically non-reactive), while **strong anions** are only chloride and anions which are not measured routinely called unmeasured anions (UA^-). Examples of UA^- are, for example, ketones, lactate, endogenous anions retained mostly during liver and kidney failure. Other plasma anions – albumin,

phosphates and HCO_3^-, are **weak anions,** which dissociates only partially and they have a buffering function.

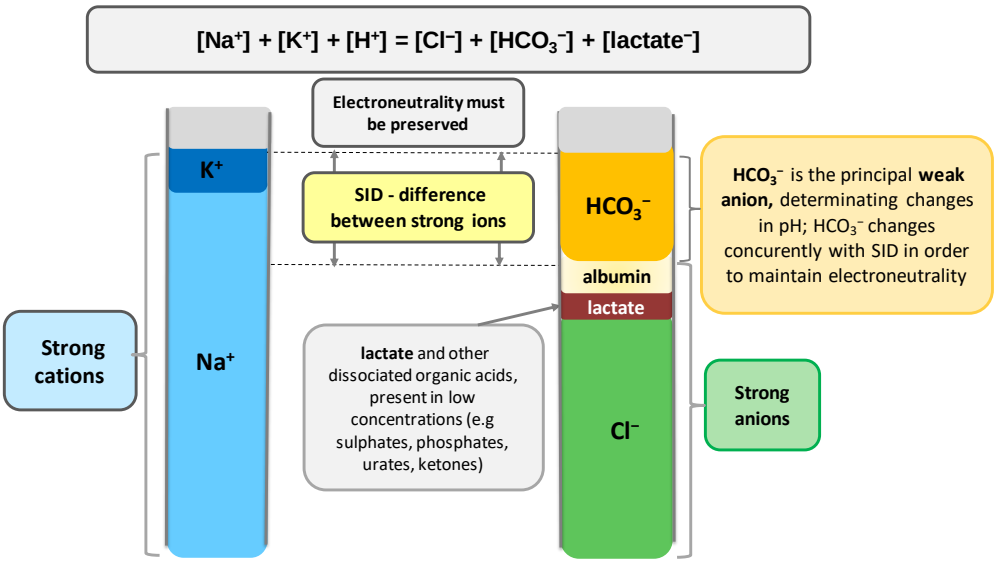


FIGURE 3.5 Concept of strong and weak ions according to Stewart and Fencl

Strong ion difference (SID) is calculated as a difference between strong cations and anions by formula **SID = $[Na^+]$ + $[K^+]$ + $[Ca^{2+}]$ + $[Mg^{2+}]$ − $[Cl^-]$ − $[UA^-]$,** where represents unmeasured $[UA^-]$. Decrease in SID has an acidifying effect, increase in SID moved pH to alkaline side.

Total weak acid (weak anions) concentration $[A_{tot}]$, represents charge on albumin and phosphate and may be calculated by formula: **$[A_{tot}] = [Alb^-] + [P_i^-]$**. Decrease in $[A_{tot}]$ has an alkalizing effect in ECF, its increase, in opposite, moves pH to acidic side.

The concentration of HCO_3^- depends on those two independent variables. Increase of strong anion (e.g. sodium) without an increase of accompanying anion, which is chloride, is followed by an increase of weak anions - HCO_3^-, in order to maintain electroneutrality. Traditional approach evaluates the acid-base disorder as metabolic alkalosis due to increase in $[HCO_3^-]$. Stewart´s approach evaluates that disorder as alkalosis, which is a consequence of SID elevation, caused by hypernatremia. By analogy, MAC results from an increase in chloride (hyperchloremic acidosis according to traditional evaluation) or strong unmeasured anions $[UA^-]$, e.g. lactate, ketones (HAGMAC).

Info 3.4 What is different in Stewart´s approach?

The still dominant concept of acid-base physiology working with Henderson-Hasselbalch equation and the Bronsted-Lowry definition of an acid is based on assumption that plasma bicarbonate is not only an indicator of acid-base status, but also a principal determinant of metabolic disorders.

In the late 1970s Peter Stewart used laws of physical chemistry, including law of electroneutrality and the Van Slyke definition of an acid, to produce a new acid-base approach. Stewart's main reason for exploring acid-base physiology was that he found the bicarbonate-centred approach confusing and inadequate in many clinical situations. His approach, using the strong ion difference (particularly the sodium chloride difference) and the concentration of weak acids (particularly albumin), pushes bicarbonate into a minor role as an acid-base indicator rather than as an important mechanism. The Stewart´s approach may offer new insights into acid-base disorders and therapies in critically ill patients.

3.6 Assessment of oxygen transport

The oxygen delivery to all tissues depends on their blood supply and on content of oxygen in the blood. Oxygen content is determined mostly by hemoglobin concentration and by its saturation with O_2, as only 2% of oxygen in the blood exists in dissolved form, which is insufficient to meet all demands of tissue metabolism. Both of O_2 forms depend directly on the partial pressure of oxygen - pO_2. The oxygen transport is facilitated by the pressure gradient between atmospheric air and intracellular compartment.

Hypoxia is a state of low oxygen content and partial pressure in cells. If metabolic needs of tissues are higher than oxygen supply, cell metabolism changes to anaerobic one which produces lactic acid. **Lactate** level in blood may inform about tissue oxygenation. Depending upon a cell type, its metabolic demands, and its ability to adapt to hypoxia, the response to various levels of hypoxia can range from substantial adaptation to cell death.

Tissue hypoxia can be caused by following general abnormalities (Table 3.10):

1. hypoxemia - low O_2 content in arterial blood, which results from:

- low O_2 content in inspired air, impaired gas exchange in pulmonary alveoli due to insufficient ventilation or diffusion (**hypoxic hypoxia**);
- low hemoglobin content and its impaired binding properties, as in case of severe anemia, COHb or MetHb (**anemic hypoxia**);
- disorders of mitochondrial function (**histotoxic hypoxia**), e.g. in sepsis;

2. hypoperfusion - low cardiac output (**stagnant hypoxia**).

TABLE 3.10 BASIC CAUSES AND MECHANISMS OF HYPOXIA

Cause (example)	**Mechanism of low arterial pCO_2**
Low pO_2 of inspired/atmospheric air	Low alveolar pO_2
Alveolar hypoventilation (supressed ventilation, neuromuscular disorders)	Low inspiring volume or frequency, Increased ventilation of dead space
Impaired diffusion (pulmonary fibrosis, lung oedema, drowning)	Normal alveolar pO_2 with low oxygenation of blood
Ventilation/perfusion mismatch (COPD, asthma)	Blood flows through non-ventilated parts of lungs
Arterial-venous shunts in lungs and heart (cyanotic heart diseases)	Mixture of arterial and venous blood
Decreased binding capacity for oxygen (insufficient/impaired function of Hb)	Severe anemia, hemoglobinopathies

Among acid-base parameters measured in arterial or arterialized capillary blood (Table 3.11), the following two ones are the most important for assessing of oxygenation:

- **partial pressure** of oxygen (pO_2) and
- hemoglobin **saturation** ($SatO_2$), reported as percentage of oxyhemoglobin.

Both parameters are measured directly on laboratory acid-base analyzers; pO_2 by using amperometry Clark´s electrode, $SatO_2$ with co-oximeter. In addition, $SatO_2$ can be monitored with non-invasive pulse oximetry based on transcutaneous measurement of signals from pulsing arterial blood. The **pulse co-oximeter** uses a fingertip sensor with several distinct wavelengths of light to measure oxygenated and reduced hemoglobin by spectrophotometry. Oxygenated and reduced hemoglobin absorb the light spectrum of different wavelength during pulse blood flow through capillaries in finger or earlobe. Their difference is a measure of hemoglobin saturation. The similar principle works also in intravascular monitors of $SatO_2$. Pulse oximetry cannot detect all pathological forms of hemoglobin (COHb, MetHb).

TABLE 3.11 PARAMETERS INFORMING ABOUT OXYGEN METABOLISM

Parameter	Values
pO_2 in atmospheric air	19.9 kPa
pO_2 in alveolar air	14.6 kPa
pO_2 in arterial blood	10.0 – 13.3 kPa
pO_2 in central venous blood	4.5 – 5.5 kPa
pO_2 in cellular cytoplasm	2.0 – 0.5 kPa
Hemoglobin	130 – 160 g/L
$SatO_2$	95 – 98%
P50 (at 37°C and pH 7.4)	3.3 – 3.9 kPa
FiO_2	21% in atmosphere, 40 – 100% on controlled ventilation
Lactate in plasma	0.5 – 2.0 mmol/L

Relationship between pO_2 and amount of oxygen bound to hemoglobin graphically describes so called dissociated curve of oxyhemoglobin (Figure 3.6). The parameter P50 represents the pO_2 value, at which 50% saturation of hemoglobin with oxygen. An increased value of P50 signalizes decreased affinity of oxygen to hemoglobin, while decreased P50 value means increased affinity of oxygen to hemoglobin.

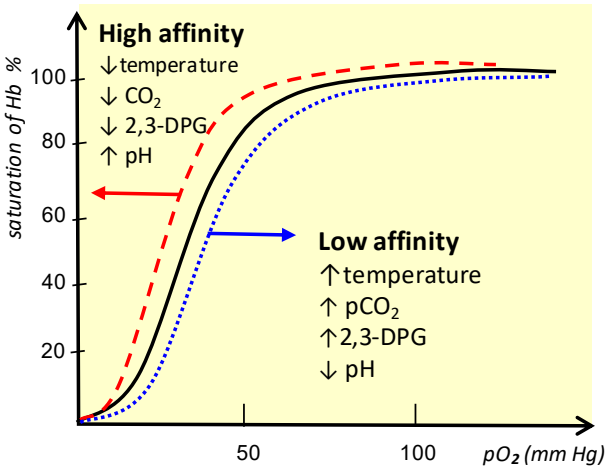


FIGURE 3.6 Dissociation curve of oxyhemoglobin

Left-sided shift (dotted line) means stronger bound of O_2 and impaired release from Hb in tissues; right-sided shift (intermittent line) means weaker bound of O_2 and its easier release from Hb in peripheral tissues

Respiratory failure

Respiratory failure is a syndrome in which the respiratory system fails in one or both of its gas exchange functions: oxygenation and carbon dioxide elimination. The value of arterial pO_2 below 8 kPa or 60 mm Hg (hypoxemia) in a patient breathing atmospheric air decreases

hemoglobin saturation with oxygen and results in decreased oxygen to peripheral tissue (hypoxia).

Hypoxemia without retention of CO_2, called **hypoxemic respiratory failure** (type I)**,** is the most frequent form of respiratory failure. It is associated with virtually all acute diseases of the lung, which generally involve fluid filling or collapse of alveolar units. An inequality between ventilation and perfusion (V/Q mismatch) or anatomic shunts are a frequent cause of a decrease in pO_2.

Hypoxemia combined with hyperkapnia due to retention of CO_2, means **hyperkapnic respiratory failure** (type II), which is caused by ventilation disorders. A decrease in alveolar ventilation can result from a reduction in overall (minute) ventilation or an increase in the proportion of dead space ventilation. Common causes include drug overdose with CNS depression, neuromuscular disease, chest wall abnormalities, and severe airway disorders (e.g. asthma and COPD). In pure hyperkapnic respiratory failure, the hypoxemia is easily corrected with oxygen therapy.

Oxygen therapy and assisted ventilation

Beside critically ill patient, evaluation and monitoring of oxygenation is important during surgery in general anaesthesia, in patients with chronic pulmonary diseases, severe anemias and hemoglobinopathies. Assessment of tissue oxygenation by laboratory tests is based on the investigation of arterial blood analysis. A complete blood cell (CBC) count may indicate anemia, which can contribute to tissue hypoxia, whereas polycythemia may indicate chronic hypoxemic respiratory failure. The biochemistry panel, particularly abnormalities in renal and hepatic function may either provide clues to etiology of respiratory failure or alert the clinician to complications associated with respiratory failure.

Laboratory values indicating the need of oxygen therapy and assisted ventilation are pO_2 <9.0 kPa and pCO_2 >6.5 kPa in arterial blood in a patient in acute condition breathing atmospheric air. The goal of oxygen therapy is to assure adequate oxygen delivery to tissues, generally achieved with an arterial pO_2 of 60 mm Hg or an arterial oxygen saturation greater than 90% (INFO 3.5).

INFO 3.5 Remarks to oxygen therapy and assisted ventilation

The use of oxygen of different concentration and its administration at different pressure sets out specific recommendations for treatment of hypoxic and hypercapnic conditions in both adults and children. In general, we choose the lowest effective concentration of oxygen in the inhaled mixture (FiO_2) to prevent toxic effects on the lung tissue. For a short term it is possible to increase FiO_2 to 40–60%.

A long-term increase in FiO_2 above 50% leads to the development of inflammatory changes in alveoli with subsequent fibrosis. Oxygen in concentrations above 60% is preferably used in controlled pulmonary ventilation. 100% oxygen is used only during acute resuscitation.

Patients with chronic hypercapnia may develop insensitivity of respiratory center to elevated pCO_2 and only persisting hypoxia maintains respiration. If therapy with too high oxygen concentrations removes the only respiratory stimulus in those patients, it results in hypoventilation, hypercapnia and RAC. During assisted or controlled ventilation, a slow decrease in pCO_2 (not faster than 0.3 – 0.7 kPa/h) is important, with continuous monitoring of blood gases. Existing renal compensation of the disorder and possible transition to metabolic alkalosis after sudden reduction of pCO_2 should be considered.

Case studies and control questions

Case 3.1

A 5-year-old boy, previously healthy, admitted for increased thirst, polyuria, weight loss, fatigue and irritability with 2 weeks of duration. On physical examination child lethargic, has dry mucous membranes of mouth and tongue, sunken eyes, tachycardia 155/min, tachypnea 40/min.

Serum	Result	RI
Glucose	22	3.3 – 5.5 mmol/L
Na^+	131	135 – 145 mmol/L
K^+	5.3	3.6 – 5.5 mmol/L
Cl^-	96	95 – 110 mmol/L
ABR		
pH	7.19	7.36 – 7.44
pCO_2	2.8	4.8 – 5.8 kPa
pO_2	11.5	9.5 – 13.9 kPa
HCO_3^-	9	22 – 26 mmol/L

Questions:

a. What type of A-B disorder is present?
b. Calculate AG.

Case 3.2

The 10-month-old girl was admitted to the hospital after 4 days of diarrhea. The child´s mother reported that the previous 2 days she had drunk only about 200 ml of milk and had an of 5-6 watery movements a day. When examined, the child was lethargic, pale, cold, with sunken eyes without tears when crying. Tachypnea 55/min. Laboratory results on admission as follow:

Serum	Result	RI
Glucose	2.8	3.3 – 5.5 mmol/L
Na^+	140	135 – 145 mmol/L
K^+	2.8	3.6 – 5.5 mmol/L
Cl^-	114	95 – 105 mmol/L
ABR		
pH	7.24	7.36 – 7.46
pCO_2	4.1	4.8 – 5.8 kPa
pO_2	12	9.5 – 139 kPa
HCO_3^-	15	22 – 26 mmol/L

Questions:

a. What type of A-B disorder is present?
b. Identify all simultaneous ion disturbances?

Case 3.3

A 38-year-old athlete is admitted to the hospital with a diagnosis of asthma. The patient complains of shortness of breath and a tight feeling in his chest and appears near panic. Breathing is strenuous, and the respiratory rate is high. An arterial blood gas analysis reveals the following:

ABR		
pH	7.49	7.36 – 7.46
pCO_2	3.3	4.8 – 5.8 kPa
pO_2	8.9	9.5 – 13.9 kPa
HCO_3^-	18	22 – 26 mmol/L

Questions:

a. What type of A-B disorder is present?
b. Identify all simultaneous ion disturbances?

Self-assessing questions

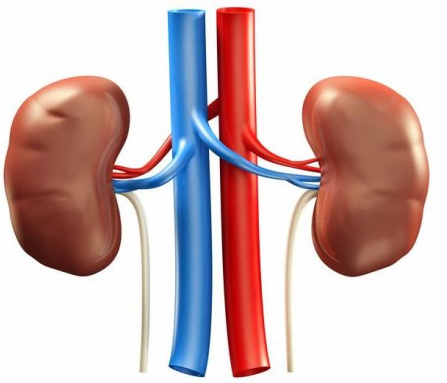
1. Explain relationship between pH and H^+ concentration.
2. Explain the term compensation of acid-base disorder.
3. How does a decrease of pCO_2 influence pH value and what type of compensatory response will follow?
4. Name causes of metabolic acidosis with high anion gap.
5. Explain, how chronic diarrhoea influences blood pH.
6. Name extra-pulmonary causes of RAL.
7. Name examples of ion shifts between ICF and ECF as a consequence of acidosis and alkalosis.

KEY INFORMATION

- ☑ The normal cellular metabolism produces excess of H^+ which concentration is maintained within narrow reference range.
- ☑ The physiological control of $[H^+]$ is maintained by three interrelated mechanisms: buffering systems, the respiratory system, and the renal system. They control the ratio $[HCO_3^-]/pCO_2$.
- ☑ Acid-base disorders can be divided into respiratory and metabolic, acute and compensated, single and mixed.
- ☑ A decrease in the ratio $[HCO_3^-]/pCO_2$ results in acidosis. Acidosis can by caused by increased H^+ production or ingestion, excessive loss of HCO_3^-, or increased retention of CO_2.

- ☑ Alkalosis is caused by increase in the $[HCO_3^-]/pCO_2$ ratio, which may result from loss of H^+, retention of HCO_3^-, ingestion of alkali, and reduction of pCO_2.
- ☑ Compensation of acid-base disturbance is the physiological process which tends to restore the blood pH to normal values, by respiratory and renal mechanisms.
- ☑ Metabolic acid-base disorders are caused by changed content (concentration) of acid and alkaline substances, which alter pH. Their concentration is elevated because of increased metabolic production, decreased elimination or exogenous input.
- ☑ Regardless of their clinical cause, metabolic acidosis is always associated with a decrease in plasma $[HCO_3^-]$. Similarly, MAL is associated with increase in $[HCO_3^-]$.
- ☑ MAC can be divided into high anion gap MAC (retention of unmeasured anions), and normal AG MAC (hyperchloremic and dilutional).
- ☑ Respiratory acid-base disorders are caused by failure of CO_2 elimination - RAC with hypercapnia or RAL with hypocapnia.
- ☑ The renal response to RAC increases plasma $[HCO_3^-]$ in order to return the $[HCO_3^-]/ pCO_2$ ratio to normal, and results in secondary hypochloridemia.
- ☑ Similarly, in RAL the increased renal excretion of HCO_3^- decreases plasma $[HCO_3^-]$, but it is relatively slow to achieve optimal effectiveness.
- ☑ Two or more single acid-base disorders may occur simultaneously. Their effect on pH may be antagonistic or complementary.
- ☑ Acid-base data have to be interpreted in connection with other laboratory and clinical findings in patients.
- ☑ Certain clinical conditions are constantly accompanied by typical acid-base disorders.
- ☑ The principal marker of pulmonary oxygenation is arterial pO_2. Capillary blood informs only about situation in peripheral tissues (ear, finger, heel in new-born).
- ☑ In chronic respiratory acidosis, a rapid decrease of pCO_2 by help of assisted ventilation may result to severe metabolic alkalosis, which threatens a patient with impaired release of oxygen from hemoglobin and vasoconstriction of cerebral vessels.

4



Kidney disorders

CONTENT

Many renal and extrarenal diseases may affect kidney function. Laboratory testing on blood and urine is integral part of diagnostics of renal function disorders. Assessment of basic renal function, as glomerular filtration rate and urine analysis, belongs to the portfolio of laboratory tests used in nearly all medical specialties.

This chapter should provide an overview of biochemical tests the most frequently used in diagnostics and monitoring of renal functions:

- assessment of glomerular filtration rate;
- urine analysis with emphasis on those finding typical for renal diseases (proteinuria, hematuria);
- basic tests for assessing of tubular function;
- laboratory findings in acute and chronic kidney failure.

4.1 Basic physiology

Kidneys represent a paired organ located behind the peritoneum at the back of the abdominal cavity. Each kidney consists from about 500 000 – 800 000 independent functional units, nephrons, although this number falls with age. The multiple renal functions can be categorised into excretory, homeostatic and endocrine (Table 4.1). Excretory and homeostatic functions are achieved through glomerular filtration and both tubular reabsorption and secretion. Glomerular filtration barrier consists of three layers - specialized endothelium in glomerular capillaries, basement membrane rich in negatively charged proteoglycans and epithelial cells, podocytes. All layers create a network with decreasing size of pores, which is permeable to water and low molecular weight substances, but impermeable to macromolecules like proteins. This permeability is determined by both size and charge of molecules, thus only proteins smaller than albumin (68 kDa) pass into ultrafiltrate and positively charged molecules are filtered more readily than those with negative charge.

Newer findings indicate, that the glomerular filtration surface is not only a simple filter with certain size of pores. They emphasize the role of basement membrane functioning rather than gel and its features influence permeability of filtration surface for different molecules.

The glomerular filtration rate (GFR) depends on:

- the difference in hydrostatic and oncotic pressures between the glomerular capillaries and the lumen of the nephron;
- the nature of glomerular basement membrane;
- total available filtration area (= number of functioning nephrons).

TABLE 4.1 OVERVIEW OF PHYSIOLOGICAL RENAL FUNCTIONS

Category	Example
Excretion of waste products and toxins	N-compounds - urea, creatinine, uric acid; drugs, endogenous metabolites
Control of ECF volume and composition	water, electrolytes
Acid-base balance regulation	H^+ excretion, reabsorption + regeneration of HCO_3^-
Endocrine	renin, calcitriol (1,25(OH)dihydroxy calciferol), erythropoietin
Metabolic	20% of gluconeogenesis (mostly during starvation)

The total volume of glomerular filtrate is about 180 L/24 h, with similar composition to plasma except of a minimal content of proteins. This complex mixture of water, ions and small molecules need to be processed during passing the renal tubules. Water and ions have to be mostly retained in the body in regulated manner, proteins are reabsorbed and catabolised, and metabolic waste products have to be excreted.

The proximal tubule is responsible for the obligatory (independent on hormones) reabsorption of major part of glomerular filtrate. This is performed by energy-dependent mechanisms resulting in reabsorption of 75% of filtered Na^+, all of the K^+, HCO_3^-, amino acids and glucose, accompanied with an iso-osmotic amount of water. In the distal tubule the further reabsorption

of Na^+ under control of aldosterone takes place and also an electrochemical gradient is generated which promotes the secretion of K^+ and H^+.

To maintain constant plasma osmolality (290 mOsm/kg), the kidneys are able to change urine osmolality in the maximal range 40 – 1 200 mOsm/kg. The concentration of urine is performed by osmotic transfer (diffusion) of water from the tubular lumen into the hyperosmolar peritubular interstitium. A special role of Henle´s loop, particularly the different permeability of its segments, contributes generating of medullary hypertonicity, on which the ability depends of kidney to create and excrete concentrated urine (Figure 4.1). The last parts of nephron, collecting ducts, pass through hypertonic renal medulla and adjust the final concentration of urine.

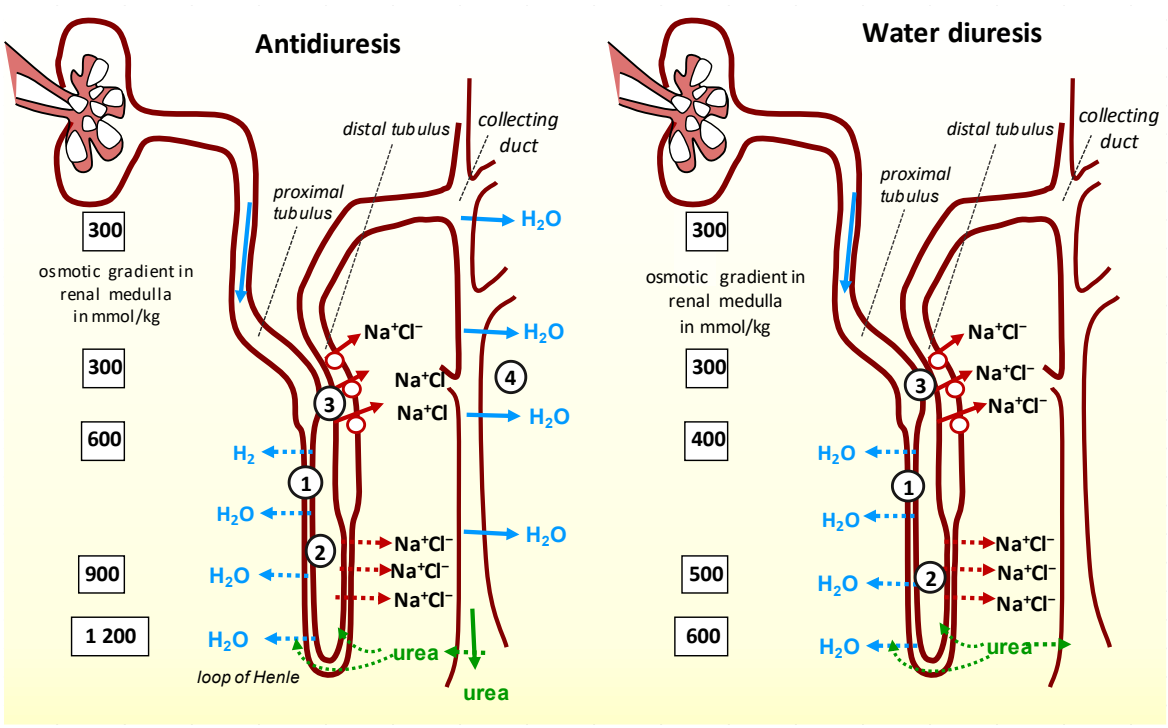


FIGURE 4.1 Role of loop of Henle in concentrating of urine

It contains three segments with different permeability: 1- permeable to water and impermeable to solutes; 2-free permeable to Na^+, Cl^- and urea (passive transport), impermeable to water; 3-active transport $Na^+/K^+/2Cl^-$ helps to maintain medullary concentration gradient; 4- reabsorption of water under influence of ADH

In the absence of ADH (vasopressin), the cell lining the collecting ducts are impermeable to water, resulting in the excretion of diluted urine with osmolality close to or lower than plasma osmolality. If ADH is secreted as a consequence of high osmolality or other non-osmotic factors, it stimulates the shift of water channels, aquaporins, into membranes of tubular cells. The subsequent passive reabsorption of water down the osmotic gradient between the tubular lumen and interstitial fluid results in formation of concentrated urine.

Different diseases may affect kidney function either selectively (for example glomerular or one of tubular functions) or several functions are affected simultaneously. Laboratory tests discussed later are useful in detecting the presence of renal disease and in assessing its progression. However, they are of less value in determining the causes of disease.

4.2 Urine analysis

Any assessment of renal function should start with urine analysis, which represent the quick, cheap and non-invasive tool for detecting kidney damage. Urine is a physiological fluid of widely varying composition and volume influenced by following processes:

- blood composition (e.g. hyperglycemia, acidosis, dehydration, drugs, toxins);
- impairment of glomerular and tubular renal function;
- urinary tract disorders (e.g. inflammation, bleeding, injury);
- other organs damage (e.g. liver disease, diabetes, inflammatory disorders);
- artificial changes during storage of urine (increase in pH after bacterial breakdown of urinary urea, breakdown of cells).

A fresh early morning specimen after overnight fasting provides the most valuable information thanks to the optimal degree of concentration and exclusion of food and medicine influence. An ideal solution is a mid-stream collection of urine and examination within 1 h which diminishes possible contamination and changes expected during storage and transportation of urine before analysis. Urine analysis consists of the assessment of its appearance and volume, chemical examination using urine dipsticks and evaluation of urinary sediment (INFO 4.1).

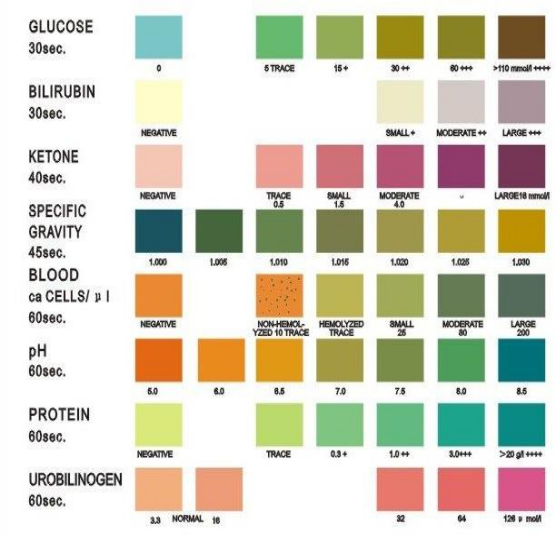
Info 4.1 Urine analysis – appearance, dipsticks

COLOR of reflects the presence of urochrome pigments and depends on concentration of urine. If urine is unusually dark or coloured, is sent for laboratory testing. Abnormal colour may be for example:

- Blue-green: methylene blue, riboflavin, Pseudomonas infection, propophol
- Pink-orange-red: hemoglobin, myoglobin, porphyrias, rifampicin, carotenes
- Red-brown-black: Hb, myoglobin, RBC, bilirubin, homogentisic acid, levodopa, methyldopa, melanin

TURBIDITY is caused by bacteriuria, hematuria or leukocyturia.

CHEMICAL ANALYSIS of urine may consist of any or all of the following parameters (only semi quantitative): pH, specific gravity, protein, glucose, ketones, blood (Hb), bilirubin, urobilinogen, nitrite, leukocytes. Multi-array urine strips are usually used in practice. For chemical principles of particular test see textbook of medical chemistry.



Chemical analysis of urine

Some pathological findings in urine discussed in following paragraph are related to renal disease. **Specific gravity** (normal range 1 003 – 1 040, mean value 1 015 – 1 025 kg/m^3) or **osmolality** (normal range 40 – 1 200, mean value 600 – 900 mmol/kg) serve as indicators of kidney concentrating or diluting ability. Reagent strips detect ionic species only, thus underestimate specific gravity if non-ionized substances are present (e.g. glucose). Specific

gravity above 1 018 kg/m^3 is a prove of preserved concentrating ability of kidney, values oscillating close to 1 010 represent so-called isosthenuria, inability to concentrate or dilute urine.

Physiological urinary **pH** varies in the range 4.6 − 8.0 in healthy individual on mixed diet is slightly acidic (5.5 − 6.0). Measurement of pH is an important part of urinary acidification tests in renal tubular acidosis or in renal stone formers.

Proteinuria, specifically the permanent one, represents an important feature of renal disease. Diagnostic urinary strips detect protein concentration >150 mg/L and they are most sensitive to albumin, less to globulins and low molecular weight proteins (e.g. free light chain of Ig = Bence-Jones proteinuria in myeloma patients). The dipstick allows only a rough estimation of protein concentration, as following example shows: trace = 0.05 − 0.2 g/L, 1+ = 0.3 g/L, 2+ = 1 g/L, 3+ = 3 g/L, 4+ = more than 5 g/L. False positive result may be present in alkaline urine, frequently present in case of bacteriuria or long storage of urine after sampling. Persistent proteinuria by dipstick needs always further testing (see section 4.3).

Positive reaction for **blood** in urine may indicate presence of erythrocytes, hemoglobin or myoglobin. Examination of urinary sediment can distinguish these possibilities along with further laboratory signs of intravascular hemolysis (anemia, reticulocyte count, bilirubin and LDH activity in serum, urobilinogen in urine) or rhabdomyolysis (information about possible muscle disorder, increased muscle proteins - myoglobin, CK). Hematuria usually indicates glomerular disease, infection, tumours, or injury of kidney or urinary tract.

Nitrites represent a screening test for significant bacteriuria and indicates urinary tract infection. Test positivity depends upon the conversion of urinary nitrates (derived from diet) to nitrites by activity of some Gram-negative bacteria (e.g. *E. coli, Proteus, Klebsiella, Pseudomonas, Staphylococcus, Aerobacter*), resulting in colour change of reagent strip. False negative result may occur due to lack of nitrates in food, short interval between urination (frequent urination, polyuria) and if infection is caused by other types of bacteria.

Leukocytes in urine usually signalize inflammatory disorders of genitourinary tract, including kidney. Leukocytes detection is based on content of their enzyme, esterase, which produce a color product.

Glucose in urine is mostly of prerenal origin (the chief cause is diabetes mellitus); renal glucosuria occurs due to decreased tubular reabsorption of glucose (plasma glucose is normal).

Urinary sediment

The next part of urine analysis is **examination of the urinary sediment** obtained by centrifugation of a fresh sample of urine. The examination is of particular value in case of blood, protein and leukocytes positivity during dipstick analysis. Urinary sediment is investigated using a conventional microscope or automated analyser, which operates on the principle of microscopy using digital cameras, taking hundreds of images with urinary elements. Increasingly, analysers that differentiate cellular elements by flow cytometry are also used for investigation of urinary sediment components.

There are several organic and inorganic bodies (elements) usually found in urinary sediment (Figure 4.2). The organic components consist of cells of different origin and cylinders. Inorganic constituents are mainly crystals which precipitate in the urine at different pH.

Red blood cells (RBC): Hematuria is the presence of abnormal amount of RBC in urine due to glomerular damage, tumors eroding urinary tract anywhere alongside its length, kidney trauma, urinary tract stones, etc. The positivity of blood in urine analysis combined with negative finding of RBC in sediment suggests presence of hemoglobinuria or myoglobinuria, which could be confirmed by serum tests (laboratory signs of intravascular hemolysis or muscle damage).

Investigation of urinary sediment by **phase-contrast microscopy** enables distinguishing between glomerular and non-glomerular origin of hematuria. Erythrocytes with changed shape - dysmorphic cells (especially acanthocytes) indicate a glomerulopathy, erythrocytes uniform in their shape and size (isomorphic) originate from subglomerular parts of nephron or urinary tract.

White blood cells (WBC): Abnormal count of leukocytes (usually granulocytes) signalizes infection in either the upper or lower urinary tract or is a marker of acute glomerulonephritis. WBC from vagina, especially in the presence of vaginal or cervical infections, or from the external urethra in men and women frequently contaminate urine.

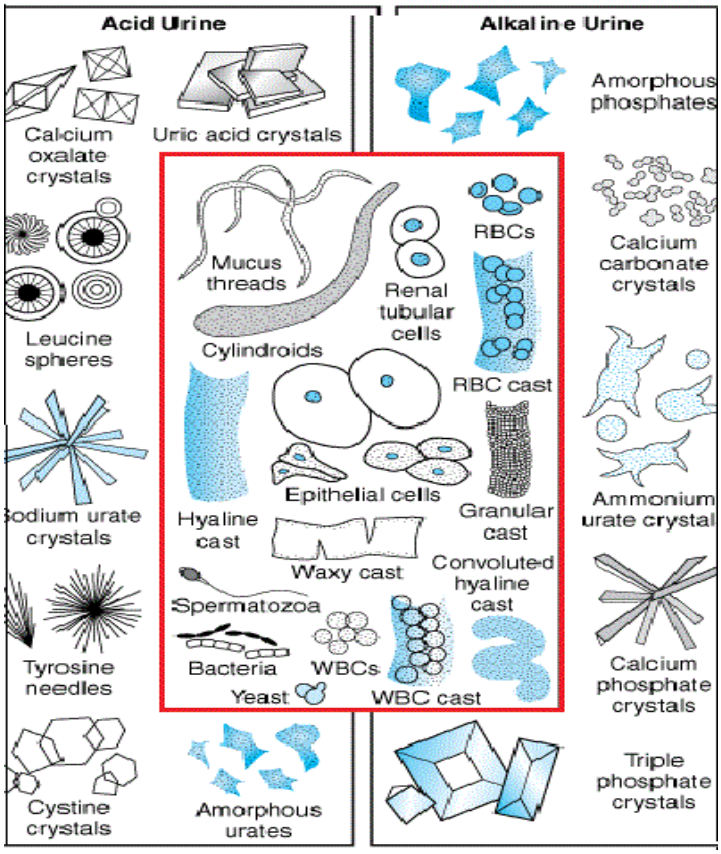


FIGURE 4.2 Organic (in red frame) and inorganic (in black frames) components of urinary sediment

Cylinders: Urinary cylinders are small cylindrical castings of tubules coming from the distal part of the nephron. Factors like low urine flow rate, high salt concentration or low pH favour protein denaturation and precipitation, thus enhance casts formation. The cylinders consist of HMW glycoprotein, physiologically synthesized by tubule cells (orosomucoid or Tamm-Horsfall protein). These, so-called hyaline cylinders may be occasionally seen in patients without renal disease, for example after fever, dehydration, severe physical activity, etc.

Hyaline cylinders may entrap material contained within the tubular lumen, such as plasma proteins or cells, resulting in the formation of pathological **protein cylinders** (granular, vaxy), or cellular (erythrocyte, leukocyte, epithelial or mixed) cylinders. The presence of large numbers of hyaline or other pathological cylinders is always evidence of severe renal disease. On the other hand, the absence of cylinders does not exclude such a disease, as they are unstable in the urine, prone to disintegration, especially in urine with low specific gravity and alkaline pH.

Interpretation of urinary sediment

Normal finding in urinary sediment consists from only **few cells** (e.g. RBC, WBC, epithelia from lower urinary tract) or crystals, and only a rare finding of hyaline cylinders. Pathologically increased number of all cells along with other types of casts and crystals may indicate acute or chronic glomerular, tubulointerstitial, vascular or metabolic kidney disease. Cells in the urinary sediment may originate from the kidneys or from any part of the urinary tracts, including the external genitalia. On contrary, cylinders are always formed only in the renal distal tubules or collecting ducts. Diagnosis of a particular disease almost always requires correlation of urinary findings with other laboratory and clinical markers (Table 4.2). For example, differential diagnosis of persistent hematuria involves a wide range of glomerular, tubulointerstitial, urological and vascular diseases.

TABLE 4.2 EXAMPLES OF INTERETATION OF URINARY SEDIMENT ABNORMALITIES

RBC	RBC casts	WBC	WBC casts	Tubular cells	Granular casts	Bacteriuria	Associated kidney disease
+	+	+	+				Acute glomerulonephritis
+	-			+	+		Hereditary nephritis, vascular nephropathy
+	-	+	-	-			Any type of urological disease
(+)		+	+				Inflammatory renal disease
(+)		+	-			+	Lower urinary tract infection

4.3 Proteinuria

Renal protein handling

Examination of urinary proteins represents one of the basic tests in nephrology, particularly in early diagnosis of kidney disease, monitoring their progression, assessing therapeutic response or estimation the risk of renal failure as well as cardiovascular risk in patients. Renal excretion of proteins mainly depends on the size of the filtration load and the effectiveness of tubular reabsorption processes. In physiological circumstances, the glomerular ultrafiltrate contains ~ 30 mg/L protein, corresponding to a total filtration load of ~5 g/24 h.

Due to effective tubular reabsorption and catabolism in tubular cells, the final protein excretion into urine is much less than a consensus value of 150 mg per day, which is considered the upper limit of physiological proteinuria. Proteins present in urine originate mainly from kidney tubules (50 – 60%, Tamm-Horsfall glycoprotein, uromodulin), plasma (20 – 40%, albumin and low molecular weight proteins (LMW), e.g. free immunoglobulin light chains, tissue degradation products) and finally from the lower urinary tract (IgA, IgG).

Renal protein excretion exhibits significant biological variability and may also increase due to extrarenal reasons, such as severe physical activity, convulsions, fever, cold, emotional stress, upright position. All mentioned situations affect glomerular hemodynamics and result in **transient or functional** proteinuria which disappears when the underlying causes are resolved. Postural or **orthostatic proteinuria** can be diagnosed by a separate examination of a nocturnal or early morning urine sample that is negative, while other random, daily urine sample is positive for the presence of proteins. Any persistent proteinuria generally suggests kidney disease.

Commonly, proteinuria is classified into **glomerular**, if the glomeruli become abnormally leaky, **tubular** proteinuria, when tubular reabsorption of proteins becomes defective, or **overflow** (prerenal) proteinuria, when filtration of excessive amounts of low MW proteins exceeds tubular reabsorption capacity (Table 4.3). This classification has limited clinical application, while different types of proteinuria commonly occur together, in particular glomerular and tubular proteinuria.

TABLE 4.3 CHARACTERIZATION OF PROTEINURIA

Type	Causes	Examples of proteins found in urine
Glomerular	Increased permeability due to damage of glomerular wall (immune complex deposition, diabetic nephropathy...): a. Loss of selectivity to charge b. Loss of selectivity to charge and size of molecule	a. Selective proteinuria: albumin, MMW - transferrin b. Nonselective: all plasma proteins including HMW (IgG)
Tubular	Decreased tubular reabsorption capacity and/or release of intracellular components (e.g. nephrotoxic drugs, anoxia) Decreased nephron number due to progressive kidney disease: increased filtered load per nephron	Predominantly physiological LMW proteins: α1-microglobulin, β2-microglobulin, retinol binding globulin; Enzymuria: NAG, ALP, glutathione-S-transferase; Tamm-Horsfall glycoprotein
Overflow	Increased plasma concentration of relatively freely filtrated protein (myeloma, rhabdomyolysis, hemolysis)	Free light chains of immunoglobulins Myoglobin, hemoglobin Amylase
Postrenal	Bleeding from subglomerular part of urinary tract	All spectrum of plasma proteins + α2-macroglobulin (non-filterable)

Testing of proteinuria

Urine analysis with testing dipstick represents screening test for detection proteinuria. In case of positive screening test (1+ and more) confirmation of proteinuria by quantitative measurement **protein to creatinine ratio (PCR)** in random urine sample should follow.

The cut-off PCR value for adult physiological proteinuria is up to 15 mg/mmol. Values above 50 mg/mmol represent significant proteinuria and values above 100 mg/mmol are typical for nephrotic range proteinuria. Physiological proteinuria varies in children depending on the age and size of the child. In children under one year of age it is customary to report proteinuria per m^2, and in older children it is also possible to use the PCR (Table 4.4).

TABLE 4.4 REPORTING OF URINARY PROTEIN EXCRETION IN CHILDREN AND ADULTS

Parameter	Age	Physiological	Nephrotic
Prot/m^2/h	<2 years	<4	>40
Prot/m^2/24 h	<1 years	<100	>1000
PCR mg/mmol	<2 years	<50	>200
PCR mg/mmol	2-15 years	20 – 25	>200
PCR mg/mmol	>15 years	<15	>200

Excretion of proteins in collected 24-hour urine (U-Prot/24 h) is a traditional method for quantifying proteinuria, particularly when monitoring the progression of kidney disease or effectiveness of treatment. However, urine collection is an inconvenient procedure for the patient and up to 1/3 of the samples in the laboratory come from incomplete urine collection.

Based on good agreement between proteinuria in single morning urine and collected 24-hour urine, more recent recommendations prefer to investigate PCR as an alternative to quantitative proteinuria. Reporting of results of proteinuria per mmol of creatinine in the form of PCR allows correction for the varying urine concentration. Examination of the first morning urine sample is also necessary to exclude transient orthostatic proteinuria.

Based on daily protein loss in urine, proteinuria is considered mild (0.15 – 1.5 g) or moderate (1.5 – 3.5 g). Proteinuria above 1 g/24 h is highly likely to have glomerular origin, tubular proteinuria usually does not exceed 1 – 1.5 g/24 h. If protein loss exceeds 3.5 g/24 h, nephrotic syndrome develops with typical findings of hypoproteinemia, hypoalbuminemia with subsequent edema, hyperlipidemia and secondary hyperaldosteronism. The extent of proteinuria in nephrotic syndrome does not always correlate with the severity of glomerular impairment. The reason is enhanced tubular catabolism of filtered proteins which have been lost from circulation but do not appear in urine.

The presence of proteinuria is the most common laboratory marker of chronic kidney disease (CKD) in addition to reduced glomerular filtration. Proteinuria is not only a result of kidney disease, but directly accelerates its progression. Numerous clinical studies have shown that proteinuria is a risk factor not only for the progression of renal disease but also for cardiovascular morbidity. In some clinical settings it is useful to identify the range of proteins

present in urine and to estimate a pattern of proteinuria. Estimation the type of proteinuria is based on qualitative or quantitative analysis of proteins excreted in urine (Figure 4.3; INFO 4.2).

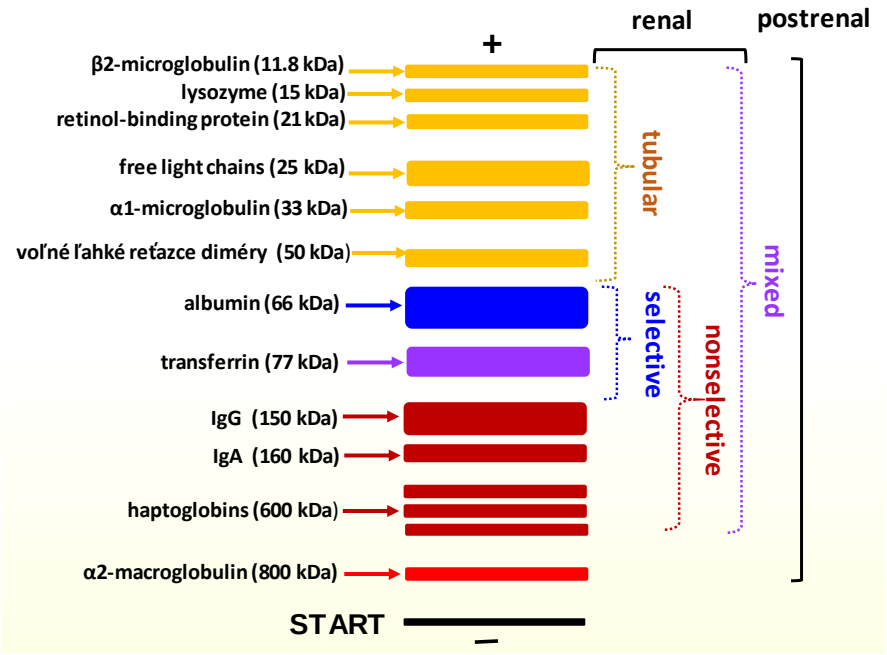


FIGURE 4.3 Protein excretion in different types of proteinuria (SDS electrophoresis – separation according to molecular mass)

INFO 4.2 Profiling of proteinuria

Electrophoretic methods provide qualitative to semi-quantitative analysis of proteins of different molecular weight indicating glomerular, tubular or mixed proteinuria. Another approach is based on the estimation of specific urinary proteins (so-called indicator proteins) by turbidimetry or nephelometry, e.g. albumin, transferrin and IgG are glomerular markers; α1-microglobulin tubular marker and α2-macroglobulin is an indicator of bleeding into the urinary tract because it is not filtered into the urine due to size of its molecule.

The pattern of their excretion (ideally in form of specific protein to creatinine ratio) helps to differentiate type of proteinuria. In addition, the most common types of pre-renal proteinuria can also be confirmed by serum tests, e.g. increased myoglobin concentration strongly indicates the presence of myoglobinuria (dark colored urine with positivity for blood and protein). Similarly, the presence of monoclonal free light chains (FLC, Bence-Jones proteinuria) is predicted or confirmed by elevated concentration of involved type free light chains in serum.

Albuminuria

Normal kidneys excrete daily less than 30 mg albumin because of intensive tubular reabsorption and catabolism of albumin to amino acids. The most frequent causes of chronic kidney disease - diabetes mellitus, hypertension and chronic glomerulonephritis are especially in their early stage connected with only slightly elevated albumin excretion, which is not detectable by using qualitative urine testing strips. **Increased albuminuria** (incorrectly called microalbuminuria) is detectable by sensitive quantitative immunochemical methods even in the early stage of glomerular damage, when testing of proteinuria by urinary strips is negative. Similar to proteinuria, a random morning urine sample is suitable for measurement **albumin**

to creatinine ratio (ACR). Due to high biological variability of daily albumin excretion, two positive tests from three analysed urine sample in the period of 3 – 6 months are necessary for confirmation of microalbuminuria. Urinary tract infection (UTI), contamination of urine with blood, dehydration or severe physical activity can cause false positivity. Table 4.5 summarizes the most frequent used forms of result reporting in random and collected urine sample. Diagnostics of increased albuminuria in patient with diabetes mellitus allows an early identification of diabetic nephropathy, and initiation of treatment measures to reduce the risk of progressive kidney damage and cardiovascular mortality.

TABLE 4.5 REPORTING AND CLASSIFICATION OF URINARY ALBUMIN EXCRETION

Parameter	Normal	Microalbuminuria	Macroalbuminuria*
ALB mg/L	<20	20 – 200	>200
ALB mg/24 h	<30	30 – 300	>300
ALB µg/min**	<20	20 – 200	>200
ACR mg/mmol	<2.8	2.28 – 22.8	>22.8

* *usually detectable by routine method for proteinuria*

*** collected urine sample is needed (overnight or 3-hrs morning urine)*

Hematuria

The presence of blood in the urine (hematuria) always requires evaluation of its duration (transient-persistent), severity (microscopic-macroscopic), presence of additional symptoms (asymptomatic-symptomatic), like proteinuria and other urinary abnormalities, and underlying causes. Hematuria accompanies plenty of renal diseases (glomerular and tubulointerstitial), however, it may be also caused by extrarenal disorders (Table 4.6).

TABLE 4.6 CHARACTERIZATION OF HEMATURIA

Type	Causes	Condition or disease
Renal - glomerular	Glomerulonephritis Systemic diseases Hereditary nephropathies	IgA nephropathy, SLE, Goodpasture syndrome, thin basement membrane disease, Alport´s syndrome
Renal - nonglomerular	Tubulointerstitial disorders Tumours, trauma, stones, febrile illnesses	Nephritis, acute tubular necrosis, Hydronephosis, Grawitz, Wilms tumour
Postglomerular	UTI, bleeding, nephrolithiasis, Prostate diseases Urogenital mucosal injuries, Metabolic causes Tendency to bleeding	Cystitis, urethritis, prostatitis, BPH, prostate biopsy, Radiotherapy, cathetrization, Hypercalciuria, hyperuricosuria Thrombocytopenia, coagulopathy

SLE - systemic lupus erythematosus, BPH - benign prostate hypertrophy

Blood originating from the nephron is termed as **glomerular hematuria**. In case of **non-glomerular hematuria** anything that disrupts the uroepithelium such as irritation, inflammation or invasion can result in normal appearing RBCs in the urine. It is possible to discriminate the origin of hematuria by examination of urinary RBC dysmorphism using the phase-contrast microscope. Usually, at least three different shapes of RBCs are present in the urine of patients with glomerular disease, giving rise to a polymorphic picture, in contrast with non-glomerular hematuria in which all red cells are similar in shape (isomorphic), resulting in a monomorphic pattern. The finding of more than 5% of acantocytes strongly suggest glomerular hematuria.

The exact cause of dysmorphic RBCs is not fully known. Probably it involves a combination of mechanical damage upon squeezing of red cells through the glomerular membrane, which is followed by exposure to the changes of osmotic environment when cells pass through the tubular system.

4.4 Testing of glomerular function

The glomerular filtration rate (GFR) is the generally accepted indicator of renal function. Examination of GFR represents an important tool for diagnostics and monitoring, which serves for assessment the severity of renal dysfunction, progression of a known renal disease or for adjustment of medicine dosing to reduced GFR. The GFR depends on three main factors, which were mentioned at the beginning of this chapter. They all may be modified by disease, but in absence of a significant change in filtration pressure or in the structure of glomerular membrane, the GFR reflects the number of functioning glomeruli and informs about the degree of renal impairment by disease.

Measurement of GFR is based on a theoretical concept of **clearance**, which means the volume of plasma, from which a substance is completely removed during one pass through kidney per time unit (minute, second). Accurate measurement of GFR requires a substance (optimally endogenous), which is produced and removed from the body with constant rate and its plasma concentration does not change during the investigation. If the substance is freely filtrated by glomeruli and is neither secreted nor reabsorbed in tubules, then the clearance value is equal to GFR. Unfortunately, no endogenous substance fulfils these criteria, but creatinine is close to them.

Creatinine clearance

Creatinine (M_w = 113 g/mol) is the endogenous product of muscle metabolism, it originates from creatine phosphate, an energy-rich compound important for muscle work. About 1 – 2% of muscle creatine, which is mainly produced in the liver, is daily converted to creatinine by non-enzymatic dehydration. Creatinine (Crea, Cr) as end-product of nitrogen metabolism is excreted into urine mainly by glomerular filtration, only small part (less than 10%) is actively secreted into urine by tubules.

If GFR falls, the contribution of tubular secretion to creatinine excretion increases up to 50%. Therefore, with a slight decrease in GF (up to 50%), serum creatinine concentration (S-Cr) does

not increase above upper limit of reference interval. The reference range of S-Cr in adults varies between 55 – 120 µmol/L depending on gender, age and analytical method. In contrary, individual subjects maintain their serum concentration within much tighter limits, inter-individual variation is low. A progressively rising serum creatinine concentration, even within the reference range, indicates declining renal function. Decreased GFR have following possible causes:

1. glomerular filtration pressure is reduced (as in circulatory shock, heart failure);
2. tubule hydraulic pressure is elevated (urinary tract obstruction);
3. plasma colloid osmotic pressure rises (severe volume depletion, hemoconcentration, hyperproteinemia);
4. permeability is reduced (diffuse glomerulopathies);
5. filtration surface/area is diminished (focal or diffuse nephron loss).

Relationship between S-Cr and creatinine clearance (C_{Cr}) has hyperbolic shape, which means that decrease in GF does not manifest as proportional increase creatininemia (Figure 4.4). The physiological value S-Cr does not exclude pathological decrease in GF in a subject with low muscle mass. In addition, progressive increase in S-Cr even within the reference interval, may indicate a decline in renal function. The GFR estimation is based on measurement of serum creatinine concentration S-Cr and its urinary excretion rate U-Cr × V (GFR = U-Cr × V/S-Cr). For details see INFO 4.3.

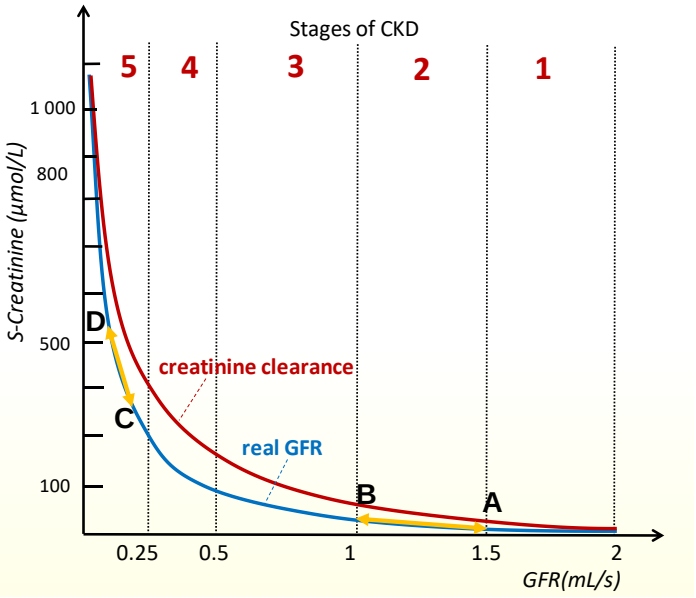


FIGURE 4.4 Relationship between S-Cr and GFR
Points A and B represent a significant decrease in GFR (from 1.5 to 1.0 mL/s), accompanied by only small rise in S-Cr. Points C and D illustrate a small decrease in GFR, which is followed by marked increase in S-Cr. Creatinine clearance tends to overestimate the real GF because of increasing tubular secretion of creatinine.

Several factors influence the determination of creatinine clearance with impact on laboratory result:

1. Laboratory method for creatinine measurement: The most commonly used Jaffe method is not specific because it also captures non-creatinine chromogens in addition to creatinine (e.g., bilirubin, glucose, ketones, ascorbic acid, pyruvate, creatine, cephalosporins, etc.). In contrast, free hemoglobin in a hemolyzed sample reduces the measured creatinine concentration. Newer enzymatic methods have fewer analytical interferences and better comparability with the reference method for creatinine determination.
2. The muscle mass size, food intake (meat) and liver function (synthesis of creatine) influence serum creatinine concentration. Changes in S-Cr due to muscle damage (trauma, dystrophy,

etc.) or pathological muscle mass (acromegalia, limb amputation, severe cachexia, etc.) can result in incorrect values in determining GFR.

3. Creatinine tubular secretion size: In patients with renal failure, the proportion creatinine secreted in tubules dramatically increases compared to people with normal GFR. Some medicines (e.g. cimetidine, trimethoprim, fenofibrate) may block tubular secretion and lead to an increase in serum creatinine concentration.
4. Incomplete urine collection or inaccurate measurement of urine volume is the most common source of errors. This procedure is restrictive situation for patients, requiring their compliance and often also supervision of a medical stuff.

INFO 4.3 Creatinine clearance – measure of GFR

For calculation of creatinine clearance as a measure of GFR four variables are needed: serum and urinary creatinine concentration (S-Cr, U-Cr), urine volume, collecting time. The sample of collected urine (usually 24 h) and blood sample obtained during collecting period is necessary for laboratory analysis. In addition, patient´s weight and height if correction for ideal body surface is made.

C_{Cr} = (U-Cr/ S-Cr) × V; V = urine volume/s

GFR_{corr} = C_{Cr} × 1.73/body surface of patient in m^2

GFR varies according to body size ranging from <0.2 mL/s in neonates up to 3 mL/s in adults with a large muscle mass. GF values depend on age and gender.

Estimated glomerular filtration rate

As has been mentioned before, measurement of creatinine clearance is influenced by several factors, which lower its reliability and accuracy. That is the reason why calculation of the **estimated GFR** (eGFR) is used in clinical practice in order to eliminate errors accompanying creatinine clearance. A number of formulae exist for calculation of eGFR from serum creatinine and additional information, such as age, sex, and ethnicity of patient (Table 4.7).

The very popular formula according to Cockroft and Gault, used in the past for adults, was replaced by newer ones recently. The formula **CKD-EPI** (Chronic Kidney Disease Epidemiology Collaboration Group) for the adult population was derived from data of ten multicentre clinical trials on more than 8000 probands. It was validated in 2009 and it offers higher accuracy of GFR estimation in patients with normal or borderline elevated serum concentration of creatinine compared to previously used MDRD (Modification of Diet in Renal Disease) equation.

The CKD-EPI Working Group reported two new equations in 2012: one based on cystatin C concentration (CKD-EPI_{cys}, 2012) and the other using both serum creatinine and cystatin C concentrations (CKD-$EPI_{Cr\text{-}cys}$, 2012). The two new equations even have been recommended by KDIGO 2012 Clinical Practice Guidelines for the Evaluation and Management of CKD as the principal tool for estimation of GFR. The **Schwartz** formula is still in use for children.

Clinical significance of eGFR is in quick evaluation of renal function in non-acute patients and mainly in screening of chronic kidney disease in an asymptomatic population. Additionally, eGFR may be helpful in some clinical situations where a quick estimation of renal function is necessary, for example before the dosage of a number of nephrotoxic drugs including cancer chemotherapy. The diagnostic cut-off value of eGFR is ≥1.5 mL/s/1.73 m^2, which means

absence of renal disease (stage G1 based on CKD classification – Table 4.14). Values <1.0 mL/s/1.73 m^2 suggest presence of CKD.

TABLE 4.7 EXAMPLES OF EQUATIONS USED FOR ESTIMATION OF GFR

Name	Formula
Cocroft &Gault	= (140-age) x weight (kg)/ S-Cr
MDRD	= 547.1535 x S-Cr $^{-1,154}$ x age$^{-0,203}$ x 1.212 (if black) x 0.742 (if female)
CKD-EPI (2009)	= 141 × min (S-Cr/κ, 1)$^{\alpha}$× max (S-Cr/κ, 1)$^{-1.209}$ × 0,993^{age} × 1,018 [if female] × 1,159 [if black]
CKD-EPI$_{Cys}$ (2012)	= eGFR =133 x min (S-Cys/0.8, 1)$^{-0.499}$ x max (S-Cys/0.8, 1)$^{-1.328}$ x 0.996^{age} x 0.932 [if female]
CKD-EPI $_{Cr-Cys}$ (2012)	= eGFR = 135 × min(S-Cr/κ, 1)$^{\alpha}$ × max(S-Cr/κ, 1)$^{-0.601}$ × min(S-Cys/0.8, 1)$^{-0.375}$ × max(S-Cys/0.8, 1)$^{-0.711}$ × 0.995^{Age} × 0.969 [if female] × 1.08 [if black]
Schwartz	= 0,55 x length/ S-Cr

κ, α – different coefficients for male and female

All calculations based on actual S-Cr concentration have eliminated the possible influence of inaccurate urine collection. However, they continue to be burdened with factors affecting serum creatinine concentration. They should not be used when S-Cr changes rapidly, in individuals with atypical diet (e.g. strict vegetarian diet or use of creatine supplements) or with non-standard muscle mass due to malnutrition, muscle wasting or amputations, or in obesity. In addition, all formulas are not recommended for pregnant women and children.

Estimation of GFR from cystatin C

Cystatin C is a small polypeptide (13 kDa) produced by all nucleated cells at a constant rate with a stable concentration in blood. Its biological function is an inhibition of cysteine proteases. The renal handling of cystatin C differs from creatinine. Although both are freely filtered by glomeruli, cystatin C, unlike creatinine, is reabsorbed and metabolized by the proximal renal ducts. Thus, under normal conditions, cystatin C does not enter the final excreted urine to any significant degree.

The serum concentration of cystatin C remains unchanged with infections, inflammatory or neoplastic states, and is not affected by body mass, diet, or drugs. Cystatin C concentration may be increased in hypercorticism or during steroid therapy, in hyperthyroidism, obesity or oncological patients with rapidly proliferating tumours. Due to immaturity of renal function, cystatin C levels are higher in neonates older than 3 months of age.

When GFR decreases, then serum cystatin C concentration increases as a consequence of decreased renal elimination. Cystatin C is considered to be the more sensitive and earlier marker of GFR in comparison to creatinine. As creatinine has a much smaller molecule than cystatin C, a small decrease in GFR does not affect its elimination and its serum concentration increases later than that of cystatin C.

Grubb´s formulas (CKD-EPI, 2012) based on cystatin C alone or in combination with S-Cr are used for estimation of GFR (Table 4.7). Cystatin C based eGFR may have advantages over

creatinine-based eGFR in certain patient groups in whom muscle mass is abnormally high or low (e.g. quadriplegics, very elderly, malnourished individuals). Cystatin C is of special value in early detection of mild decline of GFR, for example, in ongoing acute renal failure, or in monitoring patient after kidney transplantation or in potential donors of a kidney.

4.5 Examination of tubular function

The principal function of renal tubules is to modify all filtrated amounts of substances by reabsorption or secretion. Specific congenital or acquired renal disorders affecting the renal tubules may cause impaired reabsorption of amino acids, glucose, calcium, phosphate, etc., inappropriate ability to concentrate urine or to excrete acidic urine in case of acidosis. The tubular disorders may occur separately or in combinations (e.g. Fanconi syndrome or renal tubular acidosis). Tubular function tests are used if a specific tubule disorder is suspected.

Fractional excretion

The ratio of excreted to the filtered amount of any substance is called **fractional excretion** (FE). Tubular function can compensate for changes in glomerular filtration, e.g. when GFR decreases due to loss of functioning nephrons, then FE in residual nephrons compensatory increases. That mechanism of the glomerulo-tubular balance allows maintenance of diuresis and excretion of LMW substances per surviving nephrons. For example, if GFR decreases with unchanged FE water, diuresis should decrease followed by water and waste products retention. Thus, increase of FE in residual nephrons protects against that retention.

TABLE 4.8 REFERENCE VALUES AND EXAMPLES OF POSSIBLE CHANGES

Parameter	Normal value	Max. value in CKD	Examples
FE-Na^+	0.010 – 0.020	0.350	↑diuretics, osmotic diuresis in residual nephrons, disorder of tubular reabsorption ↓extrarenal losses, depletion of Na
FE-K^+	0.040 – 0.190	1.5 – 2.0	↑diuretics, hypercatabolism, hyperaldosteronism, tubular compensation of GFR decrease ↓extrarenal losses, depletion of K^+, anabolism, K^+ sparing diuretics
FE-H_2O	0.004 – 0.012	0.3 – 0.4	↑FE: water diuresis ↑FE: osmotic diuresis
FE-Osm	<0.035	≈ 0.035	Isosthenuria: FE-H_2O = FE-Osm

In clinical practice, FE of water and LMW substances (Na^+, K^+, Cl^-, Ca^{2+}, uric acid, etc.) are measured using a random or morning urine and serum sample. Fractional excretion of any substance (n) is calculated as a ratio of clearance of a substance (N) to creatinine clearance according to the following formula:

$$\textbf{FE-N} = \textbf{(U-N} \times \textbf{S-Cr)/S-n} \times \textbf{U-Cr}$$

Clinical significance of FE is in assessing, whether kidney works normally or some adaptation processes have occurred due to renal disease. In some cases, FE helps to find the cause of retention or depletion of electrolytes in the body or to distinguish between osmotic and water diuresis (Table 4.8).

Urine osmolality and tubular concentrating ability

The healthy kidney is able to change urine osmolality in the wide range **40** – **1 200 mmol/kg**. The concentrating or diluting ability of tubules depends upon water balance, availability of antidiuretic hormone, the responsiveness of tubular cells to ADH and on concentration gradient in renal medulla. Person with normal renal function is able to excrete daily solute load, which is obligatory about 600 mmol/day on mixed diet and steady state metabolism, in 500 mL in case of maximally concentrated urine or in 15 L in case of maximally diluted urine.

The diseased kidney losses its concentrating ability at a relatively late stage of the disease. It can achieve osmolality in range **250** – **350 mmol/kg** (isosthenuria). At a maximal urine concentration, diuresis close to 2 L/24 h is necessary for excretion of the obligatory daily load of solutes. This urine volume represents only about 1% of normal GFR (~2 mL/s = 170 L/24 h), but in chronic kidney disease with strongly reduced GFR (0.1– 0.05 mL/s = 4–8 L/24 h) it could take up to 50% of GFR. Consistent regulation of water intake at this stage of the disease is essential. Increased water intake in individuals with limited renal dilution capacity may lead to severe dilutional hyponatremia, while decreased intake to insufficient removal of water-soluble substances, including waste metabolic substances.

The basic laboratory examination includes **urine osmolality**, which is directly proportional to the concentrating work done by kidney and indirectly reflects activity of ADH. Urinary osmolality >800 mmol/kg in random urine sample confirms an adequate renal concentrating function and excludes the need to perform further diagnostic tests. Table 4.9 summarizes selected causes of disorders of urine concentration.

TABLE 4.9 CAUSES OF FAILURE OF KIDNEY TO CONCENTRATE URINE

Causal mechanism	Examples
Lack of vasopressin secretion	Lesions of supraoptic-hypothalamic-pituitary system (e.g. trauma, tumours)
Inhibition of vasopressin release	Psychogenic polydipsia, lesions of the thirst centre causing polydipsia
Inability to maintain renal medullary hyperosmolality	Chronic renal failure, hydronephrosis, hypokalemia, hypercalcemia, renal papillary necrosis (analgetic nephropathy)
Inability of tubules to respond to vasopressin	Renal tubular defects (nephrogenic diabetes insipidus, Fanconi syndrome)
Increased solute load per nephron	Chronic renal failure, diabetes mellitus

Renal function tests assess the ability of the kidney to produce concentrated urine in response to fluid deprivation and vasopressin administration, respectively. There are several local

differences in performing concentrating tests, which are described in details in textbooks of nephrology, but all of them measure serum or urine osmolality. Normally, there is no increase in serum osmolality (275 – 295 mmol/kg) throughout water deprivation, whereas urine osmolality increases to doubled values. If fluid restriction itself is not enough to increase urine osmolality, a synthetic analogue of vasopressin - DDAVP (1-deamino-8-D-arginine vasopressin) is administered to the patient.

After DDAVP administration, urine osmolality is measured every hour for 3 h, and values should reach maximal target value according to the age of a patient. Concentrating tests are useful in distinguishing among hypothalamic-pituitary, psychogenic and renal causes of polyuria (INFO 4.4). The DDAVP variant is preferred in practice because it is more convenient for patients. Therefore, it is used frequently, even without fluid deprivation.

INFO 4.4 Interpretation of tests for renal concentrating ability

Patients with diabetes insipidus of hypothalamic-pituitary origin produce insufficient vasopressin: they do not response to fluid deprivation, but they response to DDAVP. As a rule, these patients show an increase of S-osmolality (more than 300 mmol/kg) and a low urine osmolality (200 – 400 mmol/kg). In DDAVP test, there is a marked increase in U-osmolality to 600 or more mmol/kg.
In nephrogenic diabetes insipidus (e.g. polyuria due to inability of tubules to response to vasopressin), there is a failure to produce concentrated urine in response either to fluid deprivation or to DDAVP. In these patients, U-osmolality remains under 400 mmol/kg and S-osmolality increases as a result of fluid deprivation.

Patient with psychogenic diabetes insipidus should response both to fluid deprivation and DDAVP. However, many of them have hypoosmolality in renal medulla as a consequence of chronic suppression of physiological ADH release and they are not able to reach target urinary osmolality during tests. If fluid restriction lasts longer than 24 h and medullary osmolality is restored, they show normal response to both stimuli.

Acidification tests

Compared with plasma, urine is slightly acidic in healthy subjects on a mixed diet due to physiological overproduction of H^+ during metabolism of nutrients. An alkaline urine may be found in vegetarians and vegans, after ingesting alkali or in patient with urinary tract infection. Urine acidification is a function of distal tubules, where secretion of H^+ takes place strongly depending on the presence of urinary buffers, particularly phosphates.

At least three tubular abnormalities are responsible for acidosis of renal origin, which cannot be explained by a decrease in GF. This condition called **renal tubular acidosis** (RTA) comprises a group of both inherited and acquired disorders affecting either proximal or distal tubules. They are characterized by a **hyperchloremic, normal anion gap metabolic acidosis** with the impaired ability to excrete acidic urine, inappropriate to plasma pH. Chronic acidosis results in demineralization of bones (growth disorders in children), dehydration and ion disorders, mostly hypokalemia. To detect decreased urinary acidification a variety of provocative tests have been used in the investigation of RTAs. They should confirm decreased urinary excretion of H^+ or insufficient reabsorption of HCO_3^- (Table 4.10).

TABLE 4.10 OVERVIEW OF RENAL TUBULAR ACIDOSES

Type	Defects	Forms	Laboratory findings accept NAGMAC
Proximal RTA (type 2)	failure in bicarbonate reabsorption, normal H^+ secretion in distal tubule	isolated defect, part of Fanconi syndrome	Bicarbonaturia, normal acidification of urine after acid load, FE-HCO_3^- < 10%
Distal RTA (type1)	failure of hydrogen secretion, normal other distal tubule functions	isolated inherited defect, part of systemic disease	U-pH > 5.5 during acidification test, ↑renal K^+ losses, often hypokalemia
Hyperkalemic distal RTA (type 4)	decreased tubular secretion of H^+ and K^+	lack of aldosterone, resistance to aldosterone, hyporeninism in renal disease	Hyperkalemia, signs of hypoaldosteronism

Acidification test is used to confirm distal RTA. The test assesses the capacity of kidney to produce acidic urine after a metabolic acidosis has been introduced by administration of ammonium or calcium chloride. Urine pH is then measured on fresh urine sample at hourly intervals for 8 hours. Normally, urinary pH falls below 5.5 after load in at least one sample.

Fractional excretion of bicarbonate after alkalinisation of patient is used to confirm the diagnosis of proximal RTA. If blood HCO_3^- concentration increases to >20 mmol/L after administration of bicarbonate (oral pills), then fractional excretion of HCO_3^- is examined (from serum and urine HCO_3^- and creatinine concentrations). Calculated value of FE-HCO_3^- is normally less than 5%, above 10 −15%, whereas in most cases of distal RTA is higher than 15%.

4.6 Renal failure

Acute kidney injury

Acute kidney injury (AKI) is characterized by abrupt deterioration in kidney function, manifested by an increase in serum creatinine level with or without reduced urine output. The term acute kidney injury has largely replaced terms such as acute renal failure and acute renal insufficiency, which previously have been used to describe the same clinical condition. AKI may occur as a sudden episode of kidney failure or kidney damage develops within a few hours or a few days.

Laboratory investigations help to determine severity of the disease and to monitor its course, but usually do not help much in determining the cause of AKI. Typical clinical and laboratory findings reflect retention of nitrogenous waste products and dysregulation of extracellular volume and electrolytes. **Biochemical clues** to the development of AKI include:

- increase of creatinine, urea and uric acid;
- hyperkalemia and metabolic acidosis;
- oliguria <400 mL/24 h (AKI is nonoliguric in some cases).

The clinical assessment should reveal possible causes of AKI (Table 4.11), prerenal precipitating factors (affecting the blood supply to the kidney), intrarenal (intrinsic kidney diseases) or postrenal cause (intrarenal or extrarenal obstruction). Last two classification systems for diagnostics of AKI used in the last decades - RIFLE (Risk, Injury, Failure, Loss and End stage, 2004) and AKIN (Acute Kidney Injury Network, 2007) have been replaced by clinical practice guidelines released in 2012 by the Kidney Disease Improving Global Outcomes (KDIGO).

TABLE 4.11 CAUSES OF ACUTE KIDNEY INJURY

Cause	Example	Diagnostic tests
Prerenal	Volume depletion of different origin, Systemic vasodilatation (e.g. hypotensive, sepsis, anaphylaxis), Obstruction of renal arteria, Intra-renal vasoconstriction (hepatorenal, cardiorenal, abdominal compartment syndromes) Medication (NSAID, ACEI, AT blockers, etc.)	Assessment of hydration status, Urinary parameters
Renal	*Glomerular*: glomerulonephritis, Interstitial: medications, infectious nephritis, Tubular: ischemic, nephrotoxic (radiographic contrast agents, rhabdomyolysis, hemolysis, TLS, myeloma) Vascular: vasculitis, renal vein thrombosis, malignant hypertension, renal atheroembolic disease	Urine analysis, C3 complement, ANA, anti-dsDNA
Postrenal	*Intrarenal obstruction*: stones, clots, crystals (acyclovir, indinavir), tumours *Extrarenal obstruction:* prostate hypertrophy, bladder, prostate or cervical cancer, neurogenic bladder, wrong placed catheter	USG, other imagine studies

ANA - antinuclear antibody, dsDNA - double stranded DNA, TLS - tumour lysis syndrome

KDIGO defines AKI as any of the following:

- increase in serum creatinine by 26 µmol/L or more within 48 h or;
- increase in serum creatinine to 1.5 times baseline or more within the last 7 days or;
- urine output less than 0.5 mL/kg/h for 6 h.

When interpreting **creatininemia**, it is necessary to take into account hydration of a patient (dilutional effect of intravenous solutions), possible decreased creatinine synthesis in the liver (due to systemic inflammation or impaired synthetic liver function), increased creatinine release from damaged muscles and analytical interference in creatinine measurement (bilirubin, cephalosporins, ketone bodies).

Evaluation of **diuresis** as a diagnostic criterion of AKI has limitations in obese patients or in pregnant women (conversion to weight falsely reduces urine output) or in patients after surgery with the transient release of ADH due to non-osmotic stimulation (e.g. pain, stress, nausea). All patients at higher risk of AKI, e.g. critically ill ones with sepsis, hypovolemia, heart failure or treated with potentially nephrotoxic agents, must be monitored on a daily basis.

The diagnostic procedure for a particular patient with AKI will depend on the clinical situation, severity and duration of the condition. The basic diagnostic palette includes blood and urine tests and kidney imaging (USG). Table 4.12 shows the examinations that can be used to

distinguish oliguria from prerenal (circulatory) and renal causes. The recently studied biomarkers of AKI, which are not routinely used yet, summarizes INFO 4.5.

TABLE 4.12 TESTS HELPING IN INVESTIGATION OF LOW URINARY OUTPUT

Test	Normal	Prerenal	Renal
U-specific gravity (kg/m^3)	1 015 – 1 025	> 1 020	<1 010 (isosthenuria)
U-Na^+ (mmol/L)	> 40	< 20	> 40
FE-Na^+			
U-osmolality (mmol/kg)	> 500	> 500	< 400
U/S-urea	> 10	> 10	< 3
U/S-Cr	20 – 60	> 40	< 20
Index AKI*	2 – 3	< 1	> 3

** (U-Na^+ × S-Cr)/U-Cr*

Laboratory testing is an integral part of monitoring the course of AKI, which can be divided into three phases:

1. In initial **oliguric/anuric phase**, reduced urine production due to decrease of GFR is a hallmark (<400 mL/24 h = oliguria, <50 mL/24 h = anuria). Urine has osmolality and sodium concentration similar to plasma, since the composition of a small amount of glomerular filtrate is almost not altered by damaged tubules. Retention of urea, creatinine, uric acid (azotemia), phosphate and other waste products rises their concentrations in serum. In addition, serum urea concentration increases due to tubular reabsorption and enhanced tissue catabolism. Therefore, in AKI after trauma or surgical operation S-urea tends to rise more rapidly than in patients with renal failure due to acute glomerulonephritis. Serum sodium is usually low due to combination of factors, like water retention and increase of metabolic water from tissue catabolism. Hyperkalemia develops due to impaired renal output, increased tissue catabolism and aggravated by shift from ICF in case of metabolic acidosis. Fluid retention presenting with pulmonary oedema is the frequent complication.
2. The **diuretic phase** of AKI is connected with increase in GFR without appropriate improvement in tubular functions. Diuresis rises up to >5 L/24 hours due to:
   - osmotic effects of high urea concentration in glomerular filtrate and
   - isostenuria (composition of the urine similar to protein-free plasma) following impaired tubular concentrating function. Despite of increase in urine volume the clearance of creatinine, urea and other waste products does not improve to the same extent. During diuretic phase, it is important to monitor serum creatinine, Na^+ and K^+ and urinary excretion of Na^+ and K^+. Urinary losses of electrolytes require to be measured in collected urine for correct replacement therapy.
3. **In the recovery phase** of AKI tubular function is gradually restored and volume and composition of urine becomes close to normal eventually.

INFO 4.5 Newer biomarkers of AKI

Diagnostics of AKI using increase of S-creatinine and decrease of diuresis is sometimes questionable and interpretation of both parameters is always easy. Therefore, newer biomarkers of AKI are being sought, which should replace or supplement serum creatinine.
Intensive research in the last decade failed to discover renal 'troponin' - an early, sensitive and specific biomarker of kidney injury. This is due to pathophysiology of AKI, which is much more complex than acute that of myocardial infarction.
New biomarkers of AKI may be divided into:

- Markers of glomerular filtration: cystatin C;
- Markers of glomerular integrity: albuminuria, proteinuria;
- Markers of tubular damage: NGAL (neutrophil gelatinase-associated lipocalin), KIM-1 (kidney injury molecule-1), IGFBP7 (insulin-like growth factor binding protein 7) glutathione S-transferase, α1-microglobulin;
- Markers of intrarenal injury and inflammation: IL-18, sTREM-1 (soluble triggering receptor expressed on myeloid cells-1).

Many of these biomarkers, although they are not routinely used yet they reflect similarly to tumor markers the diverse molecular aspects of the complex pathogenesis of AZO. It cannot be excluded that some biomarkers will be able to identify patients who will benefit from a specific treatment, such as usual in oncology.

Chronic kidney disease

Chronic kidney disease (CKD) is progressive irreversible impairment of renal structure and function with decrease in the number of functioning nephrons resulting in decline of GFR lasting longer than 3 months. CKD represents a heterogeneous group of diseases with various clinical manifestations. Prevalence of CKD in adults is around 10%, but in some risk populations, it can be up to 50%. According to international guidelines for diagnostics, classification and management of CKD (KDIGO, 2012) diagnosis of CKD is based on the **decrease of GFR** below 60 ml/min/1.73 m^2 (1 ml/s/1.73 m^2) and the presence of additional **markers of kidney injury** (Table 4.13).

GFR is estimated using eGFR calculated from serum creatinine and/or cystatin C concentrations. Proteinuria is screened by the PCR (protein to creatinine ratio) or by ACR (albumin to creatinine ratio) in diabetic and hypertonic patients in random, preferably morning urine. Positive findings should be quantified by examination of proteinuria in collected urine. Other laboratory criteria for renal damage include pathological findings in urinary sediment - renal tubular cells, erythrocytes (preferably dysmorphic), leukocytes, and coarse granulated or waxy casts.

TABLE 4.13 DIAGNOSTIC CRITERIA FOR CKD

Decrease GFR	Markers of kidney injury
<60 ml/min/1.73 m^2 <1 ml/s/1.73 m^2	▪ albuminuria (ACR ≥3 mg/mmol, dU-Alb ≥30 mg/24 hours), ▪ pathological urinary sediment ▪ ion and other abnormalities due to tubular disorders, ▪ pathological histological finding (biopsy), ▪ structural abnormalities in imaging studies ▪ history of kidney transplantation

Screening of CKD should be performed especially in individuals with risk factors such as diabetes mellitus, hypertension, cardiovascular disease, acute renal impairment, polycystic kidney, recurrent nephrolithiasis, prostate hypertrophy, systemic diseases with possible renal impairment, positive family history.

The international classification based on GFR distinguishes 5 stages of CKD (Table 4.14). Stages 1 and 2 cannot be diagnosed solely based on GFR, but require the presence of additional markers of kidney disease, typically proteinuria or albuminuria, or presence of abnormalities in imaging studies (for example polycystic kidneys). CKD stages 3 – 5 require only demonstration of persistent GFR less than 1 ml/s/1.73 m^2.

TABLE 4.14 PROGNOSIS OF CKD ACCORDING TO CRITERIA KDIGO, 2012

Categories of GFR				**Persistent albuminuria categories**		
Description and range GFR				A1	A2	A3
Stage		ml/s/ 1.73m^2	ml/min/ 1.73m^2	<3 mg/mmol	3 – 30 mg/mmol	>30 mg/mmol
G1	Normal or increased	>1.5	>90			
G2	Mildly decreased	1.0 – 1.49	60 – 89			
G3a	Mildly-moderately decreased	0.7-0.99	45 – 59			
G3b	Moderately-severely decreased	0.50-0.74	30 – 44			
G4	Severely decreased	0.25 – 0.49	15 – 29			
G5	kidney failure	<0.25	<15			

Green: low risk (if no other markers of kidney disease, no CKD); yellow: moderately increased risk; orange: high risk; Red: very high risk.

The **assessment of the severity of CKD** is based on two laboratory parameters: eGFR (categories G1 to G5) and albuminuria (categories A1 to A3). Depending on categories, the patient's risk is classified as low, medium, high and very high. End-stage CKD or renal failure is associated with a decrease in GF below 0.25 mL/s (G5 stage). Patients have manifestations of uremic syndrome which includes, in particular, cardiovascular, cutaneous, endocrine, hematological, immunological, neurological and bone disorders.

Uremic syndrome, which is a clinical manifestation of advanced stage CKD, arises due to the inability to maintain the renal elimination, regulatory and endocrine functions. Metabolic disorders in CKD and their laboratory findings are as follows:

- **Retention of nitrogen compounds**: The laboratory finding of an increased serum concentration of creatinine, urea and uric acid is typical. The last two, however, may also increase from extrarenal reasons. Low protein diet and advanced liver diseases decrease urea concentration, on the other hand, gastrointestinal bleeding causes sudden increase of serum urea.
- **Ion disorders**: Potassium balance is maintained longer in same patients due to adaptation changes, particularly increased secretion in distal tubule, hyperaldosteronism, potassium

excretion into colon. Hyperkalemia is more frequent in patients with hypoaldosteronism (low renin production in diabetic nephropathy), with severe acidosis or using potassium sparing diuretics and ACEI.

- **Acid-base disorders**: Metabolic acidosis results from decreased excretion of H^+ due to reduced GFR, retention of phosphates, decreasing buffer capacity in urine, and low production of ammonia as well. In addition, an impaired reabsorption of HCO_3^- also contributes to MAC.
- **Impaired concentrating ability**: isosthenuria, urine osmolality close to plasma/serum osmolality.
- **Impaired calcium-phosphate metabolism**: Decreased synthesis of calcitriol in the kidney (inhibition of 1-hydroxylase by hyperphosphatemia, later loss of functional kidney tissue) causes impaired intestinal calcium reabsorption. Hypocalcemia leads to stimulation of PTH production and mobilization of calcium from bones (secondary hyperparathyroidism). Serum concentration of phosphate is initially maintained in normal range by increased fractional excretion in residual nephrons, in advanced stages of CKD typically develops hyperphosphatemia.
- **Decreased erythropoietin synthesis:** is one of the factors contributing to anemia. Others include bone marrow suppression by uremic toxins and cytokines, chronic blood loss, mechanical damage during hemodialysis, dilution of blood due to water retention.
- **Disorders of lipid metabolism:** 70% of patients with CKD have secondary dyslipidemia caused by hyperinsulinism (due to lower renal degradation) and insulin resistance (due to metabolic acidosis, decreased secretion of glucagon and growth hormone). Typical laboratory findings result from increased number apoB containing lipoproteins (VLDL, IDL, LDL) and include high triacylglycerol (TAG), low HDL-cholesterol (LDL-C) and frequently normal or only borderline elevated total cholesterol (TC).

Early identification and subsequent treatment of the cause leading to CKD may slow further deterioration of renal function. In some cases, however, the cause remains unidentified or incurable and patients must eventually be treated with chronic dialysis or kidney transplantation. Biochemical monitoring is essential both before and during the chronic hemodialysis program.

Case studies and control questions

Case 4.1

28-year old man with history of alcohol and drug addiction was brought to Emergency department of the hospital with unconsciousness, breathlessness and headache. His friend found him at home unconscious, where the patient had been lying for several hours. On examination, signs of dehydration, hypothermia (35.7 °C), HR 95/min and BP 90/65 mm Hg were present, CT of chest and head was negative. The urine obtained by catheter had dark brown colour, total amount diuresis for 12 hrs was only 250 mL (after IV fluid therapy of 4 L).
Laboratory results are in the following table:

Serum	Result	RI
Urea	24	2.7 – 8.0 mmol/L
Crea	255	55 – 95 µmol/L
Na^+	139	135 – 145 mmol/L
K^+	7.3	3.6 – 5.3 mmol/L
Cl^-	99	95 – 105 mmol/L
Ca^{+2}	1.66	2.1 – 2.7 mmol/L
Alb	30	36 – 50 g/L
CK	2070	<3.2 µkat/L
ABR		
pH	7.25	7.36 – 7.46
pCO_2	5.8	4.8–5.8 kPa
pO_2	9,5	9.5 – 13.9 kPa
HCO_3^-	16	22 – 26 mmol/L

Questions:

a. What is probable cause of AKI?
b. Why is patient's urine dark?
c. Which ion and acid-base disorders are present and what is their possible cause?

Case 4.2

5-year old girl was examined for face and lower extremities edema, which has occurred during previous days. Parent also noticed turbid urine. The paediatrician found proteinuria 4+ in urine analysis and sent the child to the hospital, where the following results were found:

Serum	Result	RI
Urea	3.3	2.5 – 6.6 mmol/L
Crea	48	45 – 90 µmol/L
Na^+	130	135 – 145 mmol/L
K^+	4.5	3.6 – 5.3 mmol/L
Ca^{+2}	1.61	2.1 – 2.7 mmol/L
Alb	15	35 – 48 g/L
CB	50	60 – 80 g/L
Chol	8.5	<5 mmol/L
TAG	10	<1.7 mmol/L
U-Prot/d	11.5	< 0.15 g/24 h

Questions:

a. What is probable cause of clinical and laboratory findings?
b. What are consequences of heavy proteinuria?
c. What is GFR of the child?

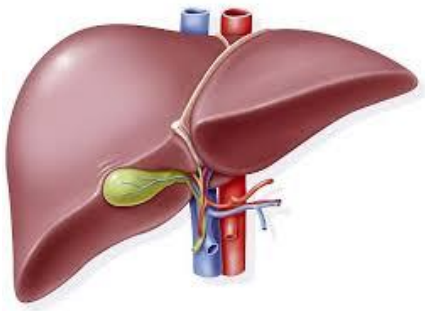
Self-assessing questions

1. Explain relationship between serum creatinine concentration and GFR.
2. Name factors interfering with creatinine method.
3. PCR of patient in the first morning urine sample is 95 mg/mmol. What does it mean?
4. Which test is suitable for early detection of nephropathy in a patient with hypertension?
5. Name typical biochemical findings in patient with stage 4 of CKD.
6. Which method for measurement GFR would you choose in patient after lower limb amputation and why?

KEY INFORMATION

- ☑ The kidneys perform a central role in the maintenance of homoeostasis of water, electrolytes, and also have a significant endocrine role.
- ☑ The general approach to kidney disease detection involves urinalysis, detection and quantification of proteinuria and assessment of GFR.
- ☑ The GFR is the best single measure of the number of functioning nephrons. It is measured by endogenous creatinine clearance, despite it overestimates true GFR (at low GFR level).
- ☑ Serum creatinine concentration and estimated GFR are used as suitable tests for assessment GFR and for diagnostics and monitoring of renal following the progress of chronic kidney disease. GFR must decline by more than 50% before serum creatinine exceeds the upper limit of the reference range.
- ☑ In clinical practice, CKD-EPI equations are the most frequently used estimation GFR derived based on serum creatinine and/or cystatin C level.
- ☑ Glomerular filtration changes during the day similarly to heart and breath frequency. Change in GFR up to 20% in two consecutive samples does not mean a significant change of renal functions. The continuous rising of S-Cr even within reference interval may signalize decrease in GFR.
- ☑ Urine test strips can detect relatively high protein concentration (>150 mg/L), therefore they are not suitable for screening of increased albuminuria in diabetic patients.
- ☑ Albumin is the predominant protein in most forms of pathological proteinuria, although it is a relatively minor component of normal urine. Increased albuminuria is a marker of early stages of a glomerulopathy.
- ☑ Protein to creatinine ratio (PCR) measured on a random urine specimen shows good correlation with protein excretion measured on a collected urine and appear to have even superior prognostic, predictive power.
- ☑ Acute kidney injury is associated with rapid onset of kidney failure and metabolic derangements. Laboratory markers of AKI involves increase of creatinine and other N-compounds in blood, hyperkalemia, metabolic acidosis and mostly decrease in diuresis per time unit.
- ☑ Chronic kidney disease may occur as a result of a variety of systemic or specific kidney diseases, in particular diabetes mellitus, hypertension, glomerulonephritis, and polycystic kidney disease.
- ☑ Chronic kidney disease is classified into 5 stages based on GFR. End stage renal disease is a point, at which renal function is no longer sufficient to support life and dialysis or transplantation is required to prevent death.

5



Biochemical tests in liver disease

CONTENT

The burden of liver disease in developed countries of the world continues to grow, especially compared to decreasing death rate from other non-communicable diseases. The specific position of the liver in the centre of metabolic functions of the human body together with a unique blood supply predispose that organ to multiple potential damage. The liver is attacked by viruses, toxins (alcohol, drugs) as well as our unhealthy and fast lifestyle, which includes physical inactivity, excess calorie intake, unhealthy fast-food or foods full of unwanted chemicals.

The currently used "liver function tests" measured in blood have been known since the 1950s, their interpretation is part of daily practice in almost all medical specialties. Liver function tests represent a cheap, affordable but non-specific tool to assess liver health and dysfunction or to monitor both acute and chronic diseases. This chapter deals with biochemical liver tests that reflect the different metabolic functions of the liver and are used to detect the presence of:

- liver damage or dysfunction of different origin,
- cholestasis and differential diagnosis of jaundice,
- acute or chronic hepatocellular damage,
- liver failure.

5.1 Basic physiology

The liver performs a lot of physiological functions, especially in intermediate metabolism. The key metabolic role of the liver results from its location. Like the skin, the liver represents a remarkable interface between the external environment represented by the digestive tract and the internal environment (blood). All substances absorbed from food (except the oral cavity and the terminal 20 cm intestine) are processed at first in the liver and then supplied the circulation. This supply responds to the current needs of the body, regardless of food intake. Communication is bi-directional, the liver retains unwanted or excess substances from metabolic processes, detoxifies them and excretes them into the external environment. The principal **metabolic function** of the liver is maintenance of sufficient blood levels of all metabolites, which is realized by:

- uptake of nutrients delivered from the digestive tract via the portal vein;
- synthesis, storage, interconversion, and degradation of metabolites;
- regulated supply of energy-rich intermediates and building blocks for biosynthetic reactions.

Biotransformation of both exogenous and endogenous substances takes place in hepatocytes. Biotransformation involves chemical transformation (e.g. hydrolysis, oxidation, reduction, conjugation) with subsequent formation of soluble conjugates secreted into bile or urine. Endogenous substances like bilirubin and most hormones (e.g. insulin, glucagon, glucocorticoids, mineralocorticoids, thyroid hormones, adrenalin, GH), and various xenobiotics (e.g. drugs, food additives) are detoxified in the liver.

Immune function of the liver is performed by a specialized form of macrophages called Kupffer cells. These cells engulf and breakdown toxic matter such as microorganisms, dead cells including old red blood cells and other foreign chemical substances. The most important biochemical processes performed in the liver are summarized in the Table 5.1.

TABLE 5.1 METABOLIC PROCESSES IN THE LIVER

Metabolism	Particular processes
Saccharide	gluconeogenesis, glycogen synthesis and breakdown, pentose cycle
Fat	synthesis and degradation of fatty acids, cholesterol, TAG; lipoprotein synthesis and clearance, bile acids synthesis; ketogenesis
Protein	synthesis and degradation of 90% plasma proteins (except immunoglobulins and proteohormones), synthesis of urea, production of glutamine in perivenous hepatocytes (mostly in acidosis)
Hormone	metabolism and excretion of steroid hormones, metabolism of polypeptide hormones, 25-hydroxylation of vitamin D
BIL	conjugation, excretion into bile
Drugs	biotransformation and excretion
Storage	glycogen, vitamin A, vitamin B12, iron, copper, blood reservoir - ~10% of blood volume
Others	metabolism of purines, synthesis of uric acid; extramedullary hematopoiesis in bone marrow disorders; thermoregulation; participation in A-B balance regulation

5.2 Liver function tests

The historical name **liver function tests (LFT)** suggests that they should inform about liver dysfunction. The set of routine liver tests consists mainly of the so-called routinely used hepatic enzymes are liver-specific and also occur in cells of other tissues. These biochemical LFTs can be divided to following (Table 5.2):

- tests reflecting hepatocyte damage - AST, ALT;
- tests reflecting disorders of bile duct and canalicular pole of hepatocytes - ALP, GGT;
- liver synthesis tests - albumin, prealbumin, cholinesterase, coagulation factors;
- tests measuring capacity to remove endogenous and exogenous substances from the circulation – bilirubin (BIL), ammonia.

TABLE 5.2 OVERVIEW OF MOST FREQUENTLY USED LIVER FUNCTION TESTS

Test	Site or significance	Clinical implication
AST	Liver, kidney, heart, muscle, brain, RBC	Hepatocellular damage
ALT	Liver, in other tissues low activities	Hepatocellular damage
BIL	Hepatic or extrahepatic disorder	Cholestasis, impaired conjugation, or biliary obstruction
ALP	Bone, intestine, liver, placenta	
GGT/GMT	With ↑ALP indicates hepatobiliary origin	Cholestasis or biliary obstruction
Alb	Diet or liver	Synthetic function
PT, INR	Liver synthesizes clotting factors	Synthetic function
NH_3	Liver synthesis of urea	Synthetic + detoxification function

Although individual LFTs are not diagnostic for specific liver diseases, they serve as an aid in the following situations:

1. making specific diagnosis based on characteristic pattern of tests;
2. establishing the severity of dysfunction or damage;
3. following the progress of disease or response to therapy.

5.2 Tests informing about integrity of hepatocytes

The activities of two enzymes **aspartate aminotransferase** (AST) and **alanine aminotransferase** (ALT) measured in serum are widely used in clinical practice as a sensitive marker of acute hepatocellular damage. Tissue specificity of aminotransferases is low; however, serum ALT measurement is more liver-specific than that of AST. Both aminotransferases possess different intracellular location (Figure 5.1). Hepatocyte contains more AST than ALT, however only 1/3 of AST is placed in the cytosol, and 2/3 in are bound in mitochondria. The content of ALT in hepatocyte is smaller, however, the whole amount is placed in the cytosol. During moderate hepatocellular damage of different origin (e.g. ischemia, shock, acute cardiac

failure, viral or toxic injury) only cytosolic fraction of aminotransferases leaks from hepatocytes into circulation, thus ALT activity in blood is higher than AST. In case severe hepatocellular damage with necrosis and breakdown the cells, both cytosolic and mitochondrial isoenzymes are released into the circulation and serum AST activity tends to be increased to a greater extent than ALT. The more severe hepatocyte damage, the more dominant is the activity of AST over ALT in serum.

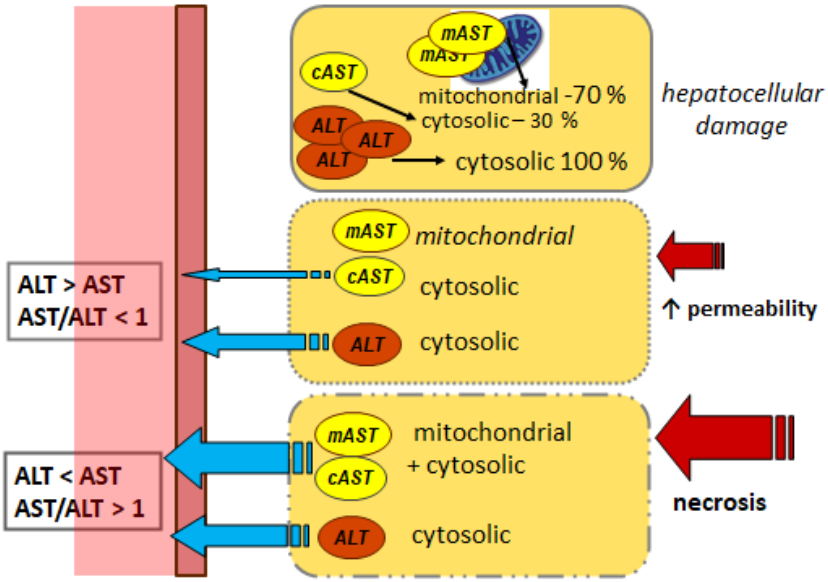


FIGURE 5.1 Location and leakage of aminotransferases during hepatocellular damage

The activity of serum aminotransferases increases mainly in hepatocellular damage, but may also be from other tissues (Table 5.3). Despite low tissue specificity of aminotransferases, the value of testing increases with the level of enzyme activity. Activity above 10-times ULRI (upper limit of reference interval) is likely caused by primary hepatocytes damage, but value less than 10-times ULRI is less specific. Normal or slightly elevated values of AST, ALT do not exclude significant hepatocellular damage. In addition, the levels of enzymes do not necessary correlate with the eventual outcome of a disease. A rapid fall of aminotransferases, especially accompanied with a rise of bilirubin and prothrombin time may signalize poor prognosis in acute liver injury.

TABLE 5.3 CAUSES OF INCREASED AMINOTRANSFERASES ACTIVITY

Liver disease	Muscle disease	Other extrahepatic
Viral hepatitis	Trauma, myositis	Myocardial infarction,
Intoxications	Prolonged compression	Heart failure
Metabolic disorders	Metabolic myopathy	Venostasis in liver
Enzymopathies	Excessive muscle exertion	Acute pancreatitis
Hepatotoxic drugs	Cramps	Multiorgan failure syndrome

The muscle or cardiac origin of AST, ALT elevation needs to be confirmed or excluded by examination of creatine kinase (CK) activity or muscle and cardiac proteins (myoglobin, troponins). The skeletal muscles account for 35 – 40% of body weight in men and 30 – 35% in women, while the liver weighs approximately 1.5 kg. During rhabdomyolysis, much more AST than ALT is released from the damaged muscle cells, not rarely AST activity tends to be above 100 μkat/L and therefore the AST to ALT ratio is greater than 1.

5.4 Tests for cholestasis

Cholestasis is the term used to describe the consequences of failure to produce and/or excrete bile. The dysfunction may be related to hepatocellular damage or to reduction in the bile flow due to obstruction of hepatic ducts. Traditional biochemical markers of cholestasis are so-called cholestatic enzyme **alkaline phosphatase** (ALP) and **gamma-glutamyl transferase** (GGT or GMT), normally attached to the internal membrane of biliary pole in hepatocytes (Figure 5.2). For this reason, ALP and GMT tends to be released only in small amounts following hepatocellular damage. On the other hand, their activity in the blood increased significantly in case of high pressure in the bile ducts – in cholestasis.

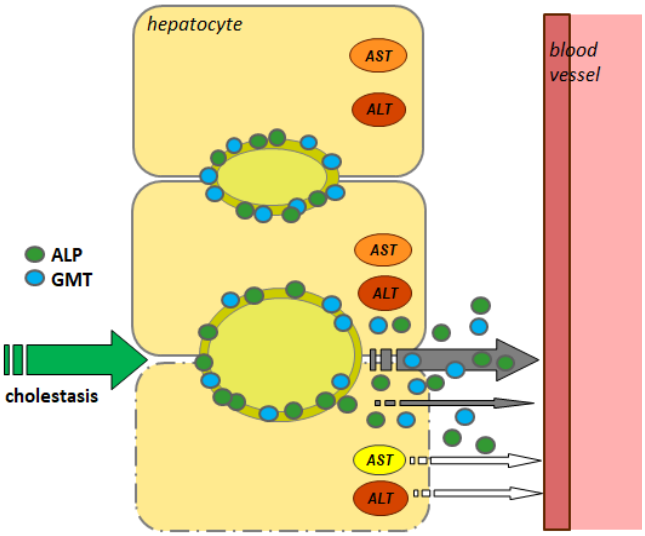


FIGURE 5.2 Location and leakage of hepatic enzymes during cholestasis

Alkaline phosphatase

Alkaline phosphatases are group of enzymes, encoded by several structural genes, which hydrolyse phosphate esters in alkaline solutions. Four different ALP isoenzymes have been distinguished in serum by using of electrophoretic methods: liver, bone, intestinal or placental iso-ALP (INFO 5.1). In the serum of healthy adult men or non-pregnant women, ALP activity is formed by bone and liver isoenzymes and a small, non-constantly present intestinal isoenzyme.

Bone and liver ALP are present in approximately equal amounts in adults, the activity of bone isoenzyme is significantly higher in children (constitutes up to 85% of total ALP), especially in periods of growth acceleration. In the third trimester of pregnancy, the placental isoenzyme ALP activity accounts for about 50% of the total serum ALP activity. Electrophoretic methods for separation of ALP isoenzymes are not freely available in routine clinical laboratories; more frequently immunochemical methods are used for measurement of bone ALP.

Liver isoenzyme of ALP is derived from interior surface of the bile pole in hepatocyte. Any obstruction of the bile duct or ductless causes an **increase in serum ALP activity** as a result of increased synthesis and consequent release into the circulation. **Intrahepatic** cholestasis is present in acute hepatocyte damage (due to liver congestion and oedema), cirrhosis and primary biliary cholangitis, intrahepatic biliary obstruction (tumours), many drugs, and also right-sided heart failure. **Extrahepatic** cholestasis is caused by compression or occlusion of the biliary tract, most commonly by gallstones and pancreatic or bile ducts tumour.

A rise in ALP activity usually precedes the onset of clinical jaundice and vice versa, after the resolution of biliary obstruction (for example surgical relief in cholestasis) normalization of bilirubin usually precedes that of ALP. It may take several days for levels return to the physiological range because of the half-life of ALP (7 – 8 days).

An isolated increase in ALP activity (GGT normal) indicates non-hepatic origin. The most common cause of isolated ALP elevation in asymptomatic individuals is **vitamin D deficiency.** A condition called benign transient **hyperphosphatasemia** can result in significantly increased serum ALP, often to several hundreds of μkat/L, persisting during 6 – 8 weeks. This condition was described in small children up to 4 – 5 year of life, but it can also occur in adults and during pregnancy. The transient elevation of ALP is associated with concurrent infection in over 60% of cases. An existence of variant forms of ALP isoenzymes (similar to bone-ALP) has been proven by electrophoretic analysis. It is believed, that changes in their carbohydrate side-chains cause failure of recognition by renal receptor and their reduced clearance, thus prolonging half-time of enzyme.

INFO 5.1 ALP isoenzymes

Four structural genes encoding ALP isoenzymes have been identified and sequenced. The tissue non-specific gene is widely expressed in osteoblasts, hepatocytes, and other cells; the tissue specific genes are found for intestinal, placental, and germ cells. Tissue specific differences in properties of the ALP originate from post-translational modification in the carbohydrate side chains.

1. Liver and bone ALP isoenzymes contribute the total serum activity of ALP in healthy individual in fasting state. Plasma ALP activity varies with age in childhood due to growth spurts, with bone formation releasing bone isoenzyme ALP. Age dependent reference ranges are therefore used up to 18 years of life. Serum activity of bone ALP isoenzyme is a marker of osteoblast activity. Slightly higher activity ALP in apparently healthy elderly people may reflect the high incidence of mild, subclinical Paget´s disease in this subpopulation. In adults it increases significantly in bone metastatic tumours.
2. Placental ALP isoenzyme may increase serum activity of ALP in third trimester pregnancy up to two- or three-fold of normal. The presence of this isoenzyme in non-pregnant women or even men may indicate a malignity (for example bronchial carcinoma or germ-cell tumours).
3. Intestinal isoenzyme: produced by enterocytes may be seen in healthy individual following food ingestion and especially in patients with blood group B and O. That increase may persist up to 12 hours. Therefore, unexplained high value of ALP should always be confirmed by a repeated fasting sample. Increased activity is found in inflammatory bowel diseases, malabsorption syndrome, dialyzed and chronic liver inflammation.

Gamma-glutamyl transferase

Gamma-glutamyl transferase (GGT or GMT) is an enzyme found on cell membranes of many tissues mainly in the liver, kidney, and pancreas. The highest concentration is in the kidney, but the liver is considered the source of normal enzyme activity in serum. The enzyme catalyzes the transfer of the gamma-glutamyl group from gamma-glutamyl peptides, such as glutathione to an acceptor such as peptides and L-amino acids. Thus, GGT is involved in the transfer of amino acids across the cellular membrane.

Activity of GGT increases in obese individuals, after alcohol and some medicines. Increased levels of GGT have low specificity for hepatobiliary diseases. The major clinical value of serum GGT is differentiation between hepatobiliary and bone causes in patients with isolated

elevation of ALP. Elevated GGT levels support the hepatobiliary source, whereas levels in the reference range strongly argue against that. GGT levels are within the reference range in bone disorders, pregnancy, and muscle disease. GGT level is a nonspecific marker of chronic alcohol intake and can be used for monitoring abstinence. After cessation of alcohol consumption, the normalization of GGT levels usually takes up to 1 month. Increased GGT levels are associated with the conditions listed in the Table 5.4.

TABLE 5.4 CAUSES OF INCREASED GGT ACTIVITY IN SERUM

Liver diseases	Extrahepatic causes
Hepatitis (acute and chronic) Cholestatic disorders Alcoholic liver disease Pancreatitis Liver metastasis and carcinoma Autoimmune liver diseases (Primary biliary cirrhosis and sclerosing cholangitis)	Drugs (carbamazepine, cimetidine, furosemide, heparin, methotrexate, oral contraceptives, phenobarbital, phenytoin, and valproic acid) Alcoholism Systemic lupus erythematosus Congestive heart failure and chronic coronary artery disease

5.5 Hyperbilirubinemia

The concentration of bilirubin (BIL) in blood and urine is a marker of the transporting capacity of liver for anions, including bile salts. Understanding the processes by which BIL is formed and removed from the blood is essential for the diagnosis of liver disease with jaundice, albeit abnormal serum concentrations of BIL from extra-hepatic origin.

Bilirubin production and metabolism

Bilirubin is a breakdown product of heme, an iron-containing protoporphyrin, mainly found in hemoglobin. About 80% of BIL arises from senescent red blood cells degraded in the macrophage-monocyte system, with the remainder coming from red cell precursors destroyed in bone marrow ('ineffective erythropoiesis'), and from other heme-containing proteins such as myoglobin and cytochromes. BIL is insoluble in water and is transported in the blood almost totally bound to albumin, but that complex is not filtered in glomeruli. The liver takes up BIL by a specific carrier mechanism into hepatocytes, and changes it to the conjugates with glucuronic acid. Resulting mono- and di-glucuronides, which are much more soluble in water than unconjugated BIL. The conjugated BIL is secreted into bile against a high concentration gradient by carrier-mediated and energy-dependent process.

The main functional constituents of the bile are bile salts, which are involved into fat digestion and absorption from the small intestine. Serum bile acid concentration is more sensitive indicator of hepatic transport function than total BIL, but it is not used commonly because of less available laboratory methods. The meaningful indication for bile acids measurement is suspicion of pregnancy-associated cholestasis in the second and third trimester, since a high level of bile acids may result in damage of fetus.

Bilirubin-glucuronides excreted via the bile into the gut are degraded by bacterial enzymes in the colon to a mixture of colourless compounds termed urobilinogen (UBG) and stercobilinogen. Next, the most of them are oxidized to urobilin and stercobilin and they are excreted by feces, contributing to coloration of stool. A small amount of UBG is absorbed from the small intestine and enters the enterohepatic circulation, cleared by the liver and repeatedly excreted into bile. The majority of UBG is excreted by stool, only small part absorbed in the terminal part of the colon reaches the systemic circulation, is filtered out by the kidney and eventually appears in the urine (Figure 5.3).

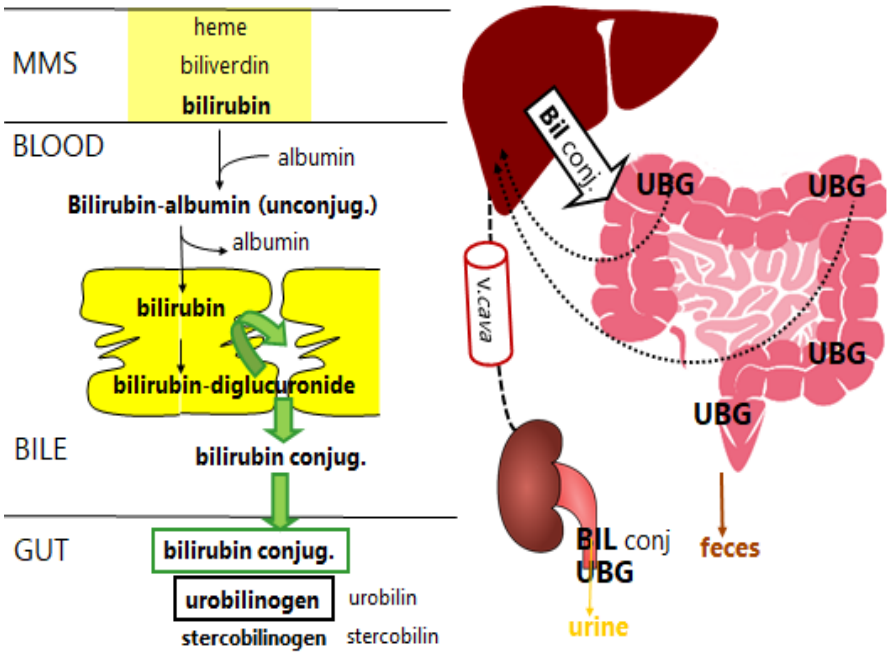


FIGURE 5.3 Metabolism of bilirubin

Normally, more than 95% of BIL in serum is unconjugated, but in liver diseases, especially those with cholestasis, the conjugated form may predominate. The measurements of **total bilirubin** (i.e. sum of unconjugated and conjugated forms) or separate measurements of **conjugated bilirubin** are available in biochemical laboratories. The BIL concentration should be interpreted in correlation with patient history, findings on physical examinations and results of urine BIL and UBG measurements.

Jaundice – differential diagnosis

Physiological BIL concentrations in serum vary slightly with age, reach maximum in adolescence, do not exceed 20 µmol/L in an adulthood. Jaundice (icterus) is a yellow coloration of the skin or sclera due to hyperbilirubinemia. It becomes clinically apparent by the naked eye when blood bilirubin exceeds 50 µmol/L, although smaller degrees of hyperbilirubinemia may be of diagnostic significance. There are three main reasons why hyperbilirubinemia occurs, which allow three causes of jaundice to be defined. Table 5.5 summarizes typical laboratory findings in the differential diagnosis of hyperbilirubinemia.

TABLE 5.5 LABORATORY DIFFERENTIAL DIAGNOSIS OF JAUNDICE

Test	Prehepatic	Hepatocellular	Posthepatic
S-unconj. BIL	↑	↑	↑
S-conj. BIL	N	↑	↑
S-AST, ALT	N	↑	N later ↑
S-ALP, GGT	N	N later ↓	↑
U-BIL	absent	present	present
U-UBG	↑	↑ (missing in total obstruction by oedema)	↑ in partial obstruction, absent in complete one

1. **Hemolysis:** The increased hemoglobin breakdown overloading the conjugating capacity of hepatocytes causes pre-hepatic or hemolytic jaundice with dominating non-conjugated hyperbilirubinemia. The frequent causes of hemolytic jaundice are:
   - physiological jaundice of newborns;
   - hemolytic jaundice of newborns (ABO, Rh);
   - intravascular hemolysis, hemolytic anemia;
   - ineffective erythropoiesis (e.g. pernicious anemia);
   - hemolysis of extra-vessel blood (e.g. hematomas).
2. **Hepatocellular diseases** (viral, toxic, ischemic or autoimmune) are connected with dysfunction of hepatocytes and failure of uptake of unconjugated BIL from the blood, conjugating mechanisms within hepatocytes and excretion of conjugated BIL into the bile. In the case of impaired liver architecture (e.g. hepatocyte necrosis) connections are established between the blood and bile ducts, and conjugated BIL is regurgitated into the blood. The hepatocellular jaundice can arise from:
   - hepatocellular damage due to infective (mostly viral) or toxic agents;
   - cirrhosis usually as a relatively late complication;
   - low activity of bilirubin UDP-glucuronyltransferase in congenital deficiency (Gilbert´s or Crigler-Najjar syndrome), premature infants or competitive inhibition of the enzyme by drugs.

   In hepatocellular damage, both forms of BIL are found in serum. The aminotransferases AST and ALT are released from the necrotic hepatocytes. Oedema, which is a part of hepatocellular diseases, causes cholestasis and rarely, even complete obstruction at the peak of a disease. Therefore, the activity of cholestatic enzymes GGT and ALP increases in serum. Failure enterohepatic circulation increases the release of UBG into the circulation and subsequently into the urine. BIL is positive in urine as a consequence of conjugated hyperbilirubinemia.
3. **Obstruction of biliary system** causes intrahepatic or extrahepatic cholestasis with post-hepatic or cholestatic jaundice. If the blockage is complete, both BIL and cholestatic enzymes (mostly ALP) are raised. There is a little or no UGB in urine and in stool (pale, acholic). After removal of obstruction, the urine again becomes positive for UBG and stool restores its color. If the blockage is only partial, UBG in urine is positive because of the failure of enterohepatic circulation. In both conditions, hyperbilirubinemia is unconjugated (uptake

disorder) as well as conjugated (increasing bile duct pressure opens up the connection between the bile and the bloodstream). The cholestatic enzymes ALP and GGT increase as first. Necrosis of hepatocytes may occur due to the pressure in the bile ducts and also hepatocellular aminotransferases enter the systemic circulation.

The **unconjugated hyperbilirubinemia** means that the majority (>80%) of total BIL exist bound on albumin (indirect BIL). This bilirubin is not measured by standard laboratory method separately; usually, it is a difference between total and conjugated (direct) BIL. It is worth mentioning here that bilirubin levels of more than 85 µmol/L in the presence of normal hepatic function cannot be explained by chronic hemolysis alone.

In the **conjugated hyperbilirubinemia**, more than 50% of total serum BIL occurs as directly measured BIL (INFO 5.2). Bilirubin levels have prognostic significance in alcoholic hepatitis, primary biliary cirrhosis, and in acute liver failure. As conjugated bilirubin is excreted in the urine, bilirubin levels rarely exceed 500 µmol/L in the absence of renal failure or hemolysis.

INFO 5.2 Remarks to different types of hyperbilirubinemia

Unconjugated hyperbilirubinemia is caused by impaired hepatic uptake and/or conjugation in the liver, which can be congenital and acquired; in addition to increased BIL formation in hemolysis. In premature neonates, the breakdown of foetal hemoglobin, along with hepatic immaturity, leads to a rapid increase in bilirubinemia. After saturating the binding capacity of albumin for unconjugated BIL, it is stored in brain structures with the risk of brain damage (so-called nuclear icterus, kernicterus). Blue light phototherapy in neonatal hyperbilirubinemia accelerates the breakdown of BIL in the skin, in severe forms (above 300 µmol/L, depending on the degree of neonatal immaturity), an exchange transfusion is necessary. An increase in conjugated BIL >25 µmol/L in neonates and infants may indicate liver disease.

Conjugated hyperbilirubinemia occurs in congenital or acquired disorders of BIL excretion into the bile. Cholestatic enzymes (especially ALP) are also increased. This finding is typical for isolated metastases or secondary liver tumours, where a preserved portion of functional liver tissue can process and secrete bilirubin. Since conjugated BIL is excreted in the urine, its concentration does not exceed 500 µmol/L, unless concomitant GFR decline is present. BIL concentrations are of prognostic importance in alcoholic liver disease, primary biliary cholangitis, and acute liver failure.

The inherited hyperbilirubinemias

These disorders caused by specific genetic defects in bilirubin transport and metabolism are characterised by unconjugated or conjugated hyperbilirubinemia with mostly normal other liver functional tests.

Gilbert´s syndrome, autosomal dominant disease, is the most common disorder from this group, which affects in average 5% of young men (seldom women). Disease is caused by decreased expression and thus activity of enzyme bilirubin UDP-glucuronyltransferase due to a genetic mutation. The unconjugated hyperbilirubinemia (<100 µmol/L) is recurrent, usually asymptomatic or connected with mild jaundice, which occurs typically after fasting, illness or increased physical activity. One of the diagnostic tests for Gilbert´s syndrome is monitoring the effect of reduced daily energy intake (400 kcal or 1.67 MJ) on serum BIL. Positive result means at least doubling of serum unconjugated BIL in a patient with Gilbert´s syndrome. The definitive diagnosis requires genetic testing in unclear situations.

Crigler-Najjar syndrome is an extremely rare condition, due to low or complete absence of activity bilirubin UDP-glucuronyltransferase. It results in severe jaundice within the first days of life and without liver transplantation often causes early death.

Rare inherited **conjugated types of hyperbilirubinemia** are known as Dubin-Johnson syndrome and Rotor´s syndrome. These are caused by a defect in the transfer of conjugated BIL into biliary canaliculus due to mutation of some genes (encoding, for example, MRP2 – multidrug resistance protein 2 in Dubin-Johnson syndrome). Usual clinical presentation is a fluctuating mild jaundice at any age of life, often after contraceptive pills or in pregnancy.

5.6 Tests for assessing the synthetic function of liver

Most of the plasma proteins originate from the liver, a minor part being synthesized by other cells - for example, lymphocytes (immunoglobulins), enterocytes (apolipoprotein B48). Protein synthesis disorders only occur in more advanced stages of liver damage. In acute liver failure, circulating protein levels decrease depending on their half-life. Coagulation factors and cholinesterase (pseudocholinesterase) have very short half-lives. Albumin has a half-life of approximately 20 days; its concentration noticeably decreases after 2 – 3 weeks of an insufficient synthesis.

Albumin

Plasma albumin concentration decreases in chronic liver disease, but tends to be normal in the early stages of acute hepatitis due to long biological half-life (~20 days) and low rate of fractional clearance of albumin. Other factors leading to hypoalbuminemia that should be taken into account when interpreting the results include:

- increased losses of albumin (renal - nephrotic syndrome, extrarenal - burns, protein losing enteropathy, loss into extravascular compartment);
- increased degradation during catabolic states (sepsis, fever, trauma, and malignancy);
- poor nutritional status (malnutrition, malabsorption) or;
- decreased synthesis as a part of acute-phase response (negative reactant).

Low oncotic pressure due to hypoalbuminemia (<30 g/L), together with increased portal pressure and sodium retention due to secondary hyperaldosteronism contribute to a formation of ascites in cirrhotic patients.

Coagulation factors

The synthesis of prothrombin and other vitamin K-dependent coagulation factors is diminished in liver disease, leading to a prolonged prothrombin time (PT). The pathological value of PT is an early feature of any acute liver disease, since some clotting factors have short half-life (e.g. factor VII only 4–6 h). Prothrombin time is often expressed as a ratio to a control value in the form of international normalized ratio (INR). A prolonged PT may also reflect of fat-soluble vitamin K deficiency, due to failure of lipid absorption. In the absence of liver disease,

parenteral administration pf vitamin K helps to normalize PT within 18 hours. However, this does not work in patients with hepatocellular damage (INFO 5.3).

INFO 5.3 Changes in hemostasis in advanced liver disease

In advanced liver diseases, both pro- and antihemostatic processes are impaired, at the level of primary hemostasis, secondary hemostasis and fibrinolytic system. The following finding are present:

- reduced synthesis of coagulation factors as well as natural blood coagulation inhibitors (antithrombin, protein C, protein S);
- slower degradation of activated coagulation factors;
- increase of the factors, which are not synthesized in hepatocytes (VIII and von Willebrand factor);
- thrombin production is maintained as in the healthy population (as opposed to prolongation of PT and APTT), the prothrombotic status develops with escalating liver damage;
- thrombocytopenia - mainly due to hypersplenism, but also decreased thrombopoietin production and immune-mediated mechanisms in autoimmune or viral hepatitis.

The traditional perception of hemostasis in advanced liver disease is overcome by a new concept of "rebalanced" hemostasis. However, this equilibrium is fragile and can easily deviate to both the bleeding side and the thrombosis side due to associated medical circumstances.

Other plasma proteins

Serum protein electrophoresis is of little value in the diagnosis of liver disease, although in certain hepatic disorders, typical patterns may be present, such as fusion of β and γ bands due to increase immunoglobulins, mostly IgA. The measurement of individual immunoglobulins is also of low diagnostic value, because the changes are of low specificity. A polyclonal increase of IgA is frequently associated with cirrhosis, particularly when the disease of autoimmune in origin; while in primary biliary cirrhosis serum IgM raises greatly. In chronic active hepatitis, serum IgG tends to be most increased. Changes of some plasma proteins, which are diagnostically helpful in liver disease, are listed in Table 5.6.

TABLE 5.6 PLASMA PROTEINS OF DIAGNOSTIC VALUE IN LIVER DISEASE

Protein	Condition/disease	Change in concentration
Albumin	chronic liver disease	↓
Immunoglobulins	cirrhosis, especially autoimmune	↑
α1-antitrypsin	α1-antitrypsin deficiency	↓
Ceruloplasmin	Wilson disease	↓
α2-fetoprotein	primary hepatocellular carcinoma	↑ greatly
Ferritin	hemochromatosis	↑
Transferrin		normal, but > 60% saturated

Ammonia

The liver is responsible not only for synthesis but also for protein degradation. In the process of urea synthesis, the liver deprives the organism of toxic ammonia. Ammonia originates from deamination of amino acids derived from degraded proteins in different organs (liver, muscles, kidneys). It is produced by bacterial metabolism of urea and food proteins in the gut and from deamination of glutamine in the small intestine. In normal circumstances, the most ammonia is cleared by liver (urea cycle) and metabolized in skeletal muscles to glutamine. The kidney also contributes to maintaining blood ammonia by excreting urea in the urine and generating ammonia in tubular cells.

Increased **ammonia concentration** is a sign of liver failure. Impaired urea synthesis manifestes with an increase in ammonia only after 90% of the hepatocytes has been eliminated. At the same time, the serum urea concentration decreases, provided that GFR is normal. Accumulation of ammonia in the blood and its passage through the blood-brain barrier is one of the main causes of hepatic encephalopathy (Figure 5.4).

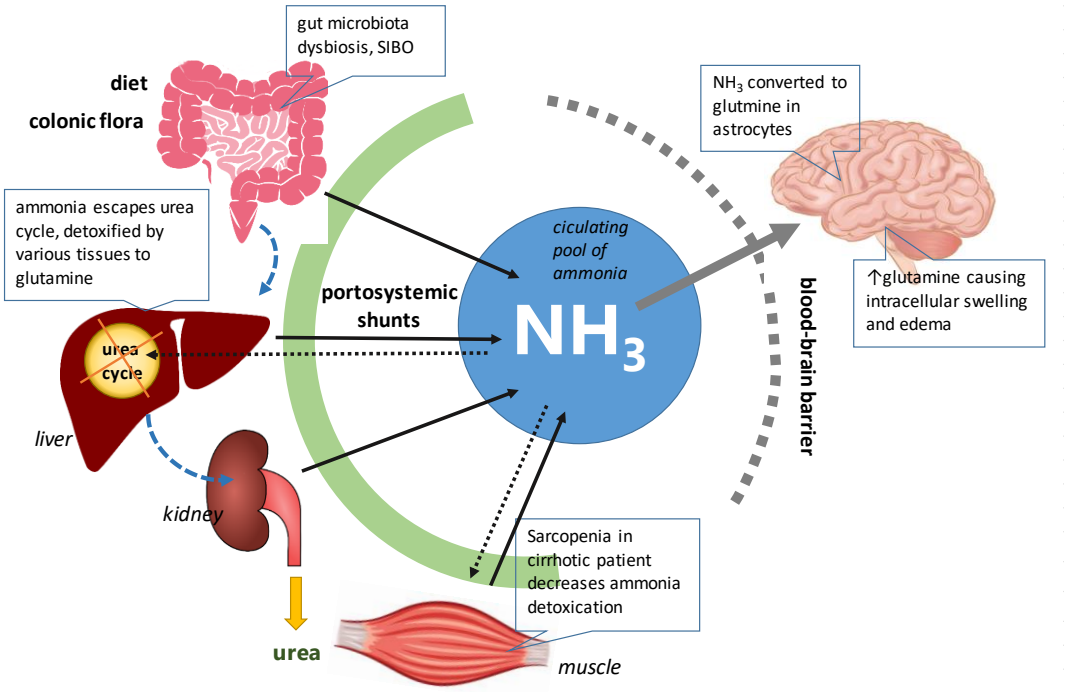


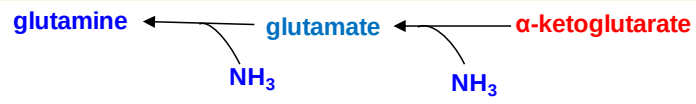
FIGURE 5.4 Contributing factors to hepatic encephalopathy

Hyperammonemia is one of the most important factors contributing development of **hepatic encephalopathy**. NH_3 easily enters the brain tissue and the only way for its elimination is synthesis of glutamine in astrocytes, which is involved in the glutamate-glutamine cycle between astrocytes and neurones. Hyperammonemia influences the function of the neural system by inhibition of exciting neurotransmission and by increase in the permeability of hemato-encephalic barrier for other compounds, which worsen encephalopathy (INFO 5.4).

In children, hyperammonemia may be due to an inherited defect of one of the urea cycle enzymes, immaturity of the neonatal liver, severe intercurrent illness (sepsis, asphyxia), intestinal bacterial overgrowth, Rey's syndrome, or some medications, e.g. valproic acid or anticancer drugs. The plasma concentration of ammonia in a healthy adult is < 45 µmol/L, the

newborns have a higher concentration (<100 µmol/L in mature and <150 µmol/L in preterm ones).

INFO 5.4 Clinical significance of hyperammonemia



The cause of hyperammonemia in liver failure is severe hepatocellular dysfunction of hepatocytes, which is manifested by reduced urea synthesis. Porto-systemic shunts and bleeding into GIT (with the resorption of proteins contained in the blood) in cirrhotic patients also contribute to the accumulation of ammonia. Skeletal muscles try to eliminate NH_3 by increased glutamine synthesis, but at the same time, the concentration of branched chain amino acids (valine, leucine, isoleucine) decreases, which inhibits proteosynthesis and enhances protein catabolism. NH_3 freely enters the CNS and the only way to eliminate it is the formation of glutamine, which is part of the glutamate-glutamine cycle between astrocytes and neurons. In this process, 2-oxoglutarate is consumed, which is sufficient and is a prerequisite for the functioning of the Krebs cycle and thus the generation of energy in the CNS cells.

Reduced energy generation in CNS leads to an inability to maintain membrane gradients and increases the permeability of the blood-brain barrier. The penetration of other substances that exacerbate encephalopathy increases. As a result of a disorder of intracellular metabolism, the formation of physiological neurotransmitters (dopamine, noradrenaline) is reduced and the synthesis of false neurotransmitters (octopamine, phenylethanolamine) from aromatic amino acids absorbed from the intestine is increased. The excitatory action of glutamine is absent, on the contrary, the formation of γ-aminobutyric acid, the inhibitory neurotransmitter, increases.

Preanalytic factors have a substantial influence on the plasma ammonia concentration. Venous blood should be collected in pre-cooled tubes containing heparin or EDTA. If this is not an emergency, the collection should be performed on an empty stomach (or at least 4 – 6 hours after a meal), the sample transported to the ice laboratory as soon as possible and analyzed immediately. The concentration of NH_3 increases in the sample due to erythrocyte metabolism as well as continuing deamination of amino acids by blood enzymes (e.g. GGT).

5.6 Liver failure

Liver failure is a clinical syndrome involving severe hepatic dysfunction that results from a massive destruction of hepatocytes. **Acute liver failure** (ALF) is a life-threatening condition occurring most commonly in adults without pre-existing liver disease. ALF typically presents with rapid evolution of deranged liver function to coagulopathy and encephalopathy. ALF is often defined according to the time interval from the development of jaundice to onset of hepatic encephalopathy (HE). The commonly used O'Grady classification categorises ALF as hyperacute (within 7 days), acute (within 28 days) or subacute (within 6 months). Worldwide, **hepatotropic viruses** (e.g. the fulminant form of hepatitis) or **hepatotoxic substances** (overdose of paracetamol and mushroom poisoning in Central Europe) are the most common causes (Table 5.7).

Acute liver failure is characterized in particular by hepatocellular dysfunction. The condition manifests by the failure of proteosynthesis (decrease of coagulation factors, later

hypoalbuminemia), disturbance of excretory function (jaundice) and disorder of detoxification, which is involved in the development of liver encephalopathy. Despite a considerable regenerating capacity of the liver, the metabolic disturbance is profound and the prognosis poor; fulminant hepatic failure with hepatic encephalopathy is often accompanied by renal failure.

TABLE 5.7 CAUSES OF ACUTE LIVER FAILURE

Cause	Example
Infection	Viral hepatitis A, B, C, D, E, Herpes simplex virus, Epstein-Barr virus, CMV, paramyxovirus, influenza virus B
Medicine	Paracetamol, halotan, isoniazid, valproic acid, rifampicin, phenytoin, NSAI, aspirin (Rey´s syndrome)
Toxins	*Amanita phalloides*, tetrachlormethane, bacterial toxins (*Bacillus cereus*), aphlatoxin, drugs (3,4-methylendioxymetamphetamine, MDMA, Extasy)
Ischemic	Right-sided heart failure, heart tamponade, septic shock, heat shock, portal vein thrombosis, Budd-Chiari syndrome
Metabolic	Wilson´s disease, α1-antitrypsin deficiency, fructose intolerance, galactosemia, tyrosinemia
Pregnancy	HELLP syndrome (hemolysis, elevated liver enzymes, low platelets)
Malignancy	Lymphomas, metastatic liver tumours
Other	Autoimmune hepatitis, primary graft failure after liver transplantation

Chronic liver failure is a long-term disease with progressive loss of functional liver parenchyma and compensatory mechanisms that solve some acute problems but create conditions for further pathological changes. Because portal hypertension leads to the formation of portosystemic anastomoses, the failure also vascular decompensation (Table 5.8). The most common cause of chronic liver failure is **liver cirrhosis**. The clinical picture depends on the underlying cause, but also on accelerating exogenous factors such as alcohol intake, high protein intake, intercurrent illnesses, hepatotoxic drugs, GIT bleeding and others.

TABLE 5.8 DIFFERENCES BETWEEN ACUTE AND CHRONIC LIVER FAILURE

Failure	Acute	Chronic
Dominant cause	Viral infections, toxins	Progression of cirrhosis of different origin
Course	Short (5 – 6 days to 6 months)	Long-term (years)
Mortality	High (up to 90%)	~30%
Damage	Hepatocellular failure	Hepatocellular + vascular decompensation
Clinical manifestation	Metabolic and neuropsychiatric	Metabolic, neuropsychiatric and vascular

Laboratory findings in liver failure

Biochemical manifestations of acute liver failure in addition to the pattern of hepatocellular and cholestatic injury in LFTs, are fasting hypoglycemia, dilutional hyponatremia and multifactorial ABR (Table 5.9).

TABLE 5.9 TYPICAL LABORATORY FINDINGS IN ACUTE LIVER FAILURE

Finding	Comment
↑↑ BIL	conjugated (regurgitation due to hepatocytes necrosis) + unconjugated (impaired uptake and conjugation)
↑↑AST, ALT (1000-times)	AST > ALT (marker of hepatocellular necrosis), do not correlate well with the severity of disease
↑↑ALP, GGT	reflect a cholestatic component of liver damage
Prolonged PT/INR	↓ FBG and other clotting factors (II, V, VII, IX, X)
↓ Alb, urea	decreased synthetic ability
↑ Ammonia	decreased elimination due to↓ urea synthesis
↓ Fasting glycemia	failure of liver gluconeogenesis, depletion of glycogen
↓ Calcemia	due to ↓ albumin (bound Ca^{2+}) and alkalosis (Ca^{2+})
↑Na^+, ↓K^+	secondary hyperaldosteronism
Mixed acid-based disturbance	lactic MAC + RAL + MAL

Hyperbilirubinemia and icterus: The change in the liver architecture in cirrhosis is the basis of the cholestatic type of icterus. As the disease progresses, both types of BIL always increase. Predominantly conjugated hyperbilirubinemia develops as the firs (leaking from necrotizing hepatocytes), and with the gradual disappearance of functional parenchyma (uptake and conjugation disorder), unconjugated BIL increases, eventually. Both BIL and UBG are positive in the urine (shunts, enterohepatic circulation failure).

Hepatic enzymes: AST, ALT are elevated with a preterminal fall, AST is significantly higher than ALT as a consequence of necrosis of hepatocytes. **GGT, ALP** are also elevated, very high especially if cholestasis is the cause of the failure (Budd-Chiari syndrome). In chronic liver failure, the activity of all four liver enzymes decreases with decreasing functional tissue, their activity to the reference extent does not exclude advanced cirrhosis.

Hypoglycemia: an acutely failing liver is not capable of gluconeogenesis (renal gluconeogenesis is not sufficient), therefore, hypoglycemia is present when glycogen stores are depleted. Chronic hepatic insufficiency also affects glucose metabolism by altering the production and breakdown of both insulin and glucostatic hormones (glucagon, steroid hormones, growth hormone). Actual blood glucose depends mainly on food intake. Postprandial hyperglycemia occurs due to glucose uptake by portocaval shunts directly into

the bloodstream. Insulin resistance may also be involved. In the fasting state, as in acute liver failure, there is a tendency to hypoglycemia.

Hypoalbuminemia: Albumin not necessarily decreases at first, due to its longer half-life, a more marked decrease indicates an escape into the extracorporeal space. In chronic liver failure, hypoalbuminemia and reduced production of coagulation factors are always present due to reduced proteosynthesis. Hypoproteinemia with hypoalbuminemia is a typical finding, hypoalbuminemia without hypoproteinemia is rarely found in the autoimmune origin of chronic hepatopathy due to increased production of immunoglobulins.

Ammonia: Insufficient urea synthesis in the liver leads to an increase in serum ammonia level.

Urea concentration in serum does not always correlate with renal function. The impairment of urea synthesis decreases serum urea level. On the other hand, catabolism (starvation, proteolysis, GIT bleeding) increases urea level. There is a marked increase in serum urea in hepatorenal syndrome.

Creatinine: In stabilized cirrhotic patients without renal impairment, creatinine tends to be within the reference interval or even lower due to muscle atrophy. If the disease progresses to the stage of vascular and parenchymal decompensation, levels of all nitrogen substances - urea, creatinine and uric acid - increase. That increase may initially signalize pre-renal renal failure (decreased GF for vasoconstriction, hypovolemia) - hepatorenal syndrome. Later, progression to intra-renal impairment (acute tubular necrosis) is possible.

Acid-base balance: In acute liver failure several mechanisms affect AB balance. As a result of a metabolic breakdown, MAC with elevated AG (lactic, ketoacidosis, uremic) is present. HAGMAC is frequently disguised by respiratory alkalosis due to irritation of the respiratory centre by accumulated toxins. Besides, metabolic alkalosis may be present as a consequence of secondary of an increase in the HCO_3^- concentration that compensates for the decrease in the negative charge of albumin.

Minerals: Hyponatremia from hemodilution (increase in ADH in hypovolemia) and redistribution in Na^+/K^+ pump failure (lack of energy), hypokalemia and hypomagnesemia in secondary hyperaldosteronism and diuretic therapy in ascites.

5.7 Chronic liver disease

If the clinical and biochemical manifestations of liver disease persist for more than 6 months, the condition is called chronic liver disease (CLD). In many cases, however, a disease exists long before clinical manifestation. The most common causes of CLD are viral hepatitis C and B, non-alcoholic fatty liver disease and alcoholic liver disease. Laboratory testing is essential for both the diagnosis and management of CLD. A typical finding of all types of CLD is an isolated increase in serum aminotransferases (AST, ALT), which reflects persistent hepatocellular damage, including point necrosis.

ALP and GGT activity generally increase at the time of disease activation or exacerbation, and they indicate intrahepatic cholestasis together with increased bilirubin. Indicators of synthetic liver function (albumin, PT, urea) remain normal until the disease progresses to cirrhosis. The

specific laboratory findings depend on the aetiology of CLD. Structural and functional changes in CLD include fibrosis, which correlates directly with the risk of cirrhosis and its complications (portal hypertension, hepatocellular carcinoma). The degree of fibrosis is the most important prognostic factor of CLD.

Non-alcoholic fatty liver disease

The obesity pandemic and increase in T2DM and metabolic syndrome have caused an increase in the incidence of non-alcoholic fatty liver disease (NAFLD). NAFLD affects a third of the European adult population, with prevalence of up to 70 – 90% in obese individuals. It is the most common cause of chronic hepatitis and pathological liver tests in asymptomatic adults. We consider NAFLD today as a hepatic manifestation metabolic syndrome. A key factor in its pathogenesis is insulin resistance, which leads to dyslipidemia and fat accumulation in the form of TAG in the liver. The disease has two phenotypes:

1. **nonalcoholic fatty liver** (NAFL) with no inflammatory component and fibrosis;
2. **nonalcoholic steatohepatitis** (NASH), where hepatocyte damage, inflammation and fibrogenesis are added to steatosis, which may later progress to cirrhosis.

The pathogenesis of NAFLD is multifactorial (Figure 5.5), with insulin resistance leading to increased synthesis and accumulation of TAG (steatosis) as an initial factor, and oxidative stress and inflammation promoted by the formation of cytokines and adipokines is another 'hit'. The main factors contributing to the disease are: hypercaloric diet, physical inactivity, genetic factors and endotoxemia from the microbiota of the digestive tract.

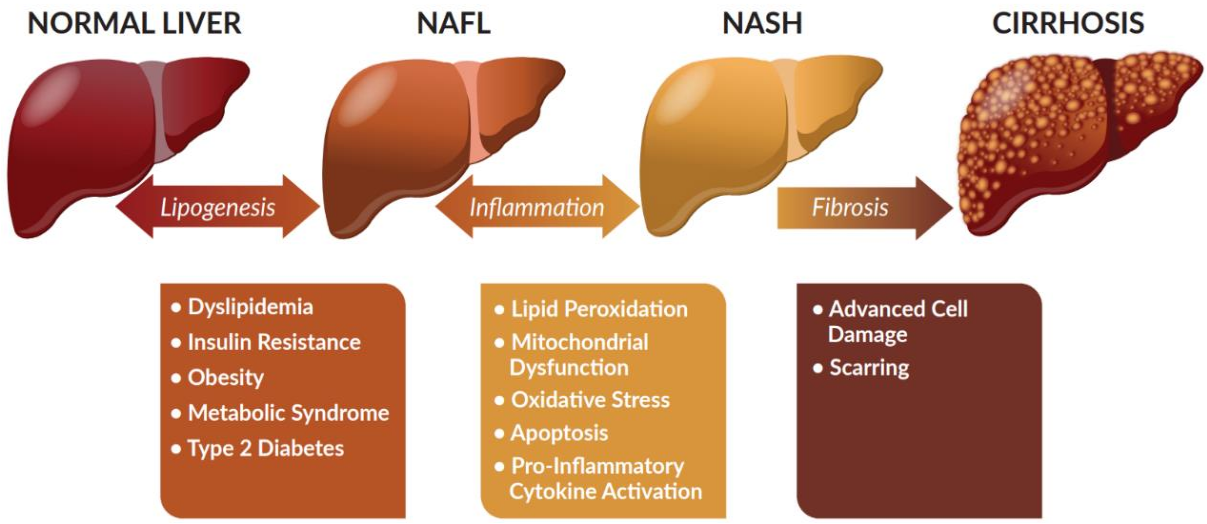


FIGURE 5.5 Pathogenesis and progression of NAFLD/NASH

A typical laboratory finding in NAFLD consists of a chronic increase in AST, ALT and GGT (max 3 times above ULRI) and the presence of other signs of metabolic syndrome, i.e. increased fasting blood glucose, central type obesity and typical dyslipidemia (increased TAG and low HDL-cholesterol). The diagnosis of NAFLD requires the presence of specific morphological criteria related to liver fat accumulation (e.g. ultrasound, CT), the absence of daily alcohol intake, and concomitant elimination of other specific causes of liver disease (Table 5.10). Liver biopsy allows definitive confirmation of advanced fibrosis and cirrhosis and the elimination of other liver diseases, but has its limitations and disadvantages.

TABLE 5.10 LABORATORY AND IMAGING TESTS IN NAFLD/NASH

Tests for confirmation	Test excluding other hepatitis
↑AST+ALT (in 10% of NASH patients normal)	Viral hepatitis
AST/ALT ratio < 1 (in alcoholic hepatitis > 2)	Alcohol steatohepatitis
↑ferritin (+ of normal transferrin saturation)	Autoimmune liver disease
Imaging test results confirming fat accumulation in the liver (ultrasound, MRI), liver biopsy	Congenital causes of chronic liver disease Drug induced hepatitis

All adults with steatosis regardless of elevated hepatic enzymes should be screened for metabolic syndrome. All individuals with persistently abnormal liver enzymes should be screened for NAFLD, because NAFLD is the main reason for unexpectedly elevated liver enzymes. In high-risk individuals diagnosed with NAFLD (age >50 years, T2DM, metabolic syndrome), it is recommended to evaluate the presence and extent of fibrosis using non-invasive laboratory markers or transient elastography (INFO 5.5).

INFO 5.5 Non-invasive markers of liver fibrosis

Considering the risks and limitations of liver biopsy, non-invasive methods for evaluating liver fibrosis and cirrhosis have been extensively studied and validated in the last two decades:
1. quantitative serum biomarkers that reflect liver changes and are indicators of fibrosis;
2. measurement of liver stiffness (elastography), which reflects the physical properties of liver tissue altered by fibrosis.
In addition to traditional liver tests, direct serum biomarkers that are directly derived from the fibrogenesis process, e.g. hyaluronic acid, PIIINP, MMP-1, -2, -9, TIMP-1. Many mathematical models - scoring systems-based markers or combination of serum markers and elastography were developed. The formulas are patented (FibroTest, Hepascore, Fibrometer) or non-patented (APRI, FIB-4). Most scoring systems can predict advanced fibrosis (Ishac > 2 or Metavir > 2) in various types of chronic liver disease, but they do not distinguish precisely the early stages of fibrosis and healthy liver sufficiently accurately compared to liver biopsy.
The use of FIB-4 or NAFLD Fibrosis Score (NFS) calculated using an on-line calculator is recommended in NAFLD patients. A rational approach to patient management in primary contact is as follows:
- low score: the patient remains under the care of the GP;
- median score: 2nd line examinations for the presence of fibrosis (ELF test, Enhanced Liver Fibrosis or elastography) are performed, and sent to a specialist for a pathological outcome;
- high score: sending to a specialized hepatology department.

Alcoholic liver disease

Chronic alcohol consumption causes alcoholic liver disease (ALD), which has three stages: steatosis, alcohol steatohepatitis (inflammation and necrosis) and liver cirrhosis. According to the EASL (European Association for the Study of the Liver) recommendations, there is no safe daily dose of alcohol. Drinking 25 g of alcohol daily increases the risk of cirrhosis. Risk factors for the development and progression of the disease are, for example, the quantity and manner of drinking, the presence of viral hepatitis C, female sex, genetic factors, obesity, nutritional deficiency, smoking. There is a clear direct relationship between the amount of alcohol consumed and the likelihood of developing ALD. Increased level of $NADH^{+}$ and acetyl-CoA

from ethanol degradation stimulates the synthesis of TAG and cholesterol. Fat accumulation is the first, reversible grade of ALD and leads to irreversible cirrhosis in the course of alcoholism.

Laboratory markers of excessive alcohol intake are indicated when ALD is suspected and also in certain groups of patients, who have to avoid drinking due to another disease (e.g. neurological, psychiatric, gastrointestinal). GGT and mean erythrocyte volume (MCV) are used as non-specific indicators of alcoholism. Carbohydrate-deficient transferrin (CDT) in serum and ethyl glucuronide in urine are more specific biochemical markers.

GGT: Alcohol induces GGT synthesis by hepatocytes, but the isolated test has low sensitivity in individuals with chronic alcohol intake (increased only by 30 – 50%) as well as specificity (also other GIT diseases increase GGT activity).

Mean cellular volume (MCV): Finding of mild macrocytosis and increased GGT activity is a better indicator of excessive alcohol intake. The disadvantage of MCV is that the increase persists for several months after the patient has stopped drinking. As with GGT, other factors affect MCV, reducing its specificity.

Carbohydrate-deficient transferrin (CDT): Alcohol induces synthesis of modified transferrin with reduced carbohydrate (sialic acid) content. The concentration of CDT in serum increases when more than 60 g of ethanol is taken daily (SP 82 – 92%, SN only 58 – 69%). CDT testing is useful for screening for excessive alcohol intake and differentiation between ALD and NAFLD.

Ethyl glucuronide is formed by the combination of unoxidized ethanol with glucuronic acid by the action of UDP-glucuronyltransferase. Only 0.02 – 0.04% of the ingested alcohol is eliminated in this way. An important fact is that ethyl glucuronide does not form in endogenous alcohol production (fermentation). For this reason, this is a specific biomarker of alcohol consumption, in monitoring compliance with abstinence, and for forensic purposes. It has less importance in the diagnosis of addiction, as it is also positive in the general consumer. The presence of ethyl glucuronide in the urine indicates ingestion of alcohol generally within 4 days of the last consumption.

5.7 Metabolic liver diseases

Many congenital metabolic diseases attack the liver as part of generalized organ damage. The liver is the major organ damaged by a metabolic disorder in the following diseases for which laboratory testing is necessary: congenital hemochromatosis (more information in the Chapter 13), α1-antitrypsin deficiency, and Wilson's disease.

α1-antitrypsin deficiency

α1-antitrypsin (AAT) deficiency is a relatively common monogenic autosomal recessive disease in the Caucasian population. The affected AAT gene, known as SERPINA 1, is located on the 14th chromosome and has over 120 variant alleles described. Clinically manifest disease is mainly caused by two of them: PiZ (Glu342Lys) and PiS (Glu264Val), with the variant ZZ (occurrence of 1 : 2 000 – 7 000 in the European population) having the most severe phenotypic involvement.

AAT is the most important natural inhibitor of serine proteases in the blood. Despite its name, more than trypsin it affects other proteases, in particular, neutrophil elastase, many tissue proteases and cathepsin G. AAT diffuses into the interstitial and alveolar lung fluid and is designed to inactivate neutrophil elastase, which is released during phagocytosis in the alveoli, thereby preventing elastin degradation. A gene defect leads to the formation of AAT with altered spatial configuration, which prevents its release into the circulation and results in its accumulation in hepatocytes with consequent damage and apoptosis.

The clinical manifestation of AAT deficiency depends on the patient's phenotype and age. Hepatic impairment occurs in young children (neonatal hepatitis, persistent jaundice with hepatosplenomegaly) as well as in adults. Chronic hepatitis gradually develops and progresses to cirrhosis with the risk of liver failure and hepatoma. Lung involvement, which is rare in children, is manifested in adults as premature (before 45 years) pulmonary emphysema and bronchiectasis with the development of chronic obstructive bronchopulmonary disease. For the pulmonary manifestation of AAT deficiency, a decrease in anti-proteinase activity below 30% of normal is required. Although the heterozygous condition does not lead to disease manifestation, it may be a factor that worsens the progression of other liver diseases, e.g. viral hepatitis C, NAFLD.

The following laboratory tests are available in laboratory diagnostics, which is an essential complement to a functional and morphological examination of the liver and lungs in AAT deficiency:

- **α1-antitrypsin** - the serum concentration of AAT has low sensitivity because it can be falsely increased for other reasons, e.g. as an acute phase reaction in inflammation, malignancy, pregnancy and oestrogen therapy, after blood derivatives containing ATT;
- **phenotyping of alleles by isoelectric focusing** is critical for diagnosis and is performed in specialized laboratories;
- **genetic testing** carried out to confirm defective bi-allelic variants of SERPINA1 if the phenotyping result is unclear.

Wilson´s disease

Wilson´s disease (WD) is a rare genetic disorder transmitted by autosomal recessive inheritance (prevalence of 1 : 30 000 in Europe) characterized by excessive accumulation of copper in the liver and other organs because of an inherited defect in biliary excretion of copper. The disease affects homozygotes with mutations of the gene for membrane protein ATP-ase type 7B (ATP7B), which is responsible for both exocytosis of copper from hepatocytes and incorporation of copper into serum glycoprotein – ceruloplasmin.

Copper accumulation causes hepatocellular damage. If the capacity of hepatocytes to store copper is exceeded or free copper is released from broken hepatocytes, the proportion of unbound copper in blood will increase, followed by accumulation in other organs, especially the brain, kidneys, heart, cornea, joints, erythrocytes (Figure 5.6).

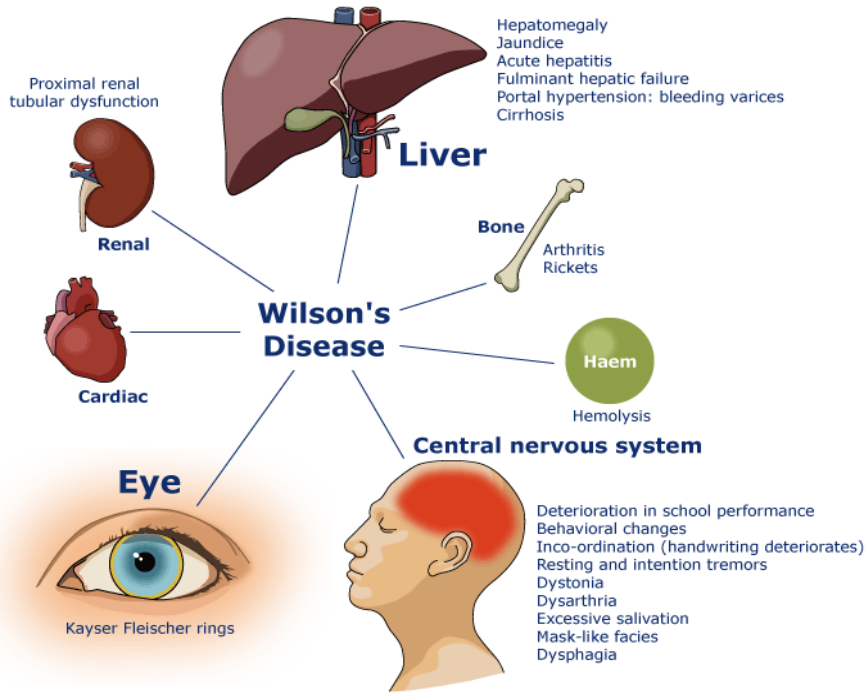


FIGURE 5.6 Organ involvement and symptoms of Wilson´s disease *Source: http://www.eurowilson.org*

The clinical manifestation of WD is **hepatic** (more common in younger than adult patients) in the form of acute or chronic hepatitis or **neuropsychiatric** manifestations resulting from degenerative changes in the basal ganglia. Hepatic involvement may present as only abnormal liver enzymes or disproportional unconjugated hyperbilirubinemia, but also in the form of acute liver failure, or cirrhosis. A typical finding is the Kayser-Fleischer ring due to copper accumulation in the eye cornea, present in up to 95% of individuals with the neurological form, but only in 30 – 50% of the hepatic form of WD.

The **diagnosis of WD** is based on clinical findings, laboratory and imaging examinations (brain NMR) and liver biopsy (Table 5.11). The serum **ceruloplasmin** measurement is not 100% specific for WD, although 95% of homozygotes have reduced serum concentrations. Spuriously "normal" levels of ceruloplasmin may occur as a result of acute-phase response in inflammatory conditions, during pregnancy and after estrogens administration. Decreased ceruloplasmin may be part of any hypoproteinemia.

The measurement of **24-hour urinary copper excretion** usually exceeds 1.5 µmol/24 h in WD and reflects the increased plasma non-ceruloplasmin bound copper. **Urinary excretion of copper**, which is also suitable for screening the disease among relatives of a WD patient, has a higher predictive value. The excretion of copper increases markedly after penicillamine administration. False high urine copper levels also occur in massive hepatocyte necrosis or nephrotic proteinuria.

Genetic testing provides evidence of ATP7B gene mutation. Currently, about 500 mutations are known, so only the most common of these are investigated. The most frequent (57%) in Central Europe is the substitution of histidine for glutamine at position 1 069 (mutation H1069Q). A negative genetic examination result does not exclude the presence of a rare mutation. Genetic screening is also recommended for siblings and offspring of a patient with WD. Asymptomatic carriers of the ATP7B gene mutation must also be treated as the disease has 100% penetration.

TABLE 5.11 DIAGNOSTIC FINDINGS IN WILSON´S DISEASE

Test	Normal	Wilson´s disease
S-ceruloplasmin	0.2 – 0.6 g/L	<0.2 g/L
U-copper	<0.6 μmol/24 h	>1.6 μmol/24 h
Copper content in the dry liver tissue (biopsy)	<250 μg/g	>250 μg/g
Kayser – Fleischer ring, sun-flower cataract	absent	Present in neurological form and in homozygotes, absent in young patients
Brain NMR	normal	Copper accumulation in the mid-brain area ("face of a giant panda")

Liver biopsy for **hepatic copper content** in dry hepatic tissue is the most precise diagnostic tool. Most homozygotes for WD have levels higher than 450 μg/g dry weight; in most of the asymptomatic heterozygotes copper content in liver exceeds 250 μg/g dry weight. Several other hepatic conditions manifesting with extreme cholestasis show elevated copper concentrations in the liver, but usually, they can be diagnosed by other clinical, serologic, or histological criteria.

Case studies and self-assessment questions

Case 5.1

A 14-year-old boy is examined by a general practitioner for 2 days of fever (more than 38.5 ° C), muscle aches, reluctance to eat. The doctor seems to have yellow sclera, the liver is neither palpable nor painful, neither BIL nor UBG is present in the urine. "Liver tests" are listed in the table. After 5 days at re-examination, temperature and jaundice subsided, S-BIL is still 30 μmol/L and ALP 8.0 μkat/L. Blood counts, including reticulocytes, were normal in both samples.

Serum	Result	RI
Alb	45	36 – 50g/L
BIL	61	<20 μmol/L
ALT	0.55	<0.8 μkat/L
ALP	8.4	1.35 – 7.5 μkat/L
GGT	0.51	<0.65 μkat/L

Questions:

a. What is the most likely cause of pathological biochemical finding?
b. How could be explained the increased activity of ALP?

Case 5.2

A 65-year-old patient repeatedly hospitalized for liver cirrhosis. Jaundice, muscle hypotrophy and ascites are present on admission. Laboratory finding is shown in the table.

Serum	Result	RI	Urine analysis	
Glucose	3.50	3.6 – 5.5 mmol/L	Protein	+
Urea	0.9	2.8 – 8.0 mmol/L	Glc	-
Creatinine	34.4	6 – 106 µmol/L	Ketones	+
Alb	28.6	32 – 48 g/L	BIL	+
BIL total	213	<20 µmol/L	UBG	+
BIL conj.	187	<5 µmol/L		
AST	2.29	<0.8 µkat/L		
ALT	0.97	<0.8 µkat/L		
GGT	10.4	<1.02 µkat/L		
ALP	4.52	0.67 – 2.15 µkat/L		
Ca^{+2} total	1.90	2.0 – 2.75 mmol/L		
K^{+}	3.2	3.5 – 5.5 mmol/L		
Ammonia	261	<50 µmol/L		

Questions:

a. What type of jaundice does the patient have?
b. Is hypoglycemia common in liver cirrhosis?
c. Why does the patient have low urea and creatinine?
d. Is decreased calcium related to other biochemical parameters?
e. What is likely cause of hypokalemia?

Self-assessing questions

1. Name liver function tests and their importance in liver function examination.
2. Which causes of unconjugated hyperbilirubinemia do you know?
3. What is an early marker of decreased hepatic proteosynthesis?
4. Name the biochemical markers of alcohol use and identify the most specific one.
5. What is the most common cause of elevated hepatic enzymes in asymptomatic individuals?
6. List the extracellular sources of elevated ALP activity.
7. Name causes of hyperammonemia?
8. What is the cause of fasting hyperglycemia in a patient with liver cirrhosis?
9. Which biochemical tests support the diagnosis of non-alcoholic fatty liver disease?
10. Which biochemical tests would you order if Wilson's disease is suspected?

KEY INFORMATION

- ☑ The liver has a central position in metabolism, being involved in carbohydrate, fat, steroid and protein synthesis; storage of vitamins and metals; and detoxification of exogenous and endogenous substances.
- ☑ Liver function tests are helpful for assessment of certain liver functions, to follow the course of disease and to monitor response to treatment. However, they do not inform about the origin of liver disease.
- ☑ A typical panel of LFTs includes enzymes ALT and AST for detection of hepatocellular damage, bilirubin to assess conjugation in hepatocytes and to detect biliary obstruction, enzyme activities of ALP and GGT for identifying cholestasis.
- ☑ Unconjugated (free) bilirubin is bound to albumin in the blood, in the liver bilirubin enters hepatocytes, where it is conjugated with glucuronic acid and excreted into the bile.
- ☑ In hyperbilirubinemia, unconjugated BIL dominates in as a consequence of hemolysis, or conjugated BIL prevails in hepatic and post hepatic disorders.
- ☑ Kernicterus is a neurological condition caused by the deposition of unconjugated bilirubin in the brain. It occurs in new-born children who have a very high level of blood bilirubin.
- ☑ The relative non-specificity of serum AST and to a lesser extent ALT, form liver disease must always be taken into consideration when interpreting results.
- ☑ ALP activity in the serum of children is higher than those of adults due to increased release of bone isoenzyme during growth phases.
- ☑ Serum GGT activity increases not only in cholestasis, but also under the influence of alcohol and drug intake.
- ☑ Prolonged prothrombin time or INR is an early marker of decreased synthetic liver function. Hypoalbuminemia signalizes relatively advanced failure of liver proteosynthesis.
- ☑ Acute and chronic liver failure and its outcome - hepatic encephalopathy is characterized by typical clinical and laboratory findings of severe coagulopathy, resulting from the failure of damaged hepatocytes to synthesize clotting factors.
- ☑ The increasing incidence of chronic liver disease in adults has three main causes: alcoholic liver disease, non-alcoholic fatty liver disease and viral hepatitis.
- ☑ Non-alcoholic fatty liver disease, which affects up to 30% of the adult population in developed countries, results from the high incidence of obesity, metabolic syndrome and T2DM.

6

Diabetes mellitus and hypoglycemia

CONTENT

Diabetes mellitus is the most common metabolic disorders with dramatically increasing incidence. The entire world is facing the pandemic of diabetes, which is being thought a serious problem in all developed countries because of its adverse micro- and macro-vascular complications. Estimates predict that by 2040, there will be 640 million people with diabetes worldwide. The average cost of diabetes in countries of EU and US exceeds 10% of the national health budget. Diabetes is undoubtedly one of the most serious diseases at all because its symptoms and complications extend to nearly every branch of medicine. Biochemical tests are particularly important in screening and diagnosing of the disease, in monitoring its control and treating its metabolic complications. It is generally supposed, that 40% of diabetics remain undiagnosed and they are potentially at risk of premature vascular complications due to accelerated atherosclerosis. Hypoglycemia occurs as an emergency complication diabetic patient and rarely from other reasons in the non-diabetic population.

In addition to basic physiological information, this chapter aims to give an overview of the biochemical tests used in:

- in disease screening and diagnosis;
- monitoring and evaluation of its compensation;
- diagnosis of both acute and chronic complications;
- differential diagnosis of hypoglycemia in non-diabetic individuals.

6.1 Basic physiology

Glucose homeostasis

The blood glucose concentration (glycemia) is constantly is tightly regulated and maintained within the physiological range, which ensures its smooth delivery to vital organs (especially the brain), while avoiding the adverse effects of hypoglycemia or renal glucose loss when the kidney threshold for glucose is exceeded. Glucose balance regulation is a process involving the intake, utilization, storage and excretion of glucose in which many organs are involved (INFO 6.1). Fasting plasma glycemia in a healthy individual is maintained in the range 3.9–5.5 mmol/L. The movement of glucose into cells depend on function of two types of transporting mechanism:

1. **SGLTs** – sodium coupled glucose co-transporters, that contribute an active transport of GLU against a concentration gradient
2. **GLUTs** – glucose transporting proteins, that mediate passive transport of glucose across cell membrane. GLUT1-2 transporters are present ubiquitous on the cell surface at all times, whereas GLUT4 in muscles, adipocytes and heart are **insulin sensitive,** e.g**.** they are translocated onto cell membrane from cytoplasm after insulin has been bound on its receptor.

INFO 6.1 Organs involved into glucose homeostasis

Liver does not need insulin to facilitate glucose uptake. However, insulin plays a key role in the regulation of glucose output by the liver. It prevents gluconeogenesis and breakdown of stored glycogen. In fed state liver removes about 70% of the glucose load that is delivered by portal vein from the gut, which is partly oxidised and partly converted to glycogen for next use as a fuel under fasting conditions. Glucose exceeding these requirements is metabolized by the liver into fatty acids and triacylglycerols (TAG), which are incorporated into very low-density lipoproteins (VLDL) and transported into adipose tissue stores. In the fasting state blood glucose concentration is maintained by liver glycogen breakdown while glycogen stores last (several hours), and then by gluconeogenesis from glycerol, lactate, pyruvate and gluconeogenic amino acids.

Muscle is responsible for the majority (~75 – 80%) of glucose uptake. Once glucose enters muscle cells, it can be used immediately for energy or stored as glycogen. Insulin activates glycogen synthase, the key enzyme regulating the production of glycogen.

Adipose tissue is the major reservoir for excess glucose storage in the form of TAG. Insulin promotes the synthesis of TAG by activation of lipoprotein lipase in the capillary walls of adipose tissue and by inhibiting hormone-sensitive lipase within adipocytes, preventing the hydrolysis of stored TAG. During fasting state muscle and adipose tissue adapt to the oxidation of fatty acids for energetic purposes.

Kidney is involved in glucose homeostasis by three mechanisms: a release of glucose into circulation via gluconeogenesis, uptake of glucose from circulation to satisfy its energy needs, and reabsorption of glucose in proximal tubules, where SGLT1-2 and GLUT 1-2 are located. More glucose may be excreted in the urine if the concentration in filtered urine exceeds the threshold of glucose reabsorption.

Hormones regulating glucose homeostasis

The regulation of glucose metabolism is under the influence of several hormones whose target tissues are liver, muscles and adipose tissue. Of these, only insulin lowers the postprandial blood glucose concentration by facilitating glucose entry into cells. In addition, it supports the synthesis of many substances, i.e. anabolic effects. The effect of glucagon, growth hormone,

cortisol and adrenaline on blood glucose is the opposite. These **counter-regulating hormones** increase their concentration in blood at low glucose concentrations and provide sufficient energy substrates from glycogenolysis, gluconeogenesis (brain), lipolysis and ketogenesis (Table 6.1).

TABLE 6.1 THE EFFECTS OF VARIOUS HORMONES ON GLUCOSE METABOLISM

Tissue and hormone	Insulin	Glucagon	Adrenalin	Cortisol	GH
LIVER					
Glycogenolysis	↓↓	↑↑	↑↑↑	None	None
Glucogenogenesis	↓↓	↑↑↑	↑	↑↑↑	↑
Ketogenesis	↓	↑↑	↑	↑	↑
MUSCLE					
Glycogenolysis	↑↑↑	None	↓	↓↓	↓
Ketone metabolism	↑	?	↓	?	?
ADIPOCYTES					
Glucose uptake	↑↑↑	None	↓	↓↓	↓
Lipolysis	↓↓↓	None	↑↑↑	↑↑	↑

Key: ↑ = stimulatory, ↓ = inhibitory, None = none effect, ? = uncertain

Insulin

Pancreatic β-cells function as glucose sensors; they secrete insulin in response to hyperglycemia and reduce insulin secretion if blood glucose decreases. The precursor of insulin in the process of its synthesis is preproinsulin (86 AK), which is progressively cleaved to proinsulin to **insulin** (51 AK) and **C-peptide** (connecting peptide) by the action of several peptidases in the endoplasmic β-cell reticulum (Figure 6.1).

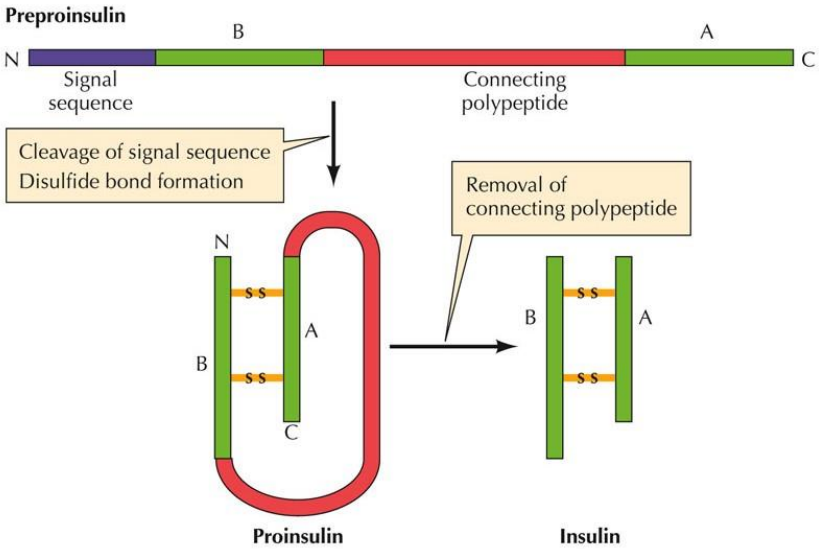


FIGURE 6.1 Scheme of insulin and C-peptide formation

Both molecules are secreted into the bloodstream in the form of secretory granules in an equimolar ratio from where they are removed from the bloodstream at different rates, resulting in their different blood ratios. Insulin concentration in the blood is lower than that of the C-peptide, mainly due to its more intensive clearance when passing through the liver. Along with insulin, the peptide hormone **amylin** is secreted from β-cells. It inhibits glucagon secretion, slows gastric emptying and functions as a signal of satiety to the brain.

Incretin hormones

Substrates other than glucose may also increase insulin secretion. Some amino acids and fatty acids present in food have also been found to increase the formation of so-called incretin or insulinotropic hormones in the small intestine. The best known are GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitor polypeptides), which are secreted after oral intake of a mixed diet.

As a result of incretin hormones, insulin secretion increases about threefold compared to an increase after parenteral glucose administration. Incretin hormones stimulate glucose-dependent insulin secretion by activating G-protein-coupled receptors on the surface of β-cells. They also increase gene expression and insulin biosynthesis, stimulate proliferation and differentiation of β-cell, and prolong their viability. Besides, GLP-1 reduces glucagon secretion by pancreatic α cells. Incretins are also part of signalling a feeling of satiety for the brain. Sulphonylurea (tolbutamide) an oral hypoglycemic medicine used for the type 2 diabetes, has also a direct stimulatory effect on β-cells. It acts by binding to a membrane sulphonylurea receptor (SUR1), which is intimately linked with K-ATP channel, and result in cell depolarization necessary for insulin secretion (Figure 6.2).

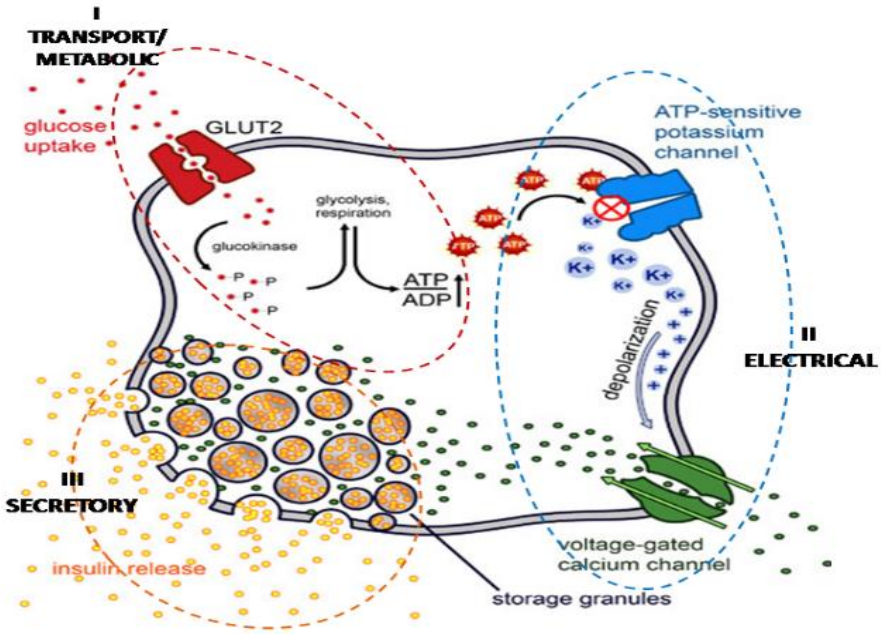


FIGURE 6.2 The main phases of glucose-induced insulin secretion

6.2 Metabolic disorders in diabetes

Diabetes mellitus, diagnosis, classification

Diabetes mellitus (DM) is a group of metabolic diseases characterized by absolute or relative insulin deficiency. In addition to chronic hyperglycemia caused mainly by reduced glucose utilization by cells, there is a disorder of fat and protein metabolism, secondarily there is a disorder of ionic and acid-base balance. The disease is complicated by acute metabolic and

chronic vascular complications, which lead to impaired function of many organs (nephropathy, retinopathy, neuropathy, accelerated atherosclerosis with its cardiovascular manifestations) and shorten the life expectancy of diabetics by more than 25%.

The disease is classified into several categories. Type 2 diabetes accounts for over 90% of cases of diabetes in the United States, Canada, and Europe; type 1 diabetes accounts for another 5-10%, with the remainder due to other causes (Table 6.2). In type 1 diabetes mellitus (T1DM), there is essentially no insulin secretion, whereas in type 2 DM (T2DM) insulin is secreted, but in amounts that are inadequate to prevent hyperglycemia, or there is resistance to its actions. In T2DM, a degree of hyperglycemia sufficient to cause pathological and functional changes in target tissues, but without clinical symptoms may be present long period before diabetes is detected. Minor portion of diabetes may be caused by specific genetic defects or may be a secondary consequence of other diseases.

TABLE 6.2 ETHIOLOGIC CLASSIFFICATION OF DIABETES MELLITUS

Category	Frequency	Features
Type 1 diabetes	5 – 10%	- β-cells destruction (immune mediated or idiopathic), - usually leading to absolute insulin deficiency - multiple genetic predispositions modified by environmental factors - prone to other autoimmune disorders - association with HLA DR3, DR4 (95%) and anti-GAD (85%)
Type 2 diabetes	90 – 95%	- may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance - strong genetic predisposition
Gestational diabetes	~7% of pregnancy	- Any degree of glucose intolerance with onset or first recognition after the 20th week of pregnancy
Other specific types	1 – 2%	- diseases of the exocrine pancreas (cystic fibrosis, chronic pancreatitis, hereditary hemochromatosis - endocrine diseases (acromegaly, Cushing´s syndrome and disease, glucagonoma, pheochromocytoma, prolactinoma…) - genetic defects of B-cell function (MODY, neonatal DM) - genetic defects in the action of insulin - drug and chemical induced DM (glucocorticoids, β-adrenergic agonists, thiazides, thyroid hormones, diazoxide, γ-interferon)

HLA DR - human leucocyte antigen DR isotype, GAD - glutamic acid decarboxylase, MODY - Maturity Onset Diabetes of Young

Hyperglycemia

The main pathogenic processes involved in the development of diabetes separately or in combination are β-cell dysfunction and insulin resistance. **Fasting and postprandial hyperglycemia** arises from combination of a reduced efficiency with which insulin can move glucose into tissues and a decrease in the number of functioning β-cells. **Insulin resistance** is term for abnormalities in insulin effects anywhere on the pathway from the receptor on the cell surface to intracellular proteins that regulate glucose transport.

Dysfunction of β-cell means their reduced ability to secrete insulin in response to hyperglycemia. The loss of β-cells is the result of their death (apoptosis or necrosis) most often due to the autoimmune process in T1DM. In T2DM, the pancreas initially tries to compensate

for the state of insulin resistance by increased insulin secretion, but later the number of functional β-cells decreases due to their increased apoptosis. Pancreatic cells are exposed to chronic hyperglycemia (= glucotoxicity) and high levels of free fatty acids (= lipotoxicity), which contribute to impaired insulin secretion and β-cell loss. Impairment in insulin secretion and defects in insulin action frequently coexist in the same patient, resulting in reduced glucose uptake into fat and muscle cells and increased glucose production by the liver.

Increased **hepatic glucose production** is the result of both insulin resistance and α-cell dysfunction (glucagon). Almost 90% of glucose comes from gluconeogenesis, which, in addition to insulin deficiency, is stimulated by elevated glucagon levels, elevated concentrations of circulating glucagon levels, elevated concentrations of circulating glucogenic precursor molecules, and elevated free fatty acid levels. Increased hepatic glucose production leads to fasting hyperglycemia.

Incretin hormones (GIP, GLP-1) increase insulin secretion in the pancreas after oral glucose and mixed dietary intake. The incretin response is almost completely absent in T2DM, resulting is insufficient stimulation of insulin secretion and inappropriate glucagon secretion, which further increases hyperglycemia.

Adipocyte dysfunction and increased visceral fat volume in T2DM also negatively affect glucose homeostasis. Elevated levels of cytokines and adipokines secreted by fat cells increase insulin resistance and increase lipolysis. Increased offer of fatty acids in muscle cells and hepatocytes is likely to contribute to the deterioration of insulin resistance in these tissues.

Impaired lipoprotein regulation and metabolic syndrome

The typical lipid finding in T2DM consists from the increased concentration of triacylglycerols (TAG), decreased HDL-cholesterol (HDL-CH), which together with an increased number of small dense LDL particles (sdLDL). All of them are independently atherogenic and create so-called **atherogenic dyslipidemia**. Hypertriacylglycerolemia is due to insulin resistance, which increases the formation of VLDL particles in the liver, as well as their slower clearance from the circulation due to low lipoprotein lipase (LPL) activity. In addition, the increased activity of hormone-sensitive lipase, which is normally suppressed by insulin, causes increased fasting and postprandial FFA production enhancing their incorporation into VLDL.

The pathological catabolism of VLDL (details in Chapter 7) leads to the formation of smaller, atherogenic sdLDL particles as well as smaller, on TAG-poorer HDL particles, which are removed more rapidly from the circulation. Insulin deficiency adversely affects the production of apolipoprotein A-I (apoA-I), thereby contributing to a reduced concentration of circulating HDL particles. As obesity develops, TAGs can also be stored in non-fat cells, especially in the liver and skeletal muscle.

A growing body of evidence has confirmed, that a combination of several metabolic abnormalities, including abdominal obesity, hyperglycemia, atherogenic dyslipidemia and hypertension, is associated with a risk of cardiovascular disease and T2DM. The listed risk factors are part of the so-called **metabolic syndrome** (MS). The main negative consequences of MS are endothelial dysfunction, which involves arterial hypertension, oxidative stress and chronic inflammation induced by pro-inflammatory cytokines (IL-6, TNF-α) released from adipose tissue macrophages as well as adipokines from adipocytes.

There are several **definitions** for metabolic syndrome. The International Diabetes Federation (IDF) and Adult Treatment Panel III (ATP III) are the most widely used. They recommend using the following five criteria, the presence of **any of three** of them qualifies for the diagnosis of metabolic syndrome (Table 6.3).

TABLE 6.3 METABOLIC SYNDROME – DIAGNOSTIC CRITERIA

Criterium	ATP III, 2005	IDF, 2009
Waist circumference	≥102 cm European men ≥88 cm women	≥94 cm European men ≥80 cm women
FPG	≥5.6 mmol/L (100 mg/dL), or treatment for hyperglycemia	≥5.6 mmol/L (100 mg/dL), or previously diagnosed T2DM
TAG	≥ 1.7 mmol/L (150 mg/dL) or specific treatment for this lipid abnormality	
HDL cholesterol	men <1.0 mmol/L (40 mg/dL); women <1.3 mmol/L (50 mg/dL) or specific treatment for low HDL	
BP	≥130/85 mm Hg or drug treatment for hypertension	

The key clinical implication of a diagnosis of MS is identification of a patient who needs an aggressive lifestyle modification focused on weight reduction and increased physical activity. Because metabolic syndrome traits co-occur, patients identified with one or just a few traits are likely to have other traits as well as insulin resistance. Available **laboratory markers of insulin resistance** are hyperglycemia and dyslipidemia (high TAG and low HDL-cholesterol). In addition, although no formal definitions of MS include glycated hemoglobin (HbA1c), abnormal HbA1c (5.7 – 6.4%) is increasingly accepted and used to define impaired glycemia in patients with metabolic syndrome.

6.3 Screening and diagnosis of diabetes mellitus

Rationale for screening

Type 2 diabetes mellitus accounts for over 90% of patients with diabetes; rates of undetected diabetes may be as high as 40 – 50%. Screening for T2DM (INFO 6.2) meets most of optimal conditions supposed for screening for any disorder:

- Diabetes is one of the major causes of early illness and death worldwide with the raising global prevalence.
- A relatively long asymptomatic period exists.
- Several screening tests are available; each can successfully diagnose asymptomatic cases of diabetes.
- Well-established treatments for T2DM and prevention of its complications exist.
- Early identification of diabetes allows interventions to prevent or limit cardiovascular complications.
- Interventions for prediabetes can prevent or delay the onset of diabetes.

INFO 6.2 Indications for screening of diabetes and prediabetes

1. Adults with a BMI >25 kg/m^2 and the following risk factors:
   - first-degree relatives of diabetics;
   - belonging to a high-risk ethnic group (Roma ethnic group in Slovakia);
   - history of gestational DM;
   - hypertension (>140/90 mmHg or treatment of hypertension);
   - HDL cholesterol <0.90 mmol/L and/or TAG 2.80 mmol/L;
   - polycystic ovary syndrome;
   - physical inactivity;
   - other conditions with increased insulin resistance (e.g. obesity, acanthosis nigricans)
2. Patients with prediabetes are tested repeatedly once a year.
3. Women with established gestational DM should be tested every 3 years.
4. Other unnamed individuals shall be examined at the age of 45 years and with a negative result every 3 years, unless their risk factors change.
5. Children and adolescents under 18 years of age with an overweight BMI >85th percentile for a given age and sex and at least one risk factor:
   - DM in the mother or GDM in the mother during the gestational development of the child;
   - family history of T2DM in 1st or 2nd-degree relatives;
   - manifestations of insulin resistance or pathological conditions associated with insulin resistance (e.g. hypertension, dyslipidemia, PCOS, low birth weight);
   - belonging to a high-risk ethnic group.

The diagnosis of diabetes is traditionally based on the identification of chronic hyperglycemia. Tests that can be used to screen are measurement of **fasting plasma glucose** (FPG), a **glycated hemoglobin** (HbA1c), and a **2-hour plasma glucose** after administration of 75 g of glucose during an oral glucose tolerance test (OGTT) – Table 6.4. In patients with clinical manifestations of the disease (thirst, polyuria, abdominal pain, fatigue, weight loss), accidental glycemia ≥ 11.1 mmol/L in venous plasma confirms the diagnosis of diabetes. If the clinical manifestations are not clearly stated, the diagnosis of diabetes requires a repeated finding of increased fasting plasma glucose or OGTT-confirmed 2-hour plasma glucose to rule out laboratory measurement uncertainty. When fasting glucose is in the range of 5.6 – 6.9 mmol/L, OGTT is performed, with glycemia being diagnostic for 2 hours after glucose administration.

TABLE 6.4 CRITERIA FOR THE DIAGNOSIS OD DIABETES AND PREDIABETES

Criterium	Diabetes mellitus	„Prediabetes“
FPG	≥7.0 mmol/L (126 mg/dL)	5.6 – 6.9 mmol/L (100-125 mg/dL)
	or	IFG: Impaired fasting glucose
2h-PG in OGTT	≥11.1 mmol/L (200 mg/dL)	7.8 – 11.1 mmol/L (140-199 mg/dL)
	or	IGT: Impaired glucose tolerance
HbA1c	≥6.5% (48 mmol/mol Hb)*	5.7 – 6.4%
	or	
Symptoms of hyperglycemia and random plasma glucose ≥11.1		mmol/L (200 mg/mL)

**test should be performed by method standardized to the DDCT assay or IFCC standard*

Since 2011, international commissions of experts and the World Health Organization (WHO) have recommended glycated hemoglobin (HbA1c) as an alternative parameter for the diagnosis of T2DM. All established criteria for the diagnosis of diabetes are listed in the Table 6.4. HbA1c is a new diagnostic parameter for T2DM provided that the test is performed by a standardized method (INFO 6.3). HbA1c ≥6.5% (48 mmol/mol) confirms the diagnosis of diabetes. However, values below 6.5% do not rule out diabetes that has been diagnosed based on glycemia. Individuals with HbA1c in the range of 5.7 – 6.4% (39 – 47 mmol/mol) have an increased risk of developing DM (prediabetes category).

HbA1c should not be used a diagnostic parameter in children, DM1 and gestational diabetes. In terms of **pre-analytical conditions**, the HbA1c test has several advantages over glycemia:

- sampling of blood is possible at any time during the day, the patient does not have to be fasting;
- an EDTA tube is required without the need to add a glycolysis inhibitor (NaF);
- HbA1c is much more stable in the tube, no immediate measurement is required.

The disadvantage is the higher price, lower availability in smaller laboratories and factors influencing its concentration. Individuals whose glycemia do not meet the criteria for DM, but are higher than normal values, fall into the category of so-called prediabetes.

Individuals, whose glucose levels do not meet criteria for diabetes, but are higher than normal were defined as having **prediabetes**, indicating the increased risk for the future development of diabetes. They are categorized as **impaired fasting plasma glucose** (IFG) **impaired glucose tolerance** (IGT) based on the abnormal value of either fasting or postprandial (2-h value in OGTT) plasma glucose level.

INFO 6.3 HbA1c measurement and reporting

Laboratory methods for the determination of HbA1c have made progress in standardization since 2009. All methods used (chromatographic - HPLC, immunochemical and electrophoretic) are calibrated to the reference method according to the IFCC (International Federation of Clinical Chemistry). IFCC methods express results in different units than older NGSP method (based on National Glycohemoglobin Standardization Program), according to the SI system - in mmol/mol (HbA1c/Hb).

For healthcare professionals and patients to get used to the new units and HbA1c values, the results are expressed in traditional percentages of the total Hb during the transition period. Reference values in nondiabetic individuals are for NGSP/DCCT assays 4 – 6% and for IFCC assays 20–42 mmol/mol.

$HbA1_c$ conversion table

Definitions	Old unit New unit	= NGSP unit = IFCC unit	= %$HbA1_c$ = mmol/mol
Conversion formulas	Old New	= 0,0915 New + 2,15% = 10,93 Old – 23,5 mmol/mol	

$HbA1_c$ Old	$HbA1_c$ New	$HbA1_c$ Old	$HbA1_c$ New
4,0	**20**	8,1	**65**
4,1	**21**	8,2	**66**
4,2	**22**	8,3	**67**
4,3	**23**	8,4	**68**
4,4	**25**	8,5	**69**
4,5	**26**	8,6	**70**
4,6	**27**	8,7	**72**
4,7	**28**	8,8	**73**
4,8	**29**	8,9	**74**
4,9	**30**	9,0	**75**
5,0	**31**	9,1	**76**
5,1	**32**	9,2	**77**
5,2	**33**	9,3	**78**
5,3	**34**	9,4	**79**
4,4	**25**	9,5	**80**
5,5	**37**	9,6	**81**
5,6	**38**	9,7	**83**
5,7	**39**	9,8	**84**
5,8	**40**	9,9	**85**
5,9	**41**	10,0	**86**
6,0	**42**	10,1	**87**

Blood and plasma glucose

Glycemia can be determined in venous or capillary whole blood or venous and capillary plasma. Plasma blood glucose levels are 10 – 15% higher than in whole blood, as erythrocytes contain less water in the same volume compared to plasma. This difference increases when blood glucose rises rapidly because the plasma and cell concentrations do not manage to equalize. Plasma provides more accurate results in this case, so measuring venous plasma glucose is recommended for the diagnosis of diabetes. The difference between capillary and venous blood could be insignificant at normal glucose concentration, however at hyperglycemic level capillary glucose may be significantly higher than venous plasma glucose. These facts should be considered when interpreting fasting glucose and OGTT results.

The fasting plasma glucose (FPG) reference values in both adults and children are 3.3 – 5.5 mmol/L. In healthy individuals, blood glucose varies slightly with age. FPG increases slightly between the 3rd and 6th decade, after the 60's the increase does not continue, but glucose tolerance deteriorates. Fasting blood glucose is always examined in the morning after an overnight fast (minimum 8 hours), as physiologically lower FPG levels in the afternoon (due to lower cortisol levels) may lead to underdiagnosing of diabetes.

If blood glucose cannot be measured immediately after collection, it is necessary to avoid a decrease in glucose concentration in the tube, which results from glucose utilization, especially by surviving erythrocytes. The rate of glycemic decline depends on glucose concentration, blood cell count and activity, and storage temperature; on average, glycemia decreases by 0.5 mmol (10 mg)/hour. In practice, we prevent the decrease of glycemia *in vitro* by using special sampling tubes containing a glycolysis inhibitor (NaF) and an anticoagulant additive (sodium citrate, K_2EDTA), which stabilize the glucose concentration for several hours.

Oral glucose tolerance test (OGTT)

The 'gold standard' for the diagnosis of diabetes is OGTT. The test informs about the ability to metabolize the administered dose of glucose. It should be performed if FPG or random blood glucose values are within an interval when DM cannot be diagnosed with certainty. The requirements for patient preparation before the examination and the contraindications for the examination are summarized in Table 6.5. A standard dose of 75 g glucose (or 1.75 g/kg body weight, but maximally 75 g in children) dissolved in 300 mL of water is given orally within 5 minutes. Blood specimens are collected before giving the glucose load and after 2 hours.

TABLE 6.5 CONDITIONS FOR THE PROPER PERFORMANCE OF OGTT TEST

How to prepare patient:	Test is not performed, if patient:
▪ 3 days before test obvious physical activity + diet without carbohydrate reduction ▪ 12 hrs fasting ▪ adequate hydration ▪ no physical activity and smoking during test	▪ has an acute illness (fever, diarrhoea, vomitus), trauma ▪ is recovering from a serious illness ▪ is not fasting ▪ has impaired intestinal resorption ▪ has vomited after ingestion of glucose

Additional diagnostic tests

Insulin and C-peptide

Pancreatic β-cells produce insulin and C-peptide in equimolar amounts. From one molecule of proinsulin, an insulin molecule and a C-peptide molecule are formed by enzymatic cleavage. Due to the rapid degradation of insulin by the liver, C-peptide concentrations are 5-fold higher compared to insulin and persist in the circulation for longer. The measured values are negatively affected by hemolysis, in which proteolytic enzymes are released from erythrocytes. Insulin and C-peptide testing is not a routine part of diabetic diagnosis or monitoring. Helps to distinguish between T1DM and T2DM in unclear cases. Patients with DM1 have low concentrations of insulin and C-peptide, which correlate with a decrease in β-cell function. Persistent, albeit low, insulin secretion slows the rate of development of organ complications, a well-known phenomenon in LADA (adult autoimmune diabetes). Patients with T2DM have normal or elevated concentrations of insulin and C-peptide, to which, however, the tissues are relatively insensitive.

TABLE 6.6 EXAMPLES OF POSSIBLE CLINICAL USE OF INSULIN/C-PEPTIDE TESTING

Purpose	Comments
- Differential diagnosis of hypoglycemia - Investigation of possible factitious hypoglycemia - Monitoring of a patient after the removal of an insulinoma	confirmation of insulinoma after administration of INS: ↑INS + N-↓CPEPT assessing the effectiveness of the surgical procedure

Insulin and C-peptide assays can also be used to decide on treatment strategies, especially the timing of switching to insulin treatment. The lower the insulin concentration before starting treatment, the more appropriate treatment with insulin or insulin secretagogues will be than first-line drugs. Further examples of the clinical use of these parameters are given in Table 6.6. Elevated concentrations of insulin or C-peptide predict the risk of cardiovascular disease, especially in patients with metabolic syndrome. From a practical point of view, however, it is more important to quantify the consequences of insulin resistance, such as glycemia and lipid parameters.

Autoimmune markers

In the majority of patients with T1DM islet β-cell are destroyed and lost by an autoimmune attack. The autoantibodies against glutamic acid decarboxylase (**GAD**), islet cell cytoplasm (**ICA),** insulin autoantibodies (**IAA**) or zinc transporter (**ZnT8**) are frequently present in patient´s blood for months or years before the onset of the disease.

Autoantibody screening is not a mandatory part of the screening or diagnosis of diabetes, mainly because there is no effective prevention or delay of DM in their carriers. It is occasionally difficult to distinguish between T1DM and atypical presentations of T2DM. Positivity of one or more of the antibodies means, that the patient should be presumed to have T1DM, and should be treated with insulin replacement therapy, as these patients respond poorly to diet and oral hypoglycemic drug therapy. The higher the number of antibodies, the higher the risk of T1DM.

Individuals with positive antibodies have also risk of other autoimmune diseases, for example, celiac disease, Graves, Hashimoto´s, Addison disease, hypogonadism, pernicious anemia (Table 6.7).

TABLE 6.7 CLINICAL SIGNIFICANCE OF AUTOANTIBODIES TESTING IN DM

Autoantibodies	Clinical condition
Positive (antiGAD)	Differentiation of adults with T2DM phenotype = diagnosis of LADA. Positive autoantibodies identify progression to insulin dependence
Positive	Differentiation of T1DM and MODY in unclear cases (especially in children)
Positive	Risk of T1DM in patients with other autoimmune disease
Negative	Neonatal DM - genetic cause
Negative	Search for donors suitable for pancreatic transplantation

Gestational diabetes mellitus

Gestational diabetes mellitus (GMD) was defined as any glucose intolerance first diagnosed during pregnancy. Because this definition may include pre-gestational diabetes caught in pregnancy, a more precise definition refers to GDM as glucose intolerance diagnosed after the 20th week of pregnancy, which usually disappears after birth. The average incidence of GDM is increasing mainly due to the increasing incidence of obesity and diseases of civilization in women of childbearing age, as well as the postponement of parenthood to a later age (6 – 10% in the Slovak Republic and the Czech Republic). During physiological pregnancy, significant hormonal changes occur in the body of the woman, which lead to increased insulin resistance in predisposed individuals, especially in the 2nd trimester (INFO 6.4).

INFO 6.4 Hormonal changes in pregnancy

In early phase of the physiological pregnancy (until the 20th GW) increased production of oestrogens, progesterone, insulin and leptin result in: ↑synthesis and storage of glycogen and TAG, ↓gluconeogenesis, and ↑GLU utilisation in peripheral tissues. In this phase, increase in insulin sensitivity is being observed, thus GDM is very rare (except in high risk women with predisposing factors).
In the next phase (since the 16th-20th week) growing placenta produces more hormones with anti-insulin effects, e.g. prolactin, human placental lactogen (HPL), human placental growth hormone (hPGH) which cause increase in insulin resistance. Consequences are increased lipolysis in expanded adipose tissue, fatty acids are oxidized by mother, while amino acids are spared for growth of placenta and fetus.

Hyperglycemia in pregnancy is associated with several known risks for:

- mother: gestational hypertension, preeclampsia, and unplanned childbirth section; T2DM after childbirth;
- foetus: macrosomia - birth weight more than 4 000 g;
- baby after birth: neonatal hypoglycemia, hyperbilirubinemia, respiratory distress syndrome.

Based on an extensive multi-centre international study (HAPO, Hyperglycemia and Adverse Pregnancy Outcome, 2008), a group of gynecology and diabetology experts proposed new, more stringent diagnostic criteria for the diagnosis of GDM that differ from those used for the general

population. The use of these criteria in the standard OGTT test significantly increases the incidence of GDM (from 5 – 6% to 15 – 20%), mainly because a single pathological value is sufficient to confirm GDM (Table 6.8).

TABLE 6.8 DIAGNOSTIC CRITERIA FOR GDM

Society	FPG	1 h post load	2 h post load
ADA	≥5.1	≥10.0	≥8.5
SDS	>5.1	≥10.0	≥7.8

ADA - American Diabetes Association, SDS - Slovak Diabetes Society

In pregnant women without risk factors, GDM screening is performed between 24 and 28 weeks of pregnancy using OGTT. Venous plasma glycemia is examined on an empty stomach, and then 1 and 2 h after glucose administration. Women with confirmed GDM should be re-examined 6 – 12 weeks after child delivery and then monitored lifelong every 3 years, as their risk of developing DM2 is higher compared to the general population (30% versus 10%).

The pandemic of obesity and type 2 diabetes increases the likelihood of undiagnosed impaired glucose tolerance in pregnancy. Risk factors for GDM are a family history of DM, a personal history of GDM, a previous birth of a child weighing more than 4 000 g, stillbirth, recurrent abortions, hypertension or preeclampsia in a previous pregnancy, age over 30 years and a BMI ≥25 kg/m^2. Pregnant women with at least two risk factors should be examined as soon as possible, ideally in the first trimester. Criteria for the non-pregnant population are used to evaluate OGTT performed in the first trimester, and if a woman meets those criteria for DM, it is previously undiagnosed pre-gestational diabetes mellitus.

6.5 Laboratory monitoring of diabetic patients

Monitoring of blood glucose

The incidence of chronic complications of DM can be reduced by strict glycemic control. Monitoring of glycemia is traditionally performed in the form of a **glycemic profile** (4 – 10 times a day) in laboratories or by patients themselves using a glucometer (self-monitoring). The frequency of monitoring is individual; it is higher in diabetics on an intensive insulin regimen. **Self-monitoring** is usually performed before and after each meal, at bedtime, occasionally before exercise, when hypoglycemia is expected, during treatment of hypoglycemia and before critical activities such as driving a motor vehicle.

A newer option is continuous blood glucose monitoring using sensors inserted subcutaneously or implanted under the skin, which can be connected wirelessly to an insulin pump. **Continuous real-time glycemic monitoring** enables to assess patient's response to treatment, identify and prevent asymptomatic hypoglycemia or hyperglycemia, and adjust insulin dosing according to actual glycemia. Despite the undeniable advantages (especially for patients), these non-laboratory methods of monitoring blood glucose also have certain limitations (INFO 6.5).

INFO 6.5 Possibilities of blood glucose examination

Examination of glycemia in the laboratory is performed by the enzyme method on biochemical analyzers (in serum, plasma) or specialized POCT devices - glucometers (in the whole capillary blood). Despite the importance of self-monitoring for the patient, it must not be forgotten that the result may be inaccurate due to insufficient training of the device operator, equipment wear, improper storage of the meter or diagnostic strips and due to insufficient accuracy of the meters at extremely low or high values.

Glucometers used at home or in medical facilities should be compared at regular intervals with laboratory methods (tolerance up to 10%). Continuous measurement of glycemia in the interstitial fluid is less accurate than measurement with a capillary blood glucose meter. The most serious shortcoming of the currently available devices for continuous blood glucose monitoring is the physiological lag of the balance of glucose concentration between the interstitial fluid and blood. It is therefore important to calibrate the device at steady blood glucose several times a day.

Assessing of patient´s compensation - $HbA1_c$

To assess the long-term compensation of a diabetic, **glycated hemoglobin (HbA1c)**, which is an indirect indicator of glycemia over the life of erythrocytes, is being tested. Glucose binds spontaneously and non-enzymatically to the amino groups of proteins forming glycated proteins. The extent of glycation depends on the average glucose concentration as well as the biological half-life of the protein. Damage to structural proteins with slow change is involved in the development of some chronic complications of diabetes. Proteins with a shorter biological half-life (e.g. hemoglobin) are also subject to extensive glycation.

Reactions with glucose, glucose-6-phosphate and similar molecules produce several forms of glycated hemoglobin. The most important form is HbA1c, which in healthy humans makes up less than 5% of total hemoglobin. HbA1c is a kind of 'perspective view' of glycemia over the last three months, with recent blood glucose levels affecting HbA1c much more than older ones. In fact, the concentration of HbA1c is most indicative of glycemia in the past 6 – 8 weeks before collection, as 50% of glycated hemoglobin is formed during the last month of RBC's life.

Target values of HbA1c in the treatment of diabetics

Numerous clinical studies have repeatedly confirmed that strict glycemic control and lower HbA1c levels reduce the incidence of micro- and macrovascular diabetic complications. Each change of HbA1c by 1% (11 mmol/mol) represents a change in mean glycemia of approximately 2 mmol/L. Reduction of HbA1c (ideally to values of close to non-diabetic), however, is associated with a higher risk of hypoglycemic episodes. The recommended target values during treatment must be determined individually for each patient (Table 6.9).

TABLE 6.9 TARGET VALUES OF HBA1C IN DIFFERENT TREATMENT GOALS

	NGSP/DCCT* (%)	IFCC* (mmol/mol)
excellent control	<6.5	<8
satisfying control	6.6 – 7.5	50 – 58
poor control	>7.5	>60

*HbA1c test standardized according to National Glycated Hemoglobin Standardization Program/Diabetes Control and Complications Trial or International Federation of Clinical Chemistry

The effort to prevent possible complications should be balanced with the possible risk of hypoglycemia. An acceptable target HbA1c for an adult diabetic is <7% (53 mmol/mol). Stricter target values for HbA1c (<6.5% and 53 mmol/mol, respectively) can be chosen in younger patients, without a tendency to hypoglycemia, with a shorter duration of disease, with T2DM treated with lifestyle change alone and metformin, without cardiovascular disease. Conversely, higher HbA1c values (<7.5% and 58 mmol/mol, respectively) can be accepted in children and adolescents, but also in patients with recurrent hypoglycemia, elderly patients, subjects with comorbidities and chronic DM complications (<8% or 64 mmol/mol).

When interpreting HbA1c values, it is necessary to know the possible interferences and limitations. All conditions that shorten the survival of RBCs in the body falsely reduce HbA1c levels, e.g. anemia (especially hemolytic), hemoglobinopathies or repeated venesections (treatment of hemochromatosis). Many other hematologic diseases associated with abnormal RBCs turnover, pathological forms of Hb (genetically or chemically modified), recent transfusions, erythropoiesis-stimulating drugs, chronic kidney disease, and pregnancy may also affect the results of HbA1c testing.

HbA1c does not provide information on glycemic variability, including hypoglycemia. In patients with severe insulin deficiency, the combination of HbA1c and glycemic self-monitoring results are used to assess compensation. Despite HbA1c measurement has become routine component of clinical management in patients with DM, there is no consensus on the **frequency of HbA1c testing**, which is ruled by national guidelines. Optimal frequency of testing is twice a year in good controlled diabetics of both types and four-times a year in patients, whose therapy has changed or who are not meeting treatment goals. In addition, HbA1c should be measured always in case of admission to the hospital, if the results of previous testing are not available. More frequent examination is indicated in diabetics after a change in treatment and in those who do not achieve treatment goals.

Urinary glucose test

In the past, testing of **glucose in urine** (glucosuria) was the main method for assessing the degree of control in a diabetic patient at home, but the interpretation of the findings is influenced by several factors. The tubular threshold for glucose is very variable and generally represents a glycemia >10 mmol/L, which is a value that can be considered as adequate compensation in some patients. Glucosuria correlates with glycemia only in patients with normal GFR. As GFR decreases, glycosuria paradoxically improves because less glucose is filtered into the urine. Besides, fluid intake and the degree of urine concentration also affect the results of urine tests. Glucosuria cannot identify normal glycemia nor possible hypoglycemia. At present, the test is replaced by self-monitoring of blood glucose. Semi-quantitative urinary glucose testing may be helpful only in situations where glucometers cannot be used, e.g. in very old or unwell patients. In some patients with T2DM, glycosuria is induced by SGLT2 inhibitors (gliflozins).

Albuminuria

Diabetes mellitus causes progressive changes in renal functions and ultimately results in diabetic nephropathy. This complication may be delayed by aggressive glycemic control. An **early sign of nephropathy** is an increase in urinary albumin excretion (see Chapter 4, Table 4.4). In addition, albuminuria is considered a marker of generalized endothelial dysfunction

that reflects the cardiovascular risk in diabetics. Interfering factors like lack of fluids casing concentrated urine, stress, physical activity, UTI, acute febrile illness, marked hypertension should be taken into account. Annual quantitative testing for albuminuria in morning spot urine after overnight fasting in all type DM is recommended. In case of positive albumin to creatinine ratio (ACR) evaluation of quantitative 24h albumin excretion together with GFR should be performed. GFR reduction below 1.0 mL/s is an independent risk factor for nephropathy progression regardless of albuminuria.

6.6 Acute complications of diabetes

A patient with poorly treated DM may develop serious metabolic complications. They can be divided into acute **hyperglycemic conditions**, including diabetic ketoacidosis (DKA), hyperglycemic hyperosmolar syndrome (HHS) and lactic acidosis (with or without hyperglycemia), and **hypoglycemia**. DKA and HHS represent two extremes in the spectrum of metabolic decompensation in diabetes. They are caused by an absolute or relative lack of insulin, which result in severe disorder of carbohydrate, fat and protein metabolism with varying degrees of osmotic diuresis, dehydration, ketosis and acidosis (Figure 6.3). Both acute complications can occur together in up to 1/3 of patients.

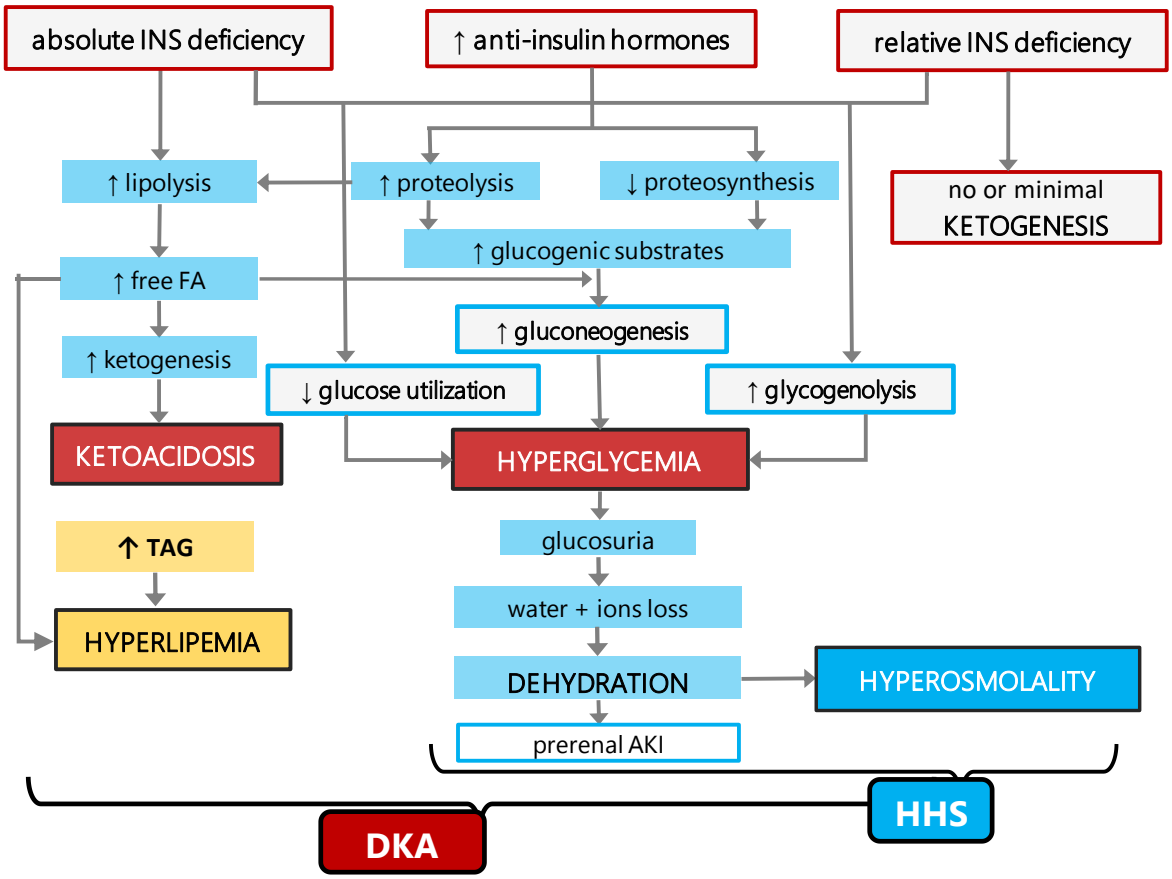


FIGURE 6.3 The simplified diagram of DKA and HHS development

Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) may be an emergency presentation in a patient with unrecognized diabetes. In patient with known diabetes it can occur due to uncontrolled disease, more frequently in T1DM, but also seen in T2DM. Common precipitating factors are inadequate insulin treatment or noncompliance, acute illness (infection, heart attack, cerebral vascular accident), rarely drugs and unusual physical or psychological stress.

The diagnosis of DKA is based on:

- the **history of symptoms** including polydipsia, polyuria, weight loss, weakness, drowsiness
- **clinical features of uncontrolled DM**: dehydration (tachycardia, hypotension, dry mucous membranes) and ketoacidosis (acetone bad breath, hyperventilation, Kussmaul breathing), impaired consciousness (coma in less than 10% of patients);
- **laboratory findings**: hyperglycemia, ketosis (presence of ketone bodies in the blood and urine) and acidosis with increased AG form a typical triad of findings.

The major metabolic abnormalities in DKA result from ketoacidosis and hyperglycemia. **Ketoacidosis** is due to severe insulin deficiency, accompanied by raised concentrations of counter-regulatory hormones, particularly glucagon, exacerbating hyperglycemia. High glucagon and low insulin levels promote lipolysis and free faty acids (FFA) release from adipose tissue. Beta oxidation of FFA yields acetyl-CoA, which is normally oxidized in the Krebs cycle to produce ATP. This pathway is overwhelmed in DKA; thus acetyl-CoA is used for acetoacetate and β-hydroxybutyrate (ketone bodies) production and accumulation, causing an acidosis.

Hyperglycemia causes extracellular hyperosmolality, which in turn results in an intracellular dehydration. Both hyperglycemia and ketoacidosis contribute to an osmotic diuresis, leading to losses of water, sodium potassium, calcium and other inorganic constituents with subsequent loss in circulating blood volume. Vomiting due to stimulation of the chemoreceptors by ketone bodies may exacerbate dehydration and ion loss. **Metabolic acidosis** is contributed by increased production of ketone bodies, but lactic acidosis and pre-renal uremia may be also present as causes of acidosis, which is associated with distributional **hyperkalemia.**

An absolute insulin deficiency, together with an increase in counter-regulatory hormones (especially glucagon), leads to hyperglycemia, which increases extracellular osmolality and consequently causes intracellular dehydration. Hormonal imbalance increases lipolysis and release of FFA from adipose tissue. Their beta-oxidation results in an excess of acetyl-CoA, which during DKA serves as a substrate for ketogenesis in the liver. **Ketoacidosis** is the result of increased formation and accumulation of ketone bodies (acetoacetic acid and β-hydroxybutyric acid), which lead to metabolic acidosis.

Both hyperglycemia and ketoacidosis contribute to osmotic diuresis, which causes water and ion losses (e.g. sodium, potassium, phosphorus) and subsequent dehydration and hypovolemia. Dehydration and ion loss are exacerbated by vomiting, which is a common manifestation of systemic acidosis. Lactic acidosis (in tissue hypoperfusion due to dehydration and vasoconstriction caused by systemic acidosis) and acidosis of renal origin (prerenal uremia) caused by low renal perfusion also exacerbates metabolic acidosis due to increased ketone body production.

Laboratory assessment and management of DKA

An initial biochemical examination performed even in-home settings are dipsticks testing of urine for glucose and ketone bodies and testing of blood glucose by glucose meters. Laboratory-based tests are needed for more precise evaluation the severity of DKA and for monitoring the effect of therapy (Table 6.10). Although it is rarely necessary to wait for laboratory results before starting emergency treatment, the further treatment should be based on precise biochemical analysis in the laboratory.

TABLE 6.10 LABORATORY PARAMETERS TYPICALLY FOUND IN DKA

Analyte	Typical value	Comment
Glucose	↑ (20 – 30 mmol/L)	Value does nor correlate with the severity of metabolic disturbance
pH	↓ under 7.3	In most cases reduced under 7.1, with compensatory reduced pCO_2
AG	↑ above 20	Variable increase, depends on rate and duration of ketones production, degradation and elimination
HCO_3	↓ under 10 mmol/L	Probable deficit 300 – 500 mmol
Na^+	N/↓	Most patient have mild hyponatremia due to urinary loss (~ 500 mmol) and water shift from cells
K^+	↑	Distributional hyperkalemia due to acidosis, probable deficit 250–800 mmol
P	N- during therapy↓	Negative phosphate balance is unmasked after treatment with insulin and volume expansion
Ketones in urine	Positivity increases during recovery of DKA	Sensitivity of dipstick only to acetoacetate
Urea	↑	Prerenal uremia due to dehydration
Creatinine	↑	Ketone bodies interfere with non-enzymatic methods
Leukocytes	↑ (< 25 x 10^9/L)	Result of stress reaction (CORT, adrenalin)
TAG	↑	Insulin deficiency + elevated levels of lipolytic hormones (adrenalin, GH, CORT, and glucagon).

Euglycemic DKA, in which the serum glucose is normal or near normal, has been described, particularly in patients with poor oral intake, treatment with insulin prior to arrival in the emergency department, or in pregnant women. In patients with sodium-glucose co-transporter 2 (SGLT2) inhibitors, the resulting glucosuria can minimize or prevent the development of hyperglycemia, despite very low insulin levels/activity and development of ketoacidosis.

Examination of urinary **ketone bodies** provides underestimated or even negative results in patients before treatment, as diagnostic strips react preferentially with acetoacetic acid (nitroprusid reaction) and do not detect β-hydroxybutyric acid, which is predominant in tissue hypoperfusion and hypoxia. The ratio of beta-hydroxybutyrate to acetoacetate, which is approximately 1:1 in normal subjects, can increase to as high as 10:1 in DKA. This ratio also

increases when lactic acidosis coexists with ketoacidosis. A more accurate marker of ketosis is direct measurement of β-hydroxybutyric acid in serum.

The frequency and timing of laboratory tests depend on the severity of DKA and the treatment method. Calculations of anion gap (see Chapter 3), serum osmolality, and hyperglycemia-corrected sodium concentration may also be useful in treatment decisions and monitoring (see Chapter 2). The aim of DKA treatment is to adjust the circulating volume of fluids, reduce hyperglycemia with its gradual normalization, suppress formation of ketone bodies and treatment of present disorders of the internal environment (INFO 6.6).

INFO 6.6 Basic therapeutic principles in DKA and HHS

- Fluid replacement. The primary goal is to adjust the circulating volume, and prevent hypoperfusion (0.9% NaCl, plasma expanders). After stabilization of blood pressure and diuresis, the goal is to gradually substitute the deficiency of total body water, including intracellular.
- Insulin administration. Serum potassium and blood pressure should be monitored and adjusted before initiating insulin administration. Intravenous insulin administration should be continued at least until serum [HCO_3^-] reaches 18 mmol/L, pH at least 7.3 and ketone bodies have disappeared (AG normalization). Patient receiving per oral food continues with s.c. insulin treatment.
- Potassium substitution is extremely important in the treatment of DKA, as severe hypokalemia can lead to fatal cardiac arrhythmias or respiratory arrest. Immediate substitution begins when the K^+ level is normal or reduced.
- Substitution of bicarbonates is not universally recommended, as it can cause: a) severe hypokalemia - rapid adjustment of MAC leads to the transfer of K^+ into cells; (b) a paradoxical decrease in CSF pH because CO_2 diffuses faster than HCO_3^-; c) sodium overload and shifting the oxyhemoglobin dissociation curve to the left, which impairs the release of oxygen in the tissues.
- Diagnosis and treatment of the underlying cause: e.g. antibiotics, thrombosis prevention.

Hyperosmolar hyperglycemic syndrome

The predominant finding in hyperglycemic hyperosmolar syndrome (HHS) is **marked hyperglycemia** (usually >45 mmol/L) without ketosis (or only with insignificant ketosis due to reduced food intake) and metabolic acidosis. Serum **osmolality** is markedly increased due to hyperglycemia and **severe dehydration**. HHS occurs in elderly patients with T2DM, sometimes as the first manifestation of the disease. Partially conserved insulin production in T2DM inhibits lipolysis and ketogenesis, but is not able to sufficiently increase glucose utilization in peripheral tissues and suppress hepatic gluconeogenesis, which is also supported by increased anti-insulin hormones.

HHS develops more slowly than DKA, although the inducing factors are similar - diuretic therapy, corticoid therapy, and neglected drinking regimens are more common in addition to inadequate insulin treatment or acute illness. The dominating clinical picture is **severe dehydration**, manifested by varying degrees of CNS dysfunction (confusion, signs imitating a vascular event) due to intracellular dehydration and reduced cerebral blood flow, as well as hypotension and tachycardia. The main differences between DKA and HHS are summarized in the Table 6.11.

TABLE 6.11 DIFFERENCES BETWEEN DKA AND HHS

Diabetic ketoacidosis	Hyperosmolar hyperglycemic syndrome
Short onset (1 – 3 days)	Long onset (many days)
Osmolality rarely >320 mOsm/kg	Osmolality frequently >320 mOsm/kg
Significant hyperketonemia and acidosis	None or low ketonemia and acidosis
Hyperglycemia relatively modest (or rarely absent	Hyperglycemia severe (can be above 45 mmol/L)
Occurs mostly in type 1 diabetes	Occurs mostly in type 2 diabetes
Mortality <6%	Mortality up to 30%

6.7 Hypoglycemia

Hypoglycemia is defined from the laboratory point of view as a fall in glycemia below the lower limit of normal for a particular age, biological material and method used. From a clinical point of view, hypoglycemia is a decrease below the lower limit of normal, which guarantees the maintenance of normal brain function. Hypoglycemic manifestations arise as a result activation of counter-regulatory hormones, including activation of the sympathetic nervous system (glucagon, adrenaline, cortisol, etc.) and critical glucose deficiency in brain cells (Table 6.12).

TABLE 6.12 SIGNS OF HYPOGLYCEMIA

Adrenergic	Neuroglycopenic	CNS disorders
tremor, nervousness, tachycardia, increased sweating, paleness, hunger, nausea, vomiting	headache, impaired concentration, behavioural changes irritability, slow reactions, extreme fatigue	severe disorientation, dysarthria, slurred speech, aphasia, convulsions, ataxia, hemiplegia, somnolence to coma
<3.6 mmol/L	<3.2 mmol/L	<2.8 mmol/L

The value of glycemia at which clinical signs appear may vary from person to person; women generally tolerate lower blood glucose levels than men. It also depends on the rate at which blood sugar decreases. Repeated or prolonged starvation develops adaptation and hypoglycemia is asymptomatic. Venous plasma values ≤2.8 mmol/L are considered hypoglycemia for all age groups (including neonates from 4th day of life).

The **Whipple triad** is used in practice to diagnose symptomatic hypoglycemia:

1. the presence of typical clinical signs of hypoglycemia,
2. low glucose concentration confirmed by laboratory method,
3. disappearance of the clinical signs of hypoglycemia after an increase in glycemia (after administration of simple carbohydrates).

In diabetics, clinical manifestations may be present at higher blood glucose levels that are still well tolerated by healthy individuals. The cause of hypoglycemia in diabetics is usually easily

identifiable: overdose of insulin (or oral antidiabetics), insufficient food intake, increased physical activity, or their combination. In the following text, we will deal with hypoglycemia in individuals without DM, which may occur after several hours of fasting (fasting hypoglycemia) or is related to food intake (reactive, postprandial hypoglycemia). Laboratory diagnostics aims to confirm hypoglycemia and identify its cause.

Fasting hypoglycemia

Fasting hypoglycemia is a large group of conditions that result from decreased glucose intake, reduced glucose production (gluconeogenesis) between meals or increased utilization (Table 6.13). One cause of hypoglycemias is **hyperinsulinism** of endogenous origin (insulinoma, congenital hyperinsulinism in children), rarely of exogenous origin, when insulin is administered to an individual without diabetes.

TABLE 6.13 FASTING HYPOGLYCEMIA

Cause	Examples
▪ Increased insulin	
Endogenous overproduction of insulin	Hyperinsulinisms of childhood, insulinoma, pancreatic tumour (MEN 1)
Exogenous insulin	Incidental or intentional overdosing
▪ Insulin normal or low	
Endocrine disorders *(failure of gluconeogenesis)*	Adrenocortical insufficiency and hypothyroidism, GH deficiency
Failure of critical organs (*defective gluconeogenesis + low degradation of insulin*	Liver failure, kidney failure
Extra pancreatic tumours (*increased glucose utilisation + production of IGF-1*	leiomyosarcoma, fibrosarcoma, mesothelioma, hepatoma, carcinoma (stomach, rectum, pancreas)
Low saccharides intake, increased demands of the organism	malnutrition, starvation, sepsis,
Drugs and toxins *(various mechanisms)*	sulphonyl urea, ethanol, salicylates, quinine, haloperidol, disopyramide, beta-blockers etc.
Inherited metabolic disorders of saccharides, fatty acids and amino acids (enzymopathies)	glycogen storage disease, galactosemia, fructose intolerance, carnitine deficiency, leucinosis, tyrosinemia, etc.

Hypoglycemia without an increase in insulin is more common. It occurs in the absence of anti-insulin hormones or for metabolic reasons, such as e.g. defective gluconeogenesis and reduced glycogen production in liver or kidney disease or congenital metabolic disorders. Non-insulin-producing **extra-pancreatic tumours** may also lead to hypoglycemia, mainly due to increased glucose uptake by tumour cells and the production of IGF-2 (insulin-like growth factor 2).

Hypoglycemia is relatively common in **neonates**; shortly after birth, their blood glucose level falls to approximately 1.7 mmol/L in preterm infants and 1.1 mmol/L in preterm infants. Especially newborns of mothers with hyperglycemia in pregnancy are at risk of postpartum hypoglycemia. The foetus responds to increased glucose supply by overproducing insulin. Other causes of prolonged hypoglycemia in neonates include congenital hyperinsulinism and metabolic causes.

Clinical and laboratory examination of an adult patient should rule out the causes of hypoglycemia, including examination of three blood samples after an overnight fast or after a prolonged fasting. In infancy and later childhood, hypoglycemia develops after excessive physical activity associated with reduced carbohydrate intake, e.g. starvation, intercurrent infections, stress or fever. These conditions can unmask an unknown inherited metabolic disease.

Clinical signs of hypoglycemia in the neonatal period are non-specific (hypotension, drowsiness, poor drinking, apathy, changes in crying, irritability, tremor, convulsions, tachypnoea, apnoea), or hypoglycemia is asymptomatic. Therefore, it is important to rely on the laboratory a blood glucose value that is shifted to 2.8 mmol/L for neonates from day 4 of life to prevent permanent brain damage. In infancy and later age, clinical signs are more specific (Whipple triad).

Reactive hypoglycemia

Postprandial hypoglycemia occurs 1.5 – 3 hours after a meal, especially with a high content of simple carbohydrates, or in patients after gastric surgery. The cause of hypoglycemia is the rapid transport of glucose into the duodenum, where the secretion of incretin hormones increases sharply, followed by an intense release of insulin (Table 6.14). In patients with a history of postprandial hypoglycemia, a meal should be used, ideally similar in composition to the meal that usually provokes the symptoms.

TABLE 6.14 CAUSES OF POSTPRANDIAL HYPOGLYCEMIA

Cause	Examples
Accelerated evacuation of stomach followed by insulin secretion	dumping syndrome in conditions after gastrectomy, gastrojejunostomy, pyloroplasty, vagotomy
Idiopathic alimentary hypoglycemia	intake of high carbohydrate food
Carbohydrate metabolism enzyme deficiency	galactosemia, congenital fructose intolerance

Laboratory differential diagnosis of hypoglycemia

Glycemia: testing should be performed in venous plasma in the sample drawn into a tube with NaF and sodium citrate.

β-OH-butyrate: ketone body produced in hypoinsulinemic hypoglycemia as a consequence of increased lipolysis. In patients with active insulinoma, lipolysis is suppressed and concentration of β-OH-butyrate is low.

Insulin and C-peptide serum levels in serum should be suppressed (low to unmeasurable) in case of hypoglycemia. Normal or even high concentrations confirm endogenous hyperinsulinism; besides, they can be present in overdosing with sulfonylurea derivates or in autoimmune hyperinsulinism. Elevated insulin without a proportional increase in C-peptide suggests exogenous origin of insulin. Undetectable insulin and C-peptide are also found in other causes of hypoglycemia.

Cortisol, ACTH, IGF-1, GH, TSH, fT4: Their low concentration suggests deficiency of hormones with anti-insulin activity.
Other tests: antibodies against insulin or its receptors, proinsulin (produced by some pancreatic tumours), toxicological screen for drugs and ethanol and metabolic examination for exclusion of inborn metabolic error.

Case studies and self-assessment questions

Case 6.1

A 78-year-old woman brought from a nursing home after 4 days of refusing food. He has 17 years of T2DM treated with peroral hypoglycemic drugs, diabetic nephropathy (CKD-3) and hypertension. Positive findings on admission: asthenic woman, dehydrated, disoriented, not talking, weaker left side, temperature 37.8° C, sinus tachycardia 124/min, blood pressure 90/58 mm Hg, tachypnoea 32/min, lung X-ray confirmed pneumonia.

Laboratory results are shown in the table.

Serum	Result	RI
Glucose	58	3.3 – 5.5 mmol/L
Urea	46	2.5 – 8.0 mmol/L
Creatinine	241	45 – 88 μmol/L
Na+	122	135 – 145 mmol/L
K^+	4.0	3.6 – 5.3 mmol/L
Cl	97	98 – 108 mmol/L
Albumin	32	34 – 48 g/L
Osmolality	365	275 – 295 mmol/kg
HbA1c	9.9	<7 %
ABR (capillary blood)		
pH	7.48	7.36 – 7.44
pCO_2	2.7	4.8 – 5.8 kPa
pO_2	10.5	10 – 13.5 kPa
HCO_3^-	8	22 – 26 mmol/L
Urine analysis: Glucose 4+, ketones 2+, RBC 50, WBC 300		

Questions:

a. What do you think about control of diabetes?
b. What type of acid-base disorder is present and what is the probable cause of it?
Why are there positive ketone bodies in the urine?
c. What is the probable cause of low [HCO_3^-]?

Case 6.1

A 25-year-old woman was admitted to the hospital for investigation of recurrent hypoglycemia, which occurs late at night, after exercise and inadequate food intake. A fasting test was performed for suspected insulinoma (column 1 in the table), but it had to be discontinued for neuroglycopenic symptoms with IV glucose (column 2)

Serum	1	2	RI
Glucose	1,8	7.2	3.3 – 5.5 mmol/L
Insulin	11	20.3	1.9 – 23 mIU/L
C-peptide	890	1860	260 – 1730 pmol/L
Urine analysis: complete negative			

Questions:

a. Are the laboratory findings (1) consistent with the presumed diagnosis of insulinoma?
b. Why are there not positive ketone bodies in the urine for hypoglycemia?
c. Name other biochemical tests that may shed light on other causes of fasting hypoglycemia

Self-assessing questions

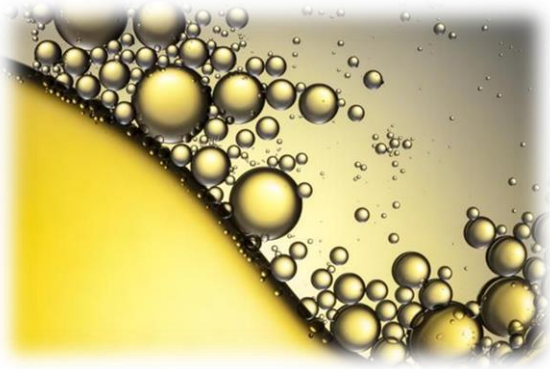
1. What effect does insulin have on gluconeogenesis and glycogenolysis?
2. What are the diagnostic criteria for "prediabetes"?
3. What does positive ketone bodies mean in diabetic urine?
4. What is HbA1c and what is its use in diabetology?
5. What are the typical biochemical findings in diabetic ketoacidosis?
6. Which biochemical parameters are useful in monitoring chronic complications DM?

KEY INFORMATION

- ☑ Blood glucose levels are tightly regulated in a narrow range. Hormonal regulation of glycemia is ensured mainly by the pancreatic hormone's insulin and glucagon. Insulin secretion is also modulated by incretin hormones, which are secreted in the small intestine by oral intake of mixed foods.
- ☑ The entry of glucose into cells depends on several transport proteins. Insulin-activated GLUT4 is found in fat and muscle cells. In contrast, cells in the brain and liver use a different insulin-independent glucose transporter.
- ☑ The liver plays a key role in the regulation of glycemia. Muscles uptake 75 – 80% of the blood glucose from the blood. The kidneys have an important function in the excretion of glucose from the body.
- ☑ Diabetes is the result of a failure of the metabolism of two main sources of energy - carbohydrates and fats. Hyperglycemia is caused by a combination of factors: decreased peripheral cell insulin (T2DM) activity and decreased pancreatic B-cell count and function (T1DM).
- ☑ Patients with both types of DM are at risk of developing chronic vascular complications, the occurrence of which can be delayed or reduced by strict glycemic control.
- ☑ A random blood glucose value of 11.1 mmol/L in patients with typical clinical symptoms or after two hours with OGTT is a criterion for confirming the diagnosis.
- ☑ Diabetic monitoring consists mainly of regular glucose measurement with a glucometer (self-monitoring). Measurement of glycated hemoglobin HbA1c is used to assess diabetes compensation.
- ☑ Increased albuminuria precedes the onset of diabetic nephropathy and should be monitored regularly in both types of DM.
- ☑ Strict control of glycemia and lipid levels can slow the onset of chronic DM complications that may result from accelerated atherosclerosis.

- ☑ Acute complications of diabetes, DKA and HHS, have relatively typical laboratory findings. Their treatment with insulin, fluids and ions is monitored by regular and initially frequent examinations of glycemia, ions (especially potassium) and ketone bodies.
- ☑ Hypoglycemia is a common and undesirable complication, especially with insulin treatment. Other causes of hypoglycemia can be identified by selected laboratory tests (e.g. C-peptide, insulin, β-OH-butyrate, cortisol, IGF-1).
- ☑ Diagnosis, treatment and monitoring of DM and its complications place great demands on health care, including laboratory diagnostics.

7



Lipids and lipoproteins

CONTENT

Lipoproteins (LP) are complex plasma particles transporting hydrofobic lipids between intestine, liver and periferal tissues for energy utilizitilization, lipid deposition, steroid hormone production, and bile acid formation. Lipoproteins consist of lipid and protein components named apolipoproteins. Impaired metabolism of lipoproteins is substantial part of atherogenesis, the process progressing in clinical atherosclerotic cardiovascular disease (ASCVD), such as coronary heart disease (CHD), stroke, and peripheral artery disease.

The chapter describes:

- types and composition of lipoproteins;
- the basic lipoprotein metabolism;
- lipoprotein disorders that can promote the development of atherosclerosis;
- laboratory test used for diagnostics and monitoring od dyslipidemias.

7.1 (Not only) basic physiology

Digestion and absorption of lipids

In a normal diet, 95% of lipids are **triacylglycerols** (TAG), the remaining 5% are all other dietary fats, including **cholesterol** (CH). They enter the bloodstream through lipoprotein particles, which are not only transporters of lipids but also other fat-soluble nutrients (e.g. vitamins A, E, D, K). Gastric lipase partially hydrolyzes TAG and released fatty acids (FA) activate contraction of the gallbladder with the subsequent expulsion of bile and pancreatic juice into the duodenum. Bile emulsifies TAG to smaller droplets which are more accessible to digestion by pancreatic lipase. Glycerol, FAs, and partly also 2-monoacylglycerol enter the enterocyte, together with free cholesterol. Short- and medium-chain FAs can be absorbed from the enterocyte into the bloodstream directly and circulate bound to albumin. The longer fatty acids in the enterocyte are used for TAG re-synthesis and cholesterol esterification.

Dietary lipids, including fat-soluble vitamins, are resorbed in the small intestinal mucosa and incorporated into lipoprotein particles - **chylomicrons** (CM). The major apolipoprotein in CM is **apolipoprotein B-48** (apoB-48), which is synthesized in enterocytes and contains only 48% of the amino acids written in the apoprotein B gene. This truncated apoprotein molecule does not contain a portion that is a ligand for the LDL receptor, so neither CM nor CM remnants bind to apoB receptors.

Lipoprotein structure

The lipoprotein (LP) particles have an approximately spherical shape, differing in size and composition (Figure 7.1). All LPs have central core storing hydrophobic molecules (TAG, cholesterol esters - CE) and surface distribution of hydrophilic components (phospholipids, apolipoproteins, unesterified cholesterol). The structural stability of the lipoprotein particle is ensured by **apolipoprotein** molecules, which, in addition to this function, are also involved in the metabolic transformations of LP particles. The different amounts and composition of lipids and apolipoproteins in lipoproteins contribute their different physical and chemical properties, e.g. molecule size, density, electrophoretic mobility.

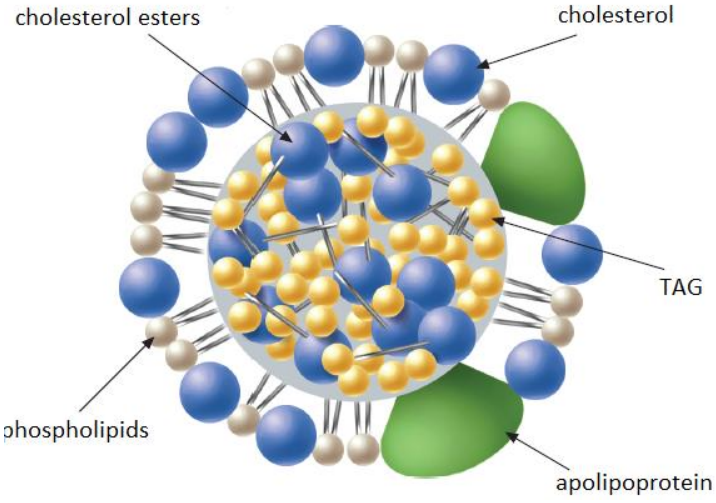


FIGURE 7.1. Shape and strukture of lipoprotein particle

Different analytical separation procedures separate lipoproteins particles according to different physical and chemical properties:

- ultracentrifugation - density of particle;
- gel electrophoresis - mobility in an electric field depending on electric charge;
- gel filtration - the size of their molecule;
- nuclear magnetic resonance spectroscoppy - different amplitude of the proton NMR signal produced by each discrete LP particle.

There are six major LPs in blood; the currently used nomenclature is based on their different density (Table 7.1).

TABLE 7.1 TYPICAL CHARACTERISTICS OF LP PARTICLES

LP	Density kg/L	% of all content					Apolipoproteins
		CE	Free C	TAG	PL	Proteins	
CM	≤0.940	3	1	90	4	2	B-48, C, E
VLDL	0.950 – 1.006	12	6	60	14	8	B-100, C, E
IDL	1.006 – 1.019	26	10	30	20	14	B-100, C, E
LDL	1.019 – 1.063	40	11	5	22	22	B-100
HDL	1.063 – 1.210	18	5	7	25	45	A, C, D, E
Lp(a)		33	9	3	22	33	B-100, (a)

Chylomicrons (CM) are formed in the small intestine and transport exogenous triacylglycerols and cholesterol. After standing plasma or serum for several hours at 4°C, CM settle on the surface in the form of a white creamy layer.

Very low-density lipoproteins (VLDL) are formed in the liver, they transport the majority of endogenously produced TAGs.

Intermediate-density lipoproteins (IDL) are formed from VLDL as transient, short-living LP particles.

Low-density lipoproteins (LDL) are products of VLDL degradation and transport most of the endogenous and exogenous cholesterol from the liver to peripheral tissues.

High-density lipoproteins (HDL) are formed in the liver and small intestine from their precursors - nascent discoid HDL particles, which acquire their definitive spherical form in the bloodstream.

Apolipoproteins

Apolipoproteins (apo) are basic structural units of LP, the composition and content of which are characteristic of individual groups of lipoprotein particles. The biochemical properties, site of synthesis, and functions of the individual apolipoproteins are summarized in the Table 7.2. Understanding the major functions of the particular apolipoproteins is clinically important because their qualitative or quantitative defects lead to abnormal lipid metabolism.

Apolipoproteins can be divided into integrals, which are a permanent part of the lipoprotein particle (e.g. apoB) and freely associated, which LP particles can exchange with each other (apoAI, AII, AIV, CI, CII, CIII, and E). Basic functions of apolipoproteins are:

1. **Structural:** In CMs the structure is stabilized by the molecule apoB-48, which remains in CM remnants until their uptake in the liver. ApoB-100 has a structural function in VLDL particles, which is also present in all lipoprotein particles derived from VLDL (VLDL-remnants, IDL, LDL). In HDL particles, apoprotein A (apo A) performs this function.
2. **Specific receptor ligand:** Part of the amino acid sequence of apoproteins is the ligand for the receptor through which the LP particle enters the cell. ApoB-100 and apoE have this function.
3. **Activation or inhibition of the activity of enzymes** involved in lipoprotein metabolism: ApoA and apoC have this function concerning lecithin-cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL).

TABLE 7.2 MAJOR APOPROTEINS AND THEIR ROLE IN LP METABOLISM

Name	Association with LP	Function
Apo A-I	HDL	Structural protein for HDL; ligand for ABCA1 transporter, activator of lecithin-cholesterol acyltransferase (LCAT).
Apo A-II	HDL	Structural protein for HDL; inhibits cellular cholesterol efflux.
Apo A-IV	CM, HDL	Activator of lipoprotein lipase (LPL) and LCAT
Apo B-48	CM	Contains 48 percent of B-100; required for assembly and secretion of chylomicrons; does not bind to LDL receptor.
Apo B-100	VLDL, LDL	Structural protein for VLDL, IDL, LDL, and Lp(a); ligand for the LDL receptor; required for assembly and secretion of VLDL.
Apo C-I	VLDL, HDL	Activator of LCAT
Apo C-II	CM, VLDL, HDL	Essencial cofactor for LPL
Apo C-III	CM, VLDL, HDL	Inhibition LPL and hepatic lipase
Apo E*	CM, VLDL, HDL	Ligand for hepatic chylomicron and VLDL remnant receptor, ligand for LDL receptor

** There are three different apoE alleles in humans: E-2, the most frequent E-3, and E-4. Compared to apoE3, apoE2 has reduced affinity and apoE4 has enhanced affinity for the LDL (apoB/E) receptor. ApoE-2 phenotype is associated with familiar dysbetalipoproteinemia with an accelerated atherosclerosis. Homozygotes for E-4 have higher risk of Alzheimer´s diseases. ABCA1 - ATP Binding Cassette*

7.2 Metabolism of lipoproteins

Basic principles

The intestine and liver play a central role in LP synthesis. The metabolism of LP is dynamic - during the circulation in the blood there is an **intensive exchange of lipids and apolipoproteins**, in which several enzymes and transport proteins are involved (Figure 7.2), especially:

Lipoprotein lipase (LPL), which is bound to the endothelium of capillaries, especially in skeletal muscle, myocardium and adipose tissue, during lactation and in the mammary gland;

Hepatic lipase (HL) is synthesized in the liver, where 95% remains bound, the remainder being found in the adrenal glands, ovaries, and macrophages. Its main task is the remodeling of CM, IDL, LDL and HDL remnants;

Acyl-CoA-cholesterol acyltransferase (ACAT) catalyzes the esterification of cholesterol;

Lecithin cholesterol acyltransferase (LCAT) is a glycoprotein and has two enzymatic activities: phospholipase A2 and acyltransferase. It is part of the HDL particle, where it catalyzes the esterification of cholesterol and is one of the limiting factors of cholesterol reverse transport;

Cholesterol ester transfer protein (CETP) provides for the exchange of cholesterol and TAG between LP particles;

Microsomal triacylglyceride transfer protein (MTP), which together with apoB participates in the synthesis of primary VLDL particles.

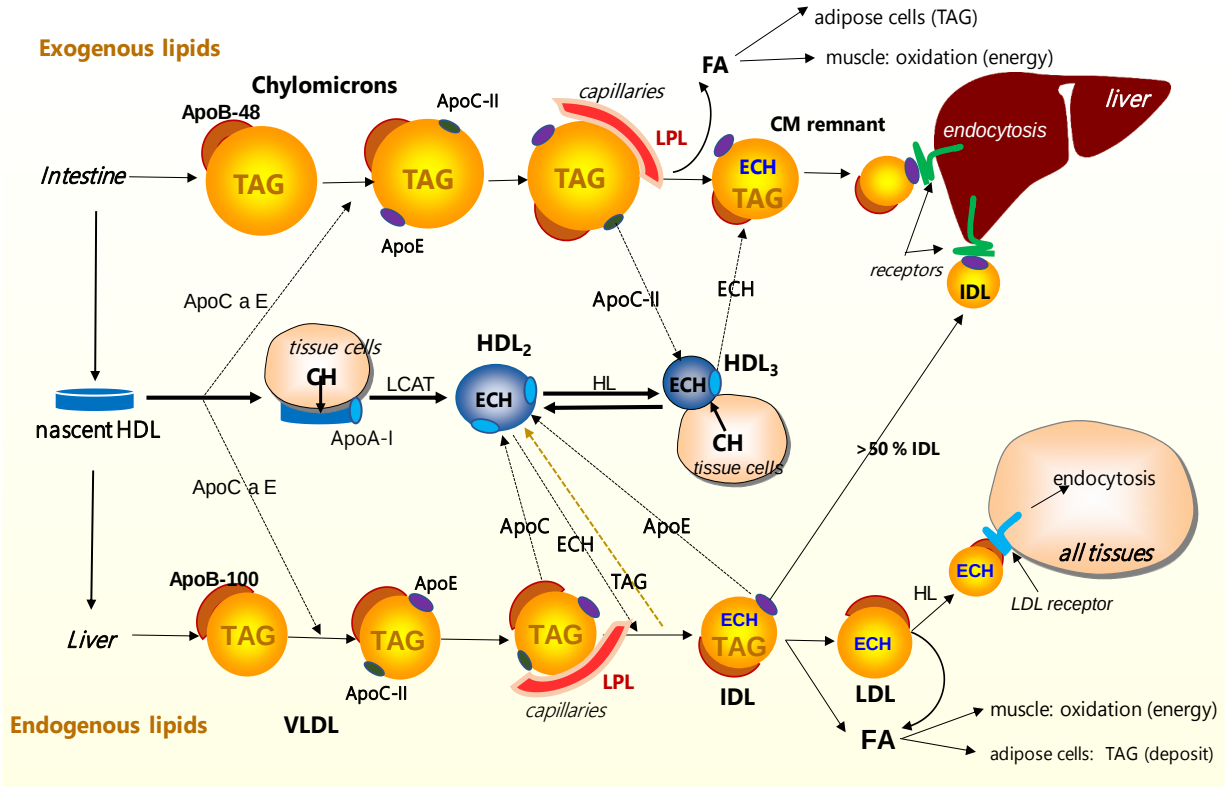


FIGURE 7.2. An overview of LP metabolism

Metabolism of apo-B containing lipoprotein particles

All apoB-containing lipoproteins <70 nm in diameter, including smaller TAG-rich lipoproteins and their remnant particles, can cross the endothelial barrier, especially in the presence of endothelial dysfunction, where they can become trapped after interaction with extracellular structures such as proteoglycans. ApoB-containing LP retained in the arterial wall provoke a complex process that leads to lipid deposition and the initiation of an atheroma.

The first of them - chylomicrons enter the bloodstream through the lymph. The highest concentration of CM in the blood occurs 3–6 hours after eating and after 10 – 12 hours of fasting are no longer present in the blood of a healthy person. In circulation, CMs receive

additional apoE and apoC from HDL particles. ApoC-II is an activator of **lipoprotein lipase** (LPL), which hydrolyzes TAG from the core of CM to form free fatty acids (FFA) and glycerol. FFAs are used by muscle cells mostly as a source of energy, while TAGs are resynthesized in adipocytes.

After hydrolysis of TAG, the CM core shrinks and smaller **chylomicron remnants** (CMR) are formed. When the amount of TAG in the nucleus drops to ∽20%, CM submits apoC-II to the HDL particles, losing the ability to further hydrolyze TAG. Excess envelope phospholipids together with apoA are incorporated into HDL particles. CMRs containing the rest of TAG and all dietary cholesterol are taken up by the liver via apoE binding receptors.

VLDL particles synthesized in the liver transport TAG (and partially cholesterol) from the liver to peripheral tissues. The rate of VLDL synthesis is limited by the rate of hepatic synthesis of apoB-100, which is their essential structural apoprotein. Increased hepatic TAG synthesis from FA or carbohydrate precursors, accelerated in metabolic syndrome, diabetes, obesity, produces **larger VLDL1** particles, while physiological metabolism produces **smaller VLDL2** particles. Simmilarly to CM, VLDL particles receive apoC and apoE from HDL particles upon entry into the circulation. Both apoC-II and insulin activate endothelial LPL, which hydrolyzes TAG in the particle core. If the content of TAG in VLDL decreases to about 30%, a transient particle - **intermediate-density lipoprotein** (IDL) is formed. IDLs bind with the receptor on hepatocytes via apoE and they are subsequently degraded, or they lose additional TAG by activity HL and change to LDL particles. IDL particles have a very short biological half-life under physiological circumstance, but are highly atherogenic.

LDL particles originate from the last stage of VLDL metabolism. They contain only 10% TAG from the original VLDL particles, but all cholesterol and structural apoB-100, the ligand for **LDL receptors** (LDL-R) both on the surface of liver cells and cells of various peripheral tissues. The size of the LDL particles allows them to pass through the endothelium into the interstitial space and can theoretically come into contact with any cell. Approximately 30 – 40% of LDL are catabolized within 24 hours, predominantly by LDL-R (2/3 of the total). The LDL particle bound to LDL-R is internalized into the cell and, after cleavage of LDL-R, is hydrolyzed to cholesterol and amino acids from the protein moiety. The released LDL-R returns to the cell membrane surface. Intracellular cholesterol is a signal for the subsequent regulation of the amount of LDL-R on the cell membrane so that the cell is not overfilled with cholesterol. The entry of cholesterol into cells via LDL-R does not depend on the level of cholesterol in the blood but the number of LDL-R on the cell surface (INFO 7.1).

LDL particles are currently considered to be the most pathogenic population of lipoproteins. LDL particles in the bloodstream are heterogeneous in composition and size. Large, lighter LDL particles containing more TAGs are less atherogenic. They are eliminated from the circulation by LDL-R; the amount entering the cell is strictly regulated. The smaller and heavier LDL particle are more atherogenic. Slowed catabolism, prolonged circulation of particles, and various pathological conditions lead to the modification of LDL particles (e.g. glycation, oxidation). **Small dense LDLs** (sdLDL) are not recognized by LDL-R, due to their size they enter the subendothelial spaces by transcytosis, where they aggregate and easily oxidize. Oxidized LDL particles are internalized into macrophages (as well as other cells) via **scavenger receptors** (SR). A macrophage in the subendothelial space crowded with LDL particles is known as a **foam cell**, which is the first step in the process of atherogenesis.

INFO 7.1 Other important molecules in lipoprotein metabolism

The LDL receptor family includes 7 cellular transmembrane proteins. LDL-R, which interacts with intact apoB-100 LDL particles, apoE3 and apoE4, play a key role in LP metabolism. Similar VLDL-R binds all isoforms of apoE, but not apoB-100, has a major function in TAG metabolism. The LDL-receptor-like protein (LRP) recognizes numerous ligands on various cells and interacts with apoE of CM remnants.

LDL receptors (LDL-R) are found on the membrane of most cells. The amount of receptors on the membrane depends mainly on the cholesterol content in the cell. If there is enough cholesterol, the number of receptors on the membrane decreases, and vice versa. LDL-R expression is regulated by SREBP (sterol regulatory element-binding protein) transcription factors. With a sufficient supply of cholesterol to the cell, the following regulations prevent the accumulation of excess cholesterol:

- hepatic cholesterol synthesis is suppressed by inhibition of HMG-CoA reductase (a key enzyme);
- LDL-R recirculation is eliminated, their number is reduced;
- LDL-R synthesis decreases followed by decreased entry of cholesterol entry into the cell;
- as a result of the activation of ACAT, the storage of EC increases.

Proprotein convertase subtilisin/kexin type 9 (PCSK9): Cells with LDL receptors simultaneously synthesize another protein, PCSK9. Upon binding of PCSK9 to the LDL-LDL-R complex, LDL-R is labeled for degradation in the endolysosome. This reduces the number of LDL-Rs on the membrane and raises the blood level of cholesterol. 1 – 3% of the population has a mutation in the PCSK9 gene. The gain-of-function mutation is associated with the overproduction of PCSK9, which reduces the recirculation of LDL-R and cholesterol in serum increases (Figure 7.5). With the loss-of-function mutation, less PCSK9 is synthesized, there is a lot of LDL-R on the membrane due to recirculation, and cholesterolemia is reduced. Currently available biological treatment for hypercholesterolemia consists of inactivating PCSK9 with a monoclonal antibody.

Scavenger receptors: Any change on the surface of a lipoprotein particle that modifies the structure of the ligand part of the apolipoprotein (glycation, oxidation) prevents the particle from binding to the LDL receptor. Modified particles are removed from the bloodstream via scavenger receptors (SRs), which are located on the surface of phagocytic cells (e.g. macrophages); they bind and internalize only the modified LDL particles. Unlike the LDL receptor, their activity is not regulated by the amount of intracellular cholesterol, so LDL can be overfilled with particles to form foam cells. Type B scavenger receptors are also present in the liver, where they bind HDL particles.

The **formation of sdLDLs** is increased in conditions with an increased supply of fatty acids (e.g., insulin resistance, obesity, increased conversion of carbohydrate precursors). The liver then synthesizes large, TAG-rich VLDL particles that are metabolized to IDL and LDL particles. In the bloodstream, all these LP particles transfer part of their TAGs to HDL particles in exchange for cholesterol via activity of CETP. Due to the action of hepatic lipase, which hydrolyzes excess TAG, these apoB-containing LP particles form extremely atherogenic, small particles rich in cholesterol - sdLDL. Similarly, HDL particle size is reduced after degradation of excess TAG by HL. The catabolism and renal clearance of these small HDL particles is faster, the amount of HDL particles and concentration of HDL-C decreases (Figure 7.3).

Lipoprotein(a) or Lp(a) is a specific form of LDL that is assembled in the liver from apolipoprotein (a) and LDL. Apo(a) links to apolipoprotein B-100 of LDL by disulfide bridges and creates several domains known as kringles (a danish pretzel-like pastry). The variable size of apo(a) can reach 300 – 800 kDa depending on the number of kringles. The kringle 4 contains regions that are homologous with the fibrin-binding domains of plasminogen.

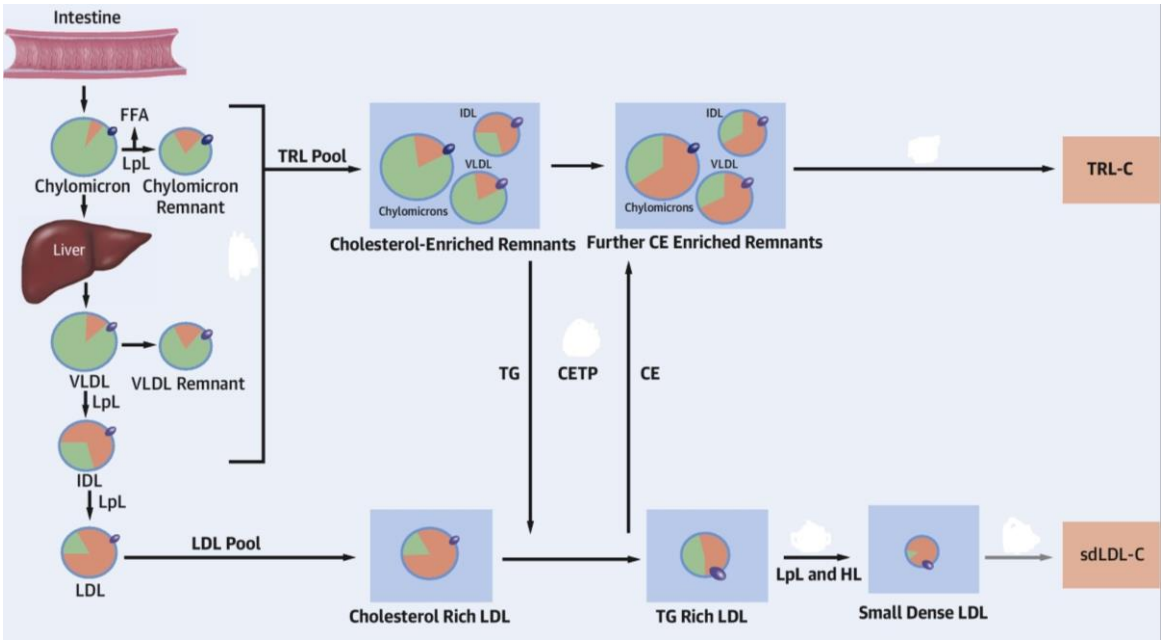


FIGURE 7.3 Formation of sdLDL particles

FFA-free fatty acids, TRL – TAG-rich lipoproteins, CE – cholesterol esters, LpL-lipoprotein lipase, HL – hepatic lipase, TG – triacylglycerols

Through this structural similarity to plasminogen, Lp(a) interferes with fibrinolysis by competing with plasminogen binding to plasminogen receptors on fibrinogen, and fibrin, leading to decreased thrombolysis. It is < 70 nm in diameter and can freely flux across the endothelial barrier, where it can become - similarly toLDL - retained within the arterial wall and thus may increase the risk of ASCVD. The concentration of Lp(a) is genetically determined in an individual and shows a stabile nonfluctuated level during life. Lp(a) has often been considered as an independent nonmodifiable cardiovascular risk factor with **atherogenic** and also **thrombogenic** potential (Figure 7.4).

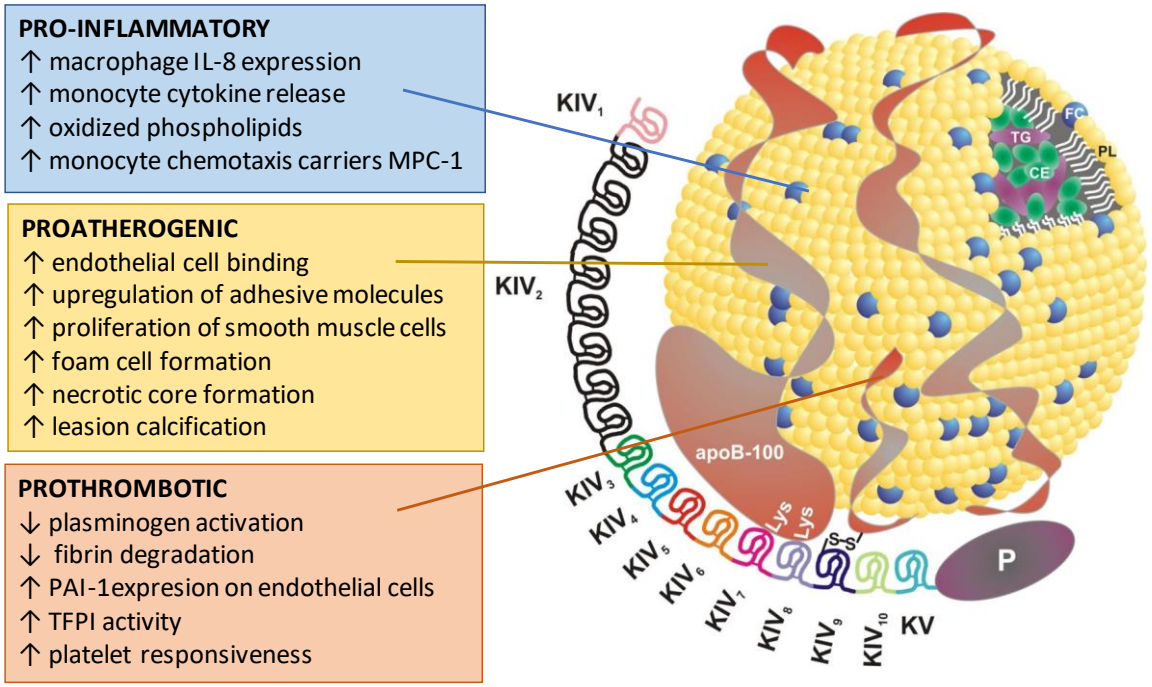


FIGURE 7.4 Atherogenic properties of Lp(a)

HDL particles

The amount of cholesterol (CH) that the intestine and liver supply to the circulation far exceeds the synthetic needs of cells. Therefore, except for conditions with an increased need for cholesterol (intensive growth or repair of damaged tissues), most of it is returned to the liver via a process called reverse transport of cholesterol mediated by HDL particles. These are formed in an immature form (nascent HDL) from phospholipids and apo A-I, especially in the liver and intestine. Nascent HDLs are formed as a phospholipid bilayer with apo A-I content and minimal cholesterol content, in the bloodstream they are enriched for the enzyme LCAT. These small, discoid particles enter the subendothelial spaces of cells with excess cholesterol by transcytosis, remove cholesterol from the cells, and after its esterification with the LCAT enzyme, they move it to the core of LP particle, thus acquiring a spherical shape (HDL2 and HDL3 particles).

Due to the involvement of HDL particles in reverse cholesterol transport, it has long been assumed that the higher the HDL-C level, the lower the cardiovascular risk. There is currently no doubt that low HDL-C is a risk factor for atherogenesis. A significant increase in HDL-C is not always connected with decreased cardiovascular risk. HDL-C level is not an indicator of the biological activity of an HDL particle. A high level of HDL-C may be caused by an accumulation of dysfunctional HDL particles related to the inflammatory state in the body. In this context, HDL particles may acquire proatherogenic properties. Excess cholesterol is transported by HDL particles back to the liver either directly via scavenger receptors (SR-B1) or indirectly via VLDL and LDL particles (Figure 7.5).

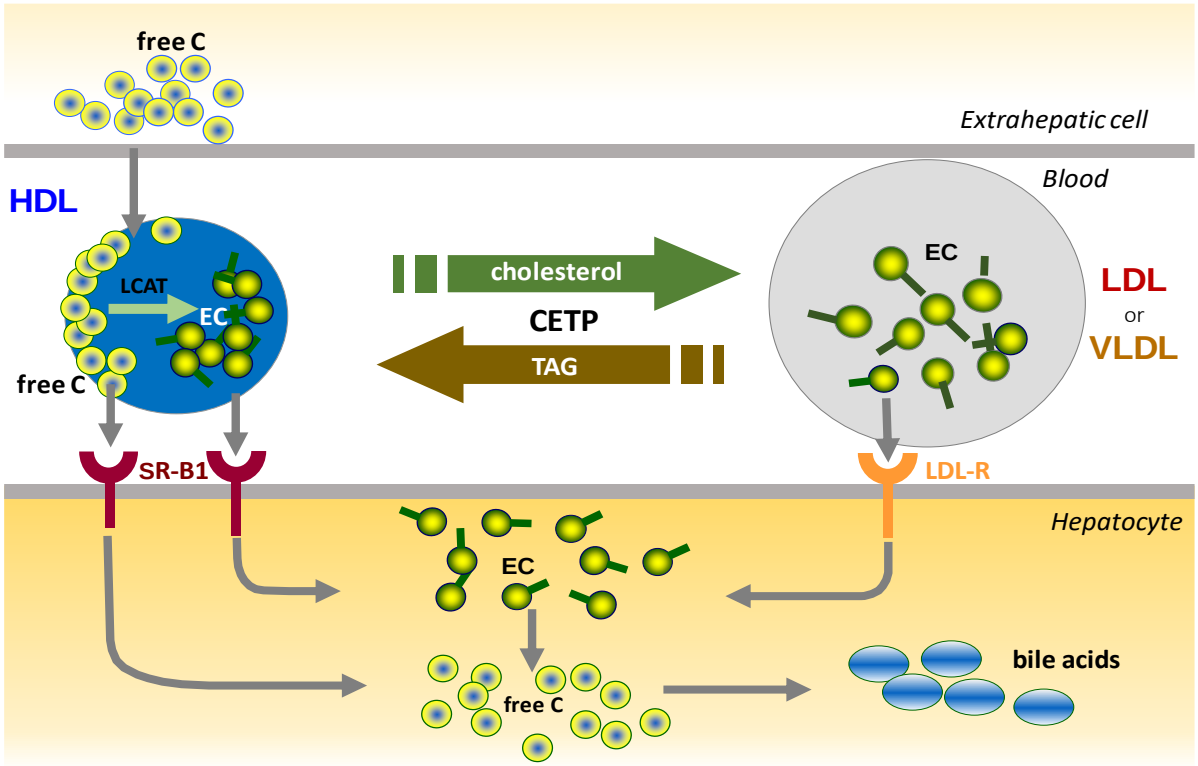


FIGURE 7.5 Reverse cholesterol transport

HDL particles also have additional physiological properties that interfere with the process of atherogenesis and thrombogenesis (INFO 7.2).

INFO 7.2 Other anti-atherogenic effect

The HDL particle, as the smallest LP particle, easily penetrates the extravascular space, interact with endothelium and subendothelial structures, especially macrophages and other LP particles. Several antiatherogenic effects of HDL particles are not related to reverse CH transport and serum HDL-C levels.

- HDL may promote maintenance of endothelial function: Increased expression of adhesion molecules on the endothelium, which are responsible for the migration of monocytes into subendothelial spaces, was demonstrated in individuals with decreased HDL-C.
- HDL protects against oxidation of LDL due to content of enzyme paraoxonase.
- HDL protects against inflammation: In non-inflammatory conditions, they serve as a complement to antioxidant enzymes that maintain the anti-inflammatory state.
- Immunomodulation: In systemic inflammation or oxidative stress (induced by CVD, diabetes, metabolic syndrome, sepsis, autoimmune disease, CKD etc.), oxidized lipids and proteins may accumulate in HDL particles and dysfunctional HDL particles are formed.
- HDL may, via a variety of actions, interfere with the thrombotic component of atherosclerosis.

7.3 Laboratory examination of lipid status

In clinical practice, the concentration of plasma lipoproteins is not usually measured directly but is estimated by measuring their CH content. Total cholesterol (TC) in humans is distributed primarily among three major lipoprotein classes: VLDL, LDL, and HDL. Smaller amounts of CH are also in two minor LP particles - IDL and Lp(a). As a rule, TC and TAG are examined in the screening. A complete examination of LP status also includes the determination of LDL-C, HDL-C, non/HDL-C, apoB-100, apoA-I, Lp(a) and, if possible, sdLDL-C. Supplementary examinations are necessary for determination of the type of primary dyslipidemia (DLP), confirmation or exclusion of secondary DLP, or influence the decision-making regarding hypolipidemic treatment. Examination of LP status is performed under **standard conditions**, i.e. after fasting, although in certain situations postprandial lipid values (INFO 7.3).

INFO 7.3 When to investigate lipoprotein status?

The concentrations of TC, HDL-C and LDL-C measured in the fasted and postprandial manner do not differ statistically significantly, the difference up is only in the level of TAG. The logical argument supporting the determination of parameters of lipoprotein metabolism at any time during the day is that currently, our metabolism is almost permanently postprandial, fasting for more than 10 hours is rare. Also, adherence to fasting and collection time can be difficult for the patient, delaying the examination, or not completing it at all. For these reasons, postprandial lipoprotein metabolism testing is permitted for general risk screening, and for assessment of cardiovascular risk by scoring systems that take into account only total cholesterol levels. For a complete examination of lipoprotein metabolism, the blood for lipids investigation should be still taken under standard conditions: after 10–12 hours of fasting, while maintaining a standard diet and physical activity 2 days before collection. There are several reasons:

- TAG levels increase after meals, in healthy people the increase is significantly lower than in individuals with insulin resistance (T2DM, metabolic syndrome).
- The TAG increase depends on the amount and composition of the diet and on the time of collection.
- Reference ranges of lipoprotein metabolism markers are derived under standard conditions.
- Recommendations from professional societies state values of markers of lipoprotein metabolism determined under standard conditions.
- The examination may also include measuring of fasting glucose and insulinemia (metabolic syndrome).

Total cholesterol

Serum total cholesterol (TC) is the sum of its content in all lipoproteins - both potentially proatherogenic apoB-containing particles, and antiatherogenic HDL. The amount of VLDL particles is low in fasting conditions; chylomicrons and pathological particles should not be present in a healthy individual. In the case of a disorder of LP metabolism, the amount of VLDL or other LPs containing cholesterol - IDL, Lp(a) - may be increased.

Cholesterol (CH) is a constant component of atherosclerotic lesions. Current scoring systems use TC to estimate a patient's general cardiovascular risk. For the general population, the optimal TC level should be currently <5.0 mmol/L. In the primary prevention of high-risk patients, a value <4.5 mmol/L is recommended, in secondary prevention TC < 4.0 mmol/L. Interventional studies have shown that a 1% reduction in blood TC leads to a reduction in the incidence of coronary heart disease of approximately 2%.

LDL cholesterol

Cholesterol content in LDL particles (LDL-C) is better marker of CV risk, although current scoring systems use TC. LDL-C is a target molecule in the **management of a patient at known risk** and in the **monitoring of hypolipidemic treatment** (Table 7.3). LDL-C can be calculated according to several equations or measured by the direct enzyme method. Calculation according to the Friedewald equation is possible: LDL-CH = TC – HDL-C - (TAG/2.2). However, this equation has inherent limitations in subjects with very low or high levels of TAG (<1.1 or >4.5 mmol/L), in patients with abnormal composition of LP particles - particular with type III hyperlipoproteinemia, with renal and liver failure, diabetes and other metabolic abnormalities. The accuracy of the enzyme methods varies according to the diagnostics used. In both, the increased concentration of TAG (lipemia) interferes.

TABLE 7.3 TARGET LDL-C ACCORDING TO THE DEGREE OF CARDIOVASCULAR RISK

Risk category	Target LDL-cholesterol values	
	2016	2019
Very high risk	<1.8 mmol/L a or >50% ↓	<1.4 mmol/L a or >50% ↓
High risk	<2.6 mmol/L or >50% ↓	<1.8 mmol/L a or >50% ↓
Moderate risk	<3.0 mmol/L	<2.6 mmol/L
Low risk	<3.0 mmol/L	<3.0 mmol/L

It should be appreciated that the population of LDL particles is heterogeneous in size and composition. Both factors play a key role in the atherogenicity of LDL. Therefore, a more promising marker is the determination of cholesterol in the most atherogenic population of small dense LDLs. The determination of sdLDL-C is a relatively new test that has not undergone extensive epidemiological studies and is not yet included in the recommendations by professional societies. An indirect indicator of LDL particle size is the simultaneous determination of LDL-C and apoB-100.

HDL cholesterol

High-density lipoprotein is a complex circulating particle with many subspecies that vary in lipid and protein composition. The amount of cholesterol contained in HDL particles can be directly measured; it is referred to as HDL cholesterol (HDL-C). According older epidemiological pupulation studies, HDL-C is a biomarker inversely associated with an increased risk of coronary heart disease events. Newer studies from the last 10 years have shown, that the mentioned association has in fact the U-shaped curve (Figure 7.6). The optimal protecting HDL-C values are close to 1.5 mmol/L, then lower and also higher levels of HDL-C are associated with an exponentionally increasing risk. There is no clear biological explanation of the association of high HDL-C and CV risk. One of hypothesis suggests functional changes of large HDL particles; after beeing enriched with cholesterol esters they fail in reverse cholesterol transport and in other anti-atherosclerotic functions. Currently, HDL-C **<1 mmol/L in men** and **<1.2 mmol/L in women** are considered as insufficient. An increase in low HDL-C of 0.39 mmol/L represents a 22% reduction in CHD risk.

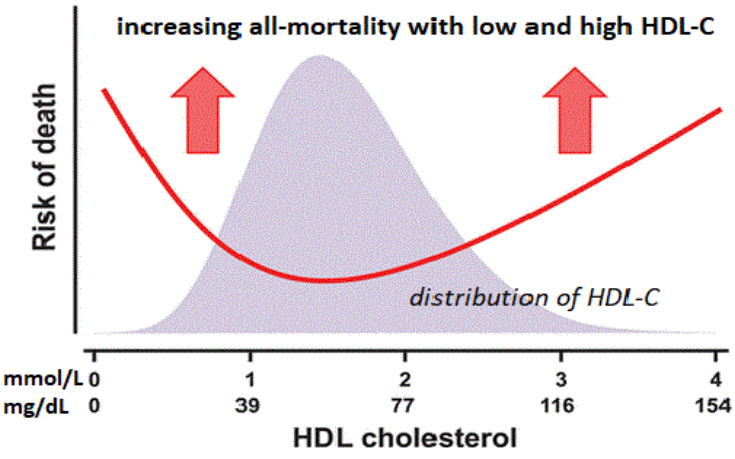


FIGURE 7.6 U-shape curve relationship between HDL-cholesterol and CV mortality

Non-HDL cholesterol

Non-HDL cholesterol (non-HDL-C) is calculated by subtracting HDL-C from TC: non-HDL-C = TC – HDL-C. The non-HDL-C represents the cholesterol content in potentially **proatherogenic apoB-containing LP particles** (VLDL, IDL, LDL, Lp (a)). It is a strong independent risk factor, and its calculation is recommended especially in patients with elevated TAG levels in whom LDL-C cannot be determined or calculated. Target concentrations of non-HDL-C depends on the degree of CV risk, similarly to LDL-C. Non-HDL-C should be used as a secondary therapeutic target in patients with mild-to-moderate hypertriglycerolemia (2 –10 mmol/L).

In a healthy individual, almost all measured TC is derived from LDL and HDL particles. Therefore, the sum of HDL-C and LDL-C is only slightly lower than TC. In the postprandial state and disorders of LP metabolism, an increased amount of CM, CMR, VLDL, and their remnants are present in the serum, all of which contain mainly TAG but also cholesterol (Figure 7.7). Therefore, the sum of HDL-C and LDL-C is significantly lower than the determined TC. The currently proposed calculation of cholesterol in remnant lipoprotein particles (**remnant lipoprotein cholesterol** - RLC) is the following: RLC = TC - (LDL-C + HDL-C). So far, there is

little experience with the interpretation of this parameter and it is not a part of the official guidelines.

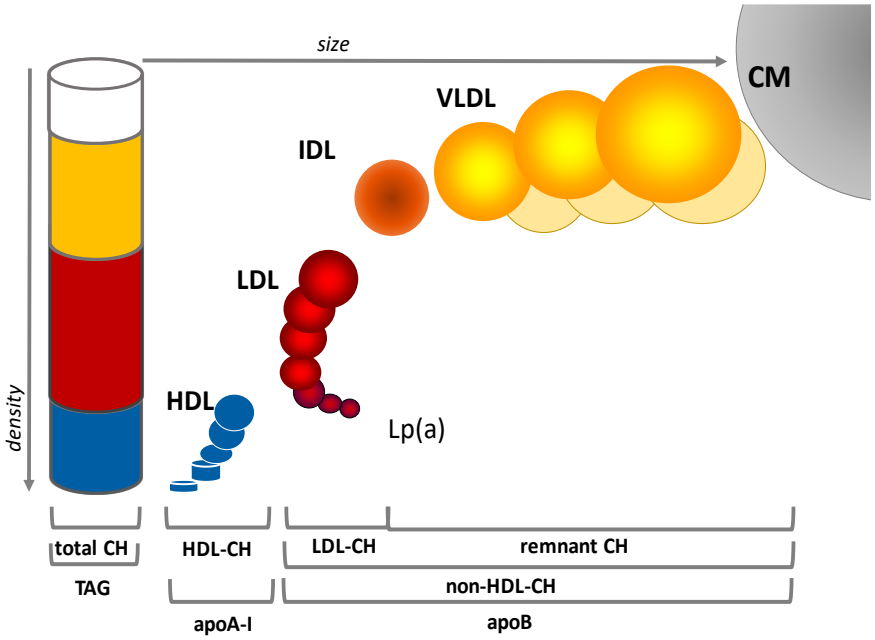


FIGURE 7.7 Distribution of cholesterol in different types of lipoprotein

Triacylglycerols (TAG)

The physiological concentration of TAG in the fasted state (10–12 hours after eating) is bellow 1.7 mmol/L. The majority of TAG exist in form of VLDL, which make up a small proportion of all LP (up to 10%); no chylomicrons are present in the fasting serum. After meals, serum TAG concentration increases due to the presence of CM, and increased hepatic TAG production, entering the bloodstream as VLDL.

TAG levels decrease with starvation and increase with excessive energy intake. Intracellular TAG in adipocytes is cleaved by hormone-sensitive lipase (HSL). Its activity is low in the presence of insulin, which is an important stabilizing hormone preventing a significant postprandial increase in TAG level. Hyperinsulinemia (T2DM, obesity) inhibits HSL in adipose tissue and slows the degradation of intracellular TAGs. On the contrary, insulin deficiency (e.g. the fasting state or T1DM) increases HSL activity.

A transient hypertriacylglycerolemia after a heavy meal or alcohol consumption is usually not proatherogenic. If hypertriacylglycerolemia is a part of the atherogenic lipoprotein phenotype, metabolic changes of LP are long-lasting and promote atherogenesis. The **atherogenic dyslipidemia** is a triad of high TAG, low HDL-C and increased circulating sdLDL. It is common in T2DM and patients with metabolic syndrome. Insulin resistance is likely to play a key role.

Lipoprotein (a)

About 20 % of the world´s population has elevated Lp(a) concentration. Because of this high incidence, and low fluctuation of Lp(a) level in time, testing should be done once in every person's lifetime. Measuring Lp(a) is recommended **in intermediate to high-risk patients** - with premature atherosclerosis, familial hypercholesterolaemia, a positive family history of CVD, recurrent AMI, and stroke, as well as in patient already treated with statins and with reccurent clinical events, and in all patients with more than 5% of the 10-year CVD risk according to the

scoring system. Lp(a) levels above the 80th percentile (>0.5 g/L, or 50 mg/dL) are associated with an increased risk of ischemic and thrombotic complications of atherosclerosis.

Apolipoprotein B

ApoB-100 is the principal apolipoprotein in all potentially atherogenic lipoprotein particles - VLDL, IDL and LDL. Each of these particles contains a single apoB molecule. Therefore, measurements of apoB represent the **total burden of the apoB-lipoprotein particles** involved in the atherosclerotic process. Usually, 85 – 90% of apoB-100 is present in LDL particles. Thus, apoB concentration indirectly reflects LDL particle concentration. Fasting is not required because even in the post-prandial state, apoB-48-containing chylomicrons typically represent <1% of the total concentration of circulating apoB-containing lipoproteins.

Furthermore, apoB may be elevated despite normal or low concentrations of LDL-CH. That disproportional elevation of apo B may be **an indicator of multiplied small dense LDLs** (Figure 7.8). Concentration of apoB <1 g/L is considered desirable in low or intermediate risk individuals. Values <0.8 g/L is desirable in high or very high risk individuals, such as those with cardiovascular disease or diabetes.

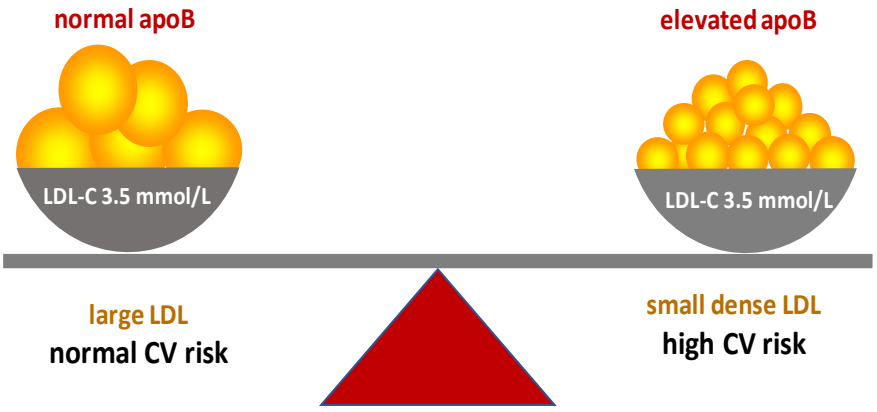


Figure 7.8 ApoB as a marker of the number of LDL particles

Apolipoprotein A-I

Apolipoprotein A-I (apo A-I) is the most important and quantitatively most abundant apolipoprotein of HDL particles. While only one apo B molecule is present in apo B-containing lipoproteins, HDL particles may have 1–5 apo A-I molecules. ApoA-I levels <1.2 g/L in men and <1.4 g/L in women correspond to undesirably low HDL cholesterol levels.

7.3 Markers of inflammation and endothelial dysfunction

Atherosclerosis is a chronic inflammatory fibro-proliferative disease affecting arteries. **Low-degree chronic inflammation** has a fundamental role in all stages of atherosclerosis, from initiation through progression and, ultimately, to the rupture of plaque and ensuing thrombotic complications of atherosclerosis. Increased inflammatory activity in atheromatous plaques leads to unstable plaques threatened by rupture (rich inflammatory infiltration, thinning fibrous

cap). The inflammatory markers encounters several problems in to identification of active, vulnerable plaques:

1. No marker specific for arteritis has been found so far. The generaly used markers increase in inflammation of any localization.
2. An increased inflammatory activity in atherosclerotic lesions increases level of markers very faintly, inflammation in other locations tends to have a more pronounced response.
3. Inflammatory markers generally increase with age, obesity, and smoking.

Therefore, more information has a negative result of the examination (there is no inflammation in the body, including blood vessels) than a positive one (it say nothing about the location of inflammation).

C-reactive protein

Prospective clinical trials have confirmed the relationship between CRP and current cardiovascular risk (presence of vulnerable plaques) as well as its predictive value for the development of cardiovascular disease in the future. The increase in CRP levels is directly proportional to the incidence of cardiovascular disease, both coronary heart disease and stroke, and peripheral arterial atherosclerosis, independently of traditional risk factors (Table 7.4).

TABLE 7.4 EVALUATION OF CV RISK BASD ON HSCRP

hsCRP	Evaluation
<1 mg/L	Low cardiovascular risk
1 – 3 mg/L	Moderate cardiovascular risk
>3 mg/L	High cardiovascular risk
>10 mg/L	Clinical inflammatory disease

Since the increase in CRP concentration with increased activity of the atherosclerotic process is slight, the used analytical method must detect low concentrations. These methods are referred to as **high sensitive CRP** (hsCRP).Based on the available evidence, hs-CRP should be measured in metabolically stable patients without known inflammatory conditions. CRP measurement is optional, it may be useful in **risk refinement** of patients without cardiovascular disease who are at intermediate risk (10 – 20%) 10-year risk for coronary heart disease) and in considering whether to intensify therapies such as lifestyle interventions, lipid-lowering drugs, antiplatelet agents, and other cardioprotective agents. In secondary prevention, the usefulness of measuring hs-CRP is limited, because the current treatment guidelines already provide for an aggressive treatment of these patients.

Albuminuria

The endothelium is one body organ, therefore generalized activation of endothelium also increases its permeability in the kidneys. Increased urinary albumin excretion is a marker of endothelial dysfunction in glomerular capillaries. This may be limited to the kidney (kidney disease) or can be generalized - for example, in sepsis, or activated inflammatory process in atherosclerotic vessels. The distinction between those two causes of endothelial dysfunction is not always clear. Increased albuminuria in diabetics may be on a local basis (nephrosclerosis) but also due to generalized activation of atherosclerosis-affected endothelium. From the practical point of view, low albuminuria is a piece of valuable INFOrmation about the patient's risk - it indicates the absence of any endothelial dysfunction. Physiological albuminuria is less than 3 mg/mmol creatinine in random morning urine or 30 mg/24 hours.

Homocysteine

Homocysteine (Hcy) is an amino acid that arises from methionine and is metabolized to cysteine. Vitamins B6, B12, and folic acid are necessary as cofactors of enzymes in that metabolism. The blood level of homocysteine depends on genetic factors and nutrition. It increases with folic acid deficiency, however, positive effects of vitamin supplementation on the development or recurrence of cardiovascular diseases has not been proven so far. Hcy concentrations <15 µmol/L are considered physiological, the interval 15 – 30 µmol/L is slightly increased, concentrations above 100 µmol/L are high. Despite many studies and meta-analyzes, the effect of Hcy on cardiovascular disease is still unclear. Proponents of its atherogenicity consider higher concentration of homocysteine to be an activator of endothelial dysfunction, which triggers atherogenesis. The incidence of elevated Hcy levels in the general population is about 5%, in patients with overt manifestations of atherosclerosis 14 – 47%. Nevertheless, homocysteine level may be measured in individuals with a personal or family history of cardiovascular disease in the absence of conventional well-established risk factors.

Additional tests for secondary dyslipidaemias

Laboratory tests useful in differential diagnosis of secondary DLP are listed in the Table 7.5. Examination of fasting and postprandial **glucose, HbA1c** and insulin will help identify patients with impaired glucose metabolism (prediabetes, diabetes mellitus, especially T2DM, metabolic syndrome). This group has a higher risk of developing complications of atherosclerosis as well as patients with previous myocardial infarction or another atherosclerotic arterial disease. Hyperglycemic conditions lead to glycation of proteins, including apolipoproteins. Glycated apoprotein lipoprotein particles are not internalized into cells by regulated transport through the LDL-R, but overwhelm the cells unregulated through scavenger receptors.

TABLE 7.5 ADDITIONAL TESTS IN SUSPICION OF SECONDARY DYSLIPOPROTEINEMIA

Parameter	Diagnostic significance
Glucose, oGTT	Diabetes mellitus, metabolic syndrome
HbA1c	Control of DM
Insulin	Insulin resistance, metabolic syndrome
Uric acid	Metabolic syndrome, other forms of hyperuricemia
LFTs	Steatosis and other disorders of liver function affecting lipoprotein metabolism and its treatment
hsCRP	Inflammatory marker
Albuminuria	Marker of endothelial dysfunction
Homocystein	Increased level associated with higher cardiovascular risk
TSH	Influence of thyroid hormones on cholesterol metabolism
Fibrinogen	Marker of hemostasis, inflammatory marker

Insulin is a regulator of both saccharide and fat metabolism. It increases LPL activity, decreases HSL activity and prevents excessive postprandial VLDL production). **Uric acid** is elevated in most of the primary dyslipopoteinemias and in metabolic syndrome. **Thyroid hormones**

potentiate synthesis of LDL receptors on hepatocytes, reduce the transfer of cholesterol from HDL to other lipoprotein particles, and increase biliary excretion of cholesterol in the bile. Hypercholesterolemia may occur in hypothyroidism. Lipoprotein particles are built, metabolized and even taken up in the liver. In case of an unhealthy diet, alcoholism or liver disease, fat can accumulate in the liver and affect liver function. Therefore, **liver function tests** (bilirubin, AST, ALT, GGT, ALP) are involved in first choice tests in patients with dyslipidemia.

Assessment cardiovascular risk

Cardiovascular disease (CVD), of which ASCVD is the major component, is responsible for >4 million deaths in Europe each year. More patients are surviving their first CVD event and are at high-risk of recurrences. In addition, the prevalence of some risk factors, notably diabetes and obesity, is increasing. **Cardiovascular risk** means the likelihood of a person developing an atherosclerotic CV event over a defined period of time. **Total CV risk** expresses the combined effect of a number of risk factors on this risk estimate. High cholesterol level is only one of many risk factors affecting the progression of the atherosclerotic process and the emergence of its non-fatal and fatal complications.

The primary prevention of cardiovascular events depends on the riskiness of the individual patient - the higher the risk, the more intensive actions he or she needs. For apparently health people in primary care, the recent ESC/EAS guidelines (2019) recommend using **scoring systems** to estimate the risk of CVD. For example, the SCORE system indicates a 10-year cummulative risk of a fatal cardiovascular event based on the presence of risk factors including age, gender, smoking, blood pressure and total cholesterol level. Risk estimates have been produced as charts for high- and low-risk regions in Europe based on the WHO data (Figure 7.9). Countries with a CVD mortality rate of ≥150/100 000 are considered to be at high-risk. Risk factor screening including the lipid profile should be considered in men >40 years old, and in women >50 years of age or post-menopausal.

A calculated SCORE ≥10% for 10-year risk of fatal CVD represents a **very high risk**; SCORE ≥5% and <10% means **high risk**, SCORE ≥1% and <5% **moderate risk** and SCORE <1% **low risk.** Risk estimation models are useless in following groups of patients with very high risk of CVD, who need active management of all risk factors:

- Patients with documented ASCVD;
- with T2DM and T1DM;
- with chronic kidney disease;
- with numerous and severe individual risk factors.

Other risk **factors can modify estimated CVD risk** obtained by the scoring system: social deprivation, psychosocial stress, mental exhaustion, severe psychiatric illness, obesity, physical inactivity, family history of premature CVD, chronic immune-mediated inflammatory conditions, treatment for HIV infection, atrial fibrillation, left ventricular hypertrophy, CKD, sleep apnoea syndrome, NAFLD. In addition, metabolic factors such as increased ApoB, Lp(a), TAG or C-reactive protein; the presence of albuminuria; the presence of atherosclerotic plaque in the carotid or femoral arteries.

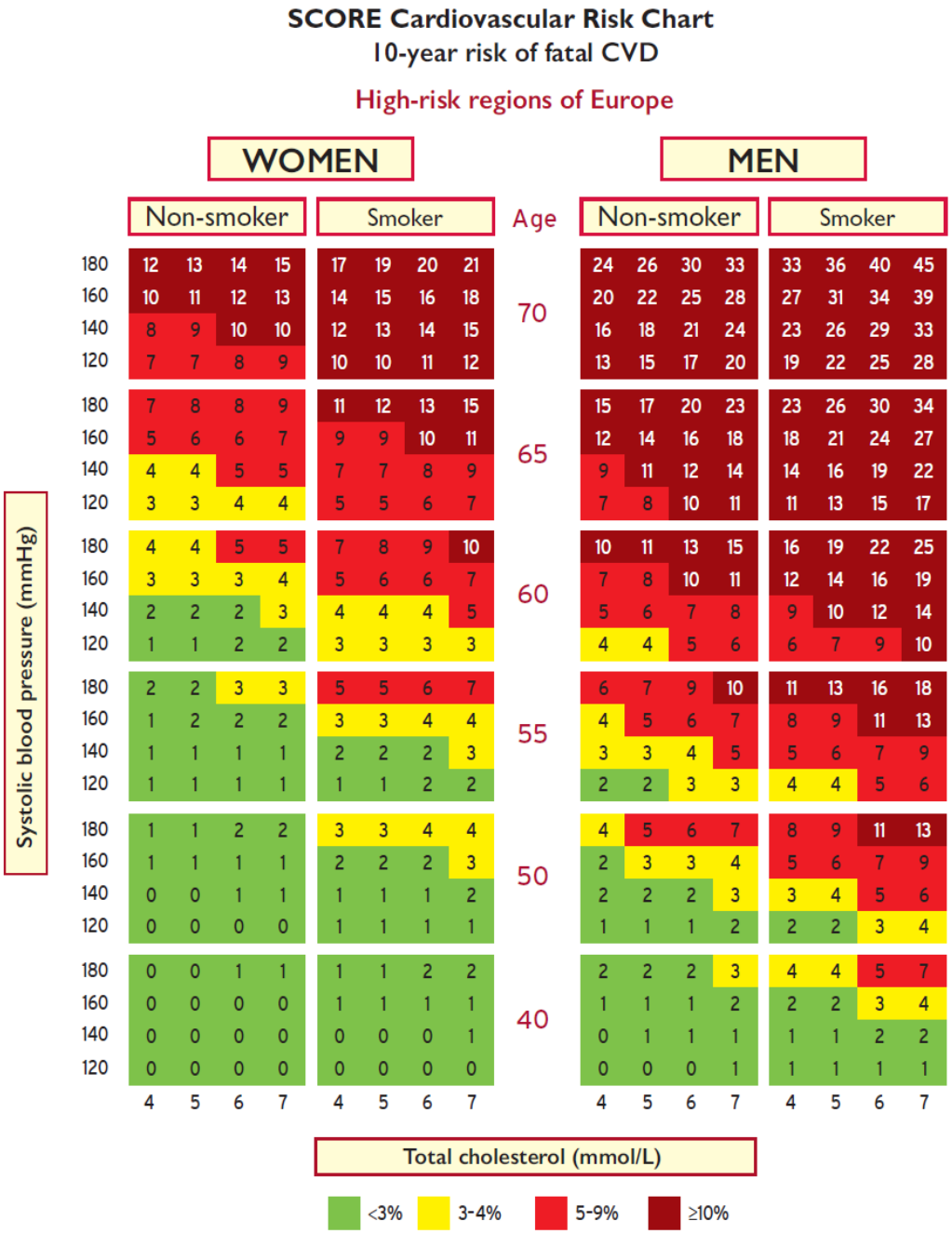


FIGURE 7.9 Example of scoring system SCORE: 10-year risk of fatal CV event for the population with high CV risk (=Slovakia) based on selected risk factors.

7.4 Dyslipoproteinemias

Dyslipoproteinemias (DLP) is a group of metabolic diseases of mass occurrence that are manifested by changes in the **quality or quantity** of serum lipoprotein particles. They have a significant impact on cardiovascular and cerebrovascular mortality, especially in developed countries. The first, generally accepted classification was the **Fredrickson classification** (1967), later adopted by the **WHO** (Table 7.6), which divided DLP according to the lipoprotein

phenotypes found in their electrophoresis, which have now been replaced by new, much more progressive diagnostic methods. The Fredrickson/WHO classification overlooked an altered levels of HDL, elevation of Lp(a), and adverse changes in the composition of LDL in hypertriglyceridemia. Therefore, the mechanism of formation of individual types of DLP will be described according to the current division.

TABLE 7.6 FREDRICKSON/WHO CLASSIFICATION OF HYPERLIPIDEMIA

Type	I	IIa	IIb	III	IV	V
Lipoprotein pattern	↑CM	↑LDL	↑LDL ↑VLDL	↑IDL or remnants	↑VLDL	↑VLDL
Cholesterol	N/↑	↑↑↑	↑↑	↑	↑	↑↑
TAG	↑↑↑	N/↑	↑	↑	↑↑	↑↑↑
Risk of AS	-	increased				
Sings of MS	-	-	central obesity, DM, hypertension, hyperuricemia			
Pancreatitis, abdominal symptoms	+	-	-	-	+	+

Etiological classification

The development of genetics and molecular biology makes it possible to divide DLP into two basic groups:
1. primary - congenital, genetically determined;
2. secondary - arising from other acute and chronic diseases, drugs ot toxo-nutritiona factors.

Most cases of lipoprotein disorders are due to an interaction between genetic and environmental factors. The same disease may not cause DLP in all patients and may lead to different DLP phenotypes in different individuals. Another possibility is that a patient with a primary disorder of lipoprotein metabolism may have a disease that results in secondary DLP, i.e., may have both primary and secondary DLP at the same time. In a patient with primary dyslipidaemia, the pattern of inheritance commonly does not suggest that there is a major single gene - monogenic disorder or more than one gene variant affecting lipoprotein metabolism (polygenic) causing the lipid abnormality.

Descriptive classification

Not every laboratory performs the full spectrum of lipoprotein metabolism tests, but they all measure cholesterol and TAG. Therefore, in 1992 the European Atherosclerosis Society (EAS) introduced a simplified phenotypic classification of DLP, which is based on levels of total cholesterol and triacylglycerols. DLP can be divided into **hypercholesterolemias, hypertriacylglycerolemias,** and **combined hyperlipidemias** (Table 7.7). The classification is simple and practical, and is usually sufficient for the prescription of non-pharmacological or pharmacological treatment. The disadvantage of the approach is that it does not take into account HDL cholesterol, the level of which can significantly affect the interpretation of total cholesterol. With the same cholesterolemia, a patient with high HDL cholesterol will have lower LDL-C, and his risk of CVD is lower than in a patient with low HDL-C.

TABLE 7.7 CLASSIFICATION OF DYSLIPOPROTEINENIAS ACCORDING EAS

1. **Isolated hypercholesterolemia** (+ normal TAG)	
Familiar monogenic hypercholesterolemia (FH)	IIa
▪ LDL receptor gene defect	
▪ apoB-100 gene defect	
▪ preproprotein convertase subtilisin/kexin 9 (PCSK9) gene defect	
Polygenic hypercholesterolemia (TC: 5 – 8 mmol/L)	IIa or IIb
2. **Isolated hypertriacylglycerolemia** (+normal cholesterol)	
Familiar hyperchylomicronemia (TAG: 20 – 200 mmol/L)	Type I
Polygenic familiar hypertriacylglycerolemia (TAG >2 mmol/L)	Type V
3. **Combined hyperlipoproteinemia** (↑ cholesterol and TAG)	
Familiar combined hyperlipoproteinemia (FCH)	Type IV
Familiar dysbetalipoproteinemia (remnant removal disease)	Type III

That clinically oriented approach to DLP set out three stages: recognition of hyperlipidemia, investigation for underlying causes (secondary hyperlipidemias) and classification of primary disorders into:

- Hypercholesterolemia due to elevated LDL levels. This includes dominantly transmitted FH and the commoner entity polygenic hypercholesterolemia. Elevated HDL also fell into this group.
- Endogenous hypertriacylglycerolemia and combined hyperlipidemia (elevated VLDL or elevated VLDL and LDL). This comprises endogenous hypertriacylglycerolemia with normalor borderline cholesterol and the phenotypic variants of FCH.
- Exogeneous hypertriacylglycerolemia, especially cases in which exacerbating factors such as diet resulted in fasting chylomicronemia.

Predominat cholesterol elevation

Familial hypercholesterolemia (FH) is a monogenic dyslipidaemia causing premature CVD due to lifelong elevation of plasma levels of LDL-C. If left untreated, men and women with *heterozygous FH* (HeFH) typically develop early coronary heart disease before the ages of 55 and 60 years, respectively. The risk of CHD among individuals with definite or probable HeFH is estimated to be increased at least 10-fold. However, early diagnosis and appropriate treatment can dramatically reduce the risk for CHD. The prevalence of HeFH in the population is estimated to be 1/200 – 250, translating to a total number of cases between 14 – 34 million worldwide.

Homozygous FH (HoFH) is a rare and life-threatening disease. The clinical picture is characterized by extensive skin and tenton xanthomas, marked premature and progressive ASCVD, and TC >13 mmol/L (>500 mg/dL), mostly in the form of LDL-C. Most patients develop coronary artery diseaae and aortic stenosis before the age of 20 years and die before 30 years of age. The frequency of HoFH is estimated to be 1/160 000–1/320 000.

FH is a monogenic disease caused by 'loss-of-function' mutations in the **LDL-R** (around 95% of FH cases) or **apoB genes**, or a 'gain-of-function' mutation in the **PCSK9 gene** (Figure 7.10). The diagnosis of FH is usually based on clinical presentation. The commonly used criteria from the Dutch Lipid Clinic Network are shown in Table 7.8.

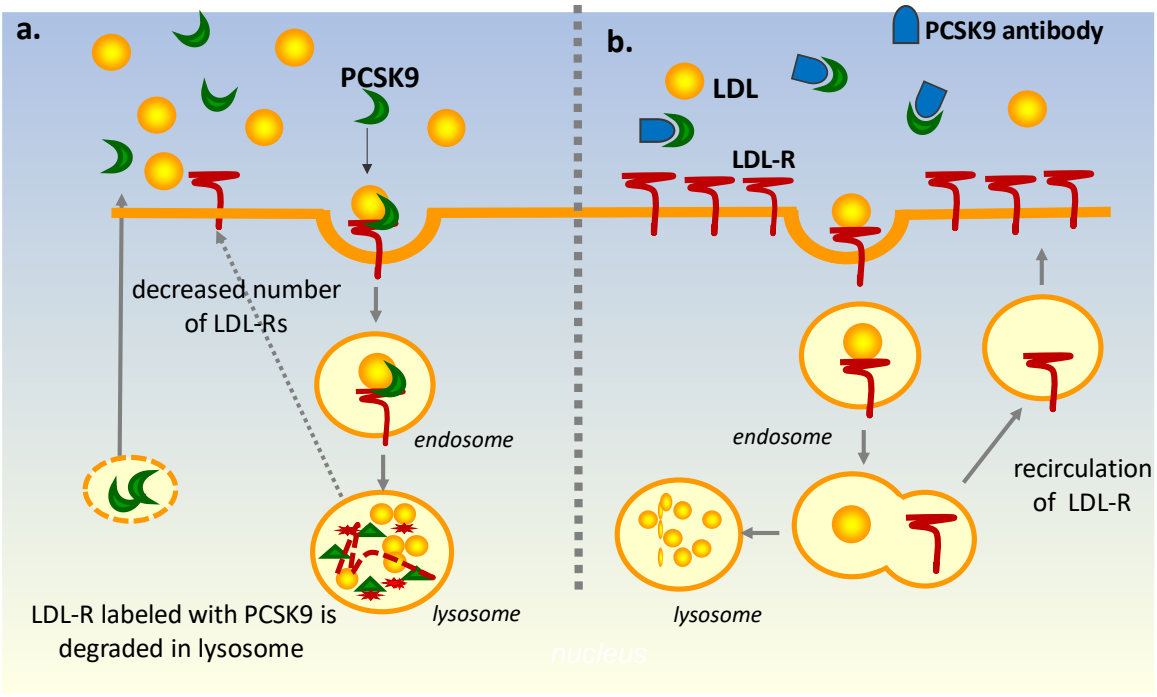


FIGURE 7.10 Role of PCSK9 in pathogenesis of monogenic FH (a.) and in biological therapy of hypercholesterolemia (b.)

TABLE 7.8 DUTCH LIPID CLINIC NETWORK DIAGNOSTIC CRITERIA FOR FH

Criteria	
LDL-C (without treatment)	>4.0 mmol/L in children, >5.0 mmol/L in adults
Family history in first degree relative: known premature (men <55 years; women <60 years) coronary or vascular disease, or LDL-C >the 95th percentile or tendinous xanthomata and/or arcus cornealis	
Clinical history: premature CHD, or cerebral or peripheral vascular disease	
Physical examination:	tendinous xantomata, or arcus cornealis before age 45 years
DNA analysis:	functional mutation in the LDLR, apoB, or PCSK9 genes

FH is the most serious disease from a clinical point of view, homozygous patients often die of CVD before their disease is diagnosed. Therefore, in heterozygous parents, children should be examined. The early identification of these children and prompt referral to a specialized clinic is crucial.

Polygenic hypercholesterolemia is caused by a combination of adverse genetic (eg, the apoE4 gene) and external (diet, obesity, alcoholism, etc.) factors. Isolated hypercholesterolemia (TC and LDL-C) tends to be lower than in FH. Skin or tendon xanthomas are not usually present. The frequency in the population is higher compared to FH, estimated at 1:100 to 1:200. The risk of cardiovascular complications is increased, in men in the fifth decade, in women in the sixth. The risk of peripheral arterial atherosclerosis is 2 – 3 times higher copmapre to the healthy population.

Combined hyperlipidemia

Familial combined hyperlipidaemia (FCH) is the most frequent dyslipidaemia (1:100 – 200) characterized by elevated levels of LDL-C, TAGs, or both, and is an important cause of premature CHD. FCH is a complex disease, and the phenotype is determined by the interaction of multiple genes and the environmental factors. The main unifying feature is an **excessive synthesis of apoB** in the liver with consequent increased production of **VLDL** particles, which are increasingly transformed into markedly proatherogenic **small dense LDL** particles. It has considerable overlap with the dyslipidaemic phenotypes of T2DM and metabolic syndrome. Even within a family, the phenotype shows high inter- and intrapersonal variability based on lipid values (TAG, LDL-C, HDL-C, and apoB). Therefore, the diagnosis is commonly missed in clinical practice; the combination of apoB >120 mg/dL and TAG >1.5 mmol/L with a family history of premature CVD can be used to identify people whomost probably have FCH.

Familiar dysbetalipoproteinemia (i.e. remnant removal disease) is rare and is generally inherited disorder with variable penetrance. Familial dysbetalipoproteinaemia produces a characteristic clinical syndrome in which both TC and TAG are elevated before treatment, usually both in the range of 7 – 10 mmol/L. In severe cases, patients develop tuberoeruptive xantomas, particularly over the elbows and knees, and yellow colouration of the palm and interdigital grooves xanthomas (xanthoma striae palmarum). There are no xanthelasma of the eyelids and arcus cornealis.

The majority of cases are homozygous for the E2 isoform of ApoE. ApoE is important for the hepatic clearance of chylomicron remnants and IDL. ApoE2 binds less readily than the E3 and E4 isoforms to hepatic receptors. The disease is characterized by a slowed metabolism of VLDL particles to LDL and an accumulation of IDL particles. The risk of CHD is very high.

Predominat triacylgycerols elevation

The genetic aetiology for hypertriacylglycerolemia seems to be very complex. Moderate elevation of TAG levels (between 2.0 – 10.0 mmol/L) is caused by the polygenic effect of multiple genes influencing both **VLDL** production and removal. The **polygenic familiar hypertriacylglycerolemia** is complex with a significant influence of external factors, especially diet. Other signs of metabolic syndrome are present (obesity, insulin resistance, glucose tolerance disorders, hyperuricaemia). Lipoprotein disorder accelerates atherosclerosis compared to a healthy population.

Monogenic familial hyperchylomicronemia causes severe HTG due to chylomicronaemia. Thus far, mutations in six genes (LPL, apoC2, apoA5, LMF1, GPIHBP1, and GPD1) with monogenic effects have been recognized to lead to severe HTG due to disruption of the chylomicron removal pathways. Lipemic serum with a very high level of TAG and the formation of a creamy layer of CM on the surface of the serum after 24 hours in the cold are typical findings. Both profound defects in the catabolism of chylomicrons and VLDL results in TAG levels >11.2 mmol/L (>1000 mg/dL), with turbid and milky serum. Cholesterol is normal, or above normal with significantly elevated TAGs. Although CM particle contains only a little cholesterol, with their large amounts, cholesterol can be borderline to slightly elevated). Clinically, HTG is manifested by cutaneous eruptive xanthomas and especially abdominal symptoms - colic to pancreatitis, hepatosplenomegaly. Visual impairment due to hyperchylomicronemia (retinal lipemia) may be the first clinical manifestation of the disease. The **risk of pancreatitis** is clinically significant if TAGs are >10 mmol/L (880 mg/dL), particularly when occurring in association with familial chylomicronaemia. Any factor that increases VLDL

production can aggravate the risk of pancreatitis, with alcohol consumption being the most common contributing factor. This DLP has not yet been shown to affect atherogenesis.

7.5 Secondary dyslipoproteinemias

Secondary DLPs arise from another underlying disease or are caused by drugs or toxic/nutritional factors (Table 7.9). As in primary DLPs, changes in lipoprotein status can be quantitative or qualitative. Regardless of the origin, DLP has the same effect on atherogenesis. Secondary DLP can additionally worsen the course of the primary disease. If we can treat the underlying disease, therapy of DLP is not necessary (e.g. thyroid disease). If we are not able to cure the underlying disease, treatment is often indicated as prevention of exacerbation of the underlying disease (e.g. chronic kidney disease).

TABLE 7.9 SECONDARY DYSLIPOPROTEINEMIAS

Possible causes	
Endocrine disorders	Drugs
Diabetes mellitus Thyreoid disorders Pituitary disorders Hypercorticism Pregnancy	Beta blockers Thiazide diuretics Steroid hormones Immunosuppressive drugs Retinic acid derivates
Liver disease	Nutrition disorders
Hepatocellular diseases Cholestastatic diseases Metabolic hepatopathy	Obesity Mental anorexia Alcohol abuse
Kidney disease	Inflammatory diseases
CKD Nephrotic syndrome	Acute and chronic Autoimmune diseases
Immunoglobulins overproduction	Others
Systemic lupus erythematosus Macroglobulinemias	Malignancies Glycogen storage diseases

Diabetes mellitus

Insulin increases LPL activity, accelerates VLDL catabolism and slightly potentiates the formation of HDL particles. By inhibiting hormone-sensitive lipase, insulin reduces the supply of FA to the liver and thus the production of VLDL. The defective catabolism of TAG rich lipoproteins (CM remnants and VLDL) seems to be a more significant contributor to hypertriacylglycerolemia than increased production of remnant particles. The pathogenesis of DLP significantly differs in T2DM and T1DM.

The lipid profile **in T1DM** patients strongly depends on glycemic control. In patients with well-controlled DM, lipids are 'super normal', characterized by subnormal TAG and LDL-C levels, whereas HDL-C levels are usually normal or slightly elevated. Subcutaneous administration of insulin that stimulates LPL activity in adipose tissue and skeletal muscle, and consequently

increases the turnover rate of VLDL particles may explain the findings. However, an absolute lack of insulin results in a dramatical increase of hepatic VLDL production, supported also by increased lipolysis in adipose tissue. There is a direct relationship between hyperglycemia and hypertriacylglycerolemia. An adequate therapy of T1DM normalizes DLP within days or weeks. T1DM is associated with high CVD risk, in particular in patients with microalbuminuria and renal disease.

The direct relationship between glycemic control and DLP is not so close in **T2DM**. The principal issue is insulin resistance with relative lack of insulin leading to accelerated lipolysis in adipocytes followed by overproduction of large VLDL in the liver. Decreased activity of LPL and increased activity of CEPT also participate in a sequence of events that generate atherogenic VLDL remnants, small dense LDL, and TAG-rich small dense HDL particles. The last particles are rapidly hydrolyzed by HL and LPL and apoA-I is catabolized by the kidney.

So-called **atherogenic triad in T2DM** consists of high TAG level, low HDL-C and normal to slightly increased TC. Increased number of sdLDL particles can be measured indirectly – as apoB concentration or sdLDL-C, if available. Hypertension, dyslipidaemia, abdominal obesity, and NAFLD often coexist with T2DM and further aggravate the CV risk. Besides, hyperglycemia and increased production of free oxygen radicals (glycation or oxidation of apoB) are involved in the formation of modified LDL particles, which are not cleared by LDL-R. DM increases CVD risk about two-fold on average, even more in women than in men.

Metabolic syndrome and abdominal visceral obesity

Metabolic syndrome (MetS) is a complex of clinical and laboratory risk factors for the development of cardiovascular disease. MetS is not a disease, but primarily a response to environmental influences - physical inactivity, overeating, stress, smoking and other factors of civilization. To date, no genes for MetS have been identified, although there are many candidate genes for the individual components of MetS (INFO 7.4).

The original definition of prof. Reaven was based on insulin resistance, while newer definitions emphasize abdominal obesity and visceral fat. Unlike subcutaneous fat, visceral adipose tissue is a metabolically active organ. The metabolic activity of visceral adipose tissue manifests as inflammation, dyslipidemia and accelerated atherosclerosis, insulin resistance and diabetes, liver steatosis, thrombosis (Figure 7.11).

A new definition of MetS proposed by a common consensus of an international and European diabetes society (IDF and EASD, 2005). The necessary criterion for the diagnosis of MetS is the waist circumference, which is defined for different ethnicities, plus the presence of at least two other parameters:

- Fasting glycemia ≥5.6 mmol/L or 2nd h OGTT glycemia 7.8 – 11.1 mmol/L;
- S-TAG >1.7 mmol/L;
- S-HDL-C <1.1 mmol/L in women and <0.9 mmol/L in men;
- Hypertension >130/85 mmHg.

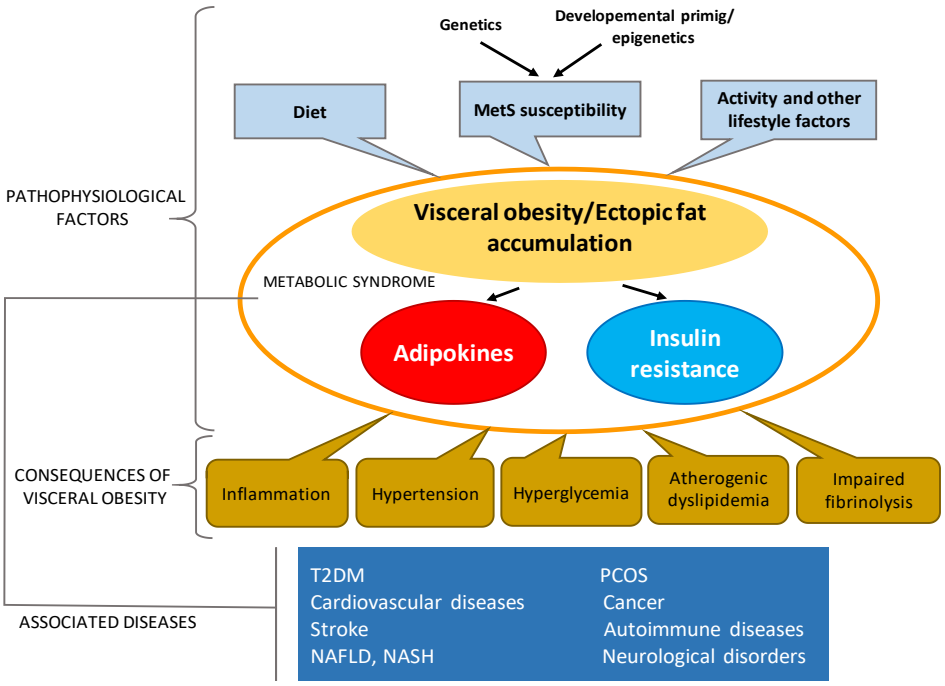


FIGURE 7.11 Pathophysiology and consequences of metabolic syndrome

MetS components have different weights. The presence of T2DM increases cardiovascular risk more significantly than the presence of several other components together. Most components of MetS are atherogenic before they reach a diagnostic target (so-called pre-metabolic syndrome). MetS is now considered an inflammatory disease, systemic inflammation has its origin in the mere inflammatory effects of adipokines of abdominal adipose tissue. The intestinal microbiome and endotoxemia appear to play an important role.

INFO 7.4 Insulin resistance and MetS from a historical point of view

A genetic predisposition to MetS is thought, but so far no putative "thrifty gene" has been identified favouring people with hyperinsulinemia. There are several theories as to why hyperinsulinemia has been beneficial in human evolution.

Insulin is a hormone that takes care of long-term human survival as it generally favours synthetic reactions - glycogenesis, lipogenesis and proteosynthesis. It increases glucose (Glc) uptake in cells, increases glycogen synthesis in the liver and muscles and inhibits its breakdown. It increases glycolysis with the formation of acetyl-CoA, FAs and ultimately TAG, and promotes proteosynthesis in muscle tissue. Glucagon has opposite effects (glycogenolysis, lipolysis, proteolysis), which ensure current survival thanks to sufficient available energy sources - Glc, FA).

Human survival during alternating periods of starvation and an abundance of food depended on the ability to store energy in the form of fat. Consumption of food in times of abundance led to hyperinsulinemia and the formation of fat reserves. If a person wanted to survive, they could not lose muscle mass during the starvation period, which gave them the ability to take care of food. Insulin resistance in muscle leads to a decreased Glc uptake, Glc remains as an energy source for the CNS. This Glc conservation minimizes proteolysis in muscle to obtain glucogenic amino acids as a source for gluconeogenesis.

Kidney disease

Dyslipidemia is frequent finding in patients with **chronic kidney disease**. Inhibition of LPL and HL by uremic toxin, which causes impaired catabolism of VLDL to LDL, is thought as a cuase of

DLP. Because the underlying kidney disease cannot be cured, DLP accelerates atherosclerosis and atherosclerosis exacerbates renal damage. Patients with CKD are considered to be at high (stage 3 CKD) or very-high risk (stage 4-5 CKD or on dialysis) of CVD, and there is no need to use risk estimation models in these patients. They require intensive management of all risk factors for atherosclerosis including DLP. In the initial stages of CKD, TG levels are specifically elevated and HDL-C levels are lowered. LDL subclasses display a shift to an excess of small dense LDL particles. The kidney plays a role in Lp(a) catabolism and that Lp(a) levels are increased in association with kidney disease.

The largest lipid changes in renal disease are found in **nephrotic syndrome** (severe proteinuria with subsequent hypoproteinemia, hypalbuminemia, and hypercholesterolemia). Most theories about the cause of hypercholesterolemia consistently suggest increased hepatic apoB synthesis during enhanced proteosynthesis, which is response to hypoproteinemia (Figure 7.12). The increased supply of ApoB is the cause of the increased production of VLDL, which are metabolized in the bloodstream to LDL particles. Because the metabolism of VLDL is relatively rapid, usually neither VLDL nor IDL particles accumulate in the serum, and TAG level usually does not increase. Severe hypercholesterolemia is also corrected by treating the underlying disease.

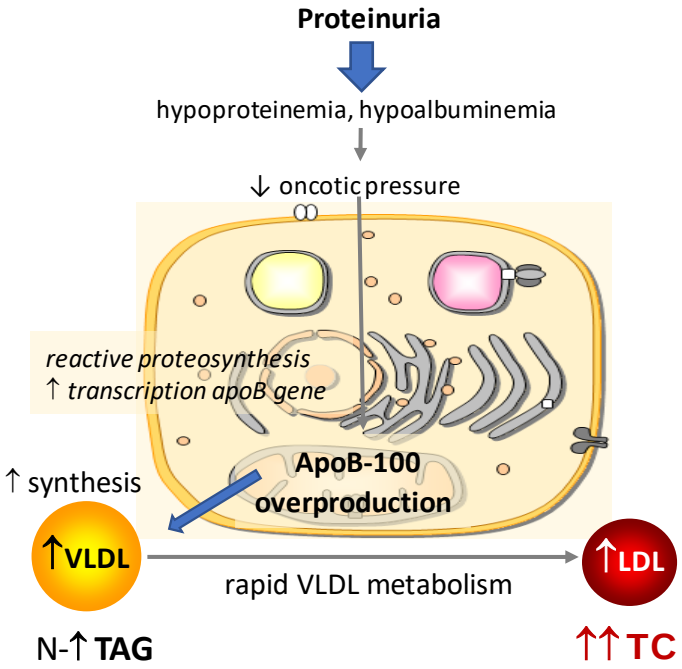


FIGURE 7.12 Dyslipidemia in nephrotic syndrome

Liver disease

The liver is a major organ in the synthesis and degradation of lipoprotein particles. Therefore, during acute liver disease, examination of lipoprotein metabolism is not appropriate, it does not represent metabolism in health. In **hepatocellular diseases**, the activity of HL and LCAT enzymes decreases, and the clearance of apoB-containing LP particles rich in TAG decreases. The production of pathological VLDL, LDL and HDL particles increases, in the blood we most often find increased TAG.

In **cholestatic diseases**, pathological LpX lipoprotein particles, formed predominantly of albumin, phospholipids and free cholesterol, are formed and accumulated. They are an indicator of bile regurgitation in the blood due to impaired liver architectonics. The decrease in clearance of remnant LP particles is also due to their competition with pathological LpX particles at receptors. Elevated cholesterol and TAG are present in the serum.

Disorders of thyroid function

Thyroxine affects lipoprotein metabolism in particular by potentiating LDL-R synthesis in the liver, increasing biliary cholesterol excretion, and inhibiting cholesterol transfer from HDL particles to other apoB lipoprotein particles. All three mechanisms result in hypocholesterolemia in hyperthyroidism and hypercholesterolemia in hypothyroidism. Thyroxine accelerates the catabolism of VLDL, but also accelerates the mobilization of FA from adipocytes and increases the production of VLDL. As this is an adverse effect, the level of TAG can be reduced, increased even in the reference range.

Mental anorexia

Long-term reduction in caloric intake, often in combination with intensive exercise, leads to a disorder of lipoprotein metabolism, which is manifested by hypercholesterolemia. When the glycogen supply is depleted, the body obtains additional glucose by gluconeogenesis. The response to a catabolic state with a lack of energy resources is a decrease in the synthesis of thyroid hormones, which induces energy savings. In LP metabolism, the following changes occur: decrease in the expression of LDL-R on hepatocytes, decrease in biliary transport of cholesterol into the bile, and increase in the transfer of cholesterol from HDL particles to apoB LP particles (CETP). In the terminal stages of anorexia, energy is obtained by proteolysis of muscle to organ protein and proteosynthesis, including enzyme synthesis (CETP), is in deep attenuation, and cholesterol levels fall.

Pregnancy

Hormonal changes in pregnancy from 8 to 12 weeks modify a woman's metabolism. Fetal growth becomes a priority, which requires a sufficient supply of energy. From the second trimester, the mother develops an insulin-resistant condition, and the consumption of glucose received by the fetus decreases. Total, LDL and HDL cholesterol, as well as TAG, rise in the mother's blood. During physiological pregnancy, cholesterol increases by approximately 50%, TAG by 100 – 200%. Metabolism returns to normal within six weeks postpartum. Women with low cholesterol during pregnancy give birth more often to hypotrophic children.

Transplantation

Dyslipidaemias are very common in patients who have undergone heart, lung, liver, kidney, or allogenic haematopoietic stem cell transplantation, and predispose such patients to an increased risk of developing ASCVD and transplant arterial vasculopathy. Immunosuppressive drug regimens may have adverse effects on lipid metabolism leading to increases in TC, VLDL, and TGs, and in the size and density of LDL particles. Several potential drug interactions must also be considered, especially with cyclosporin, which is metabolized through CYP3A4, and may increase systemic statin exposure and the risk of myopathy. Cyclosporin increases the blood levels of all statins. Tacrolimus is also metabolized by CYP3A4, but appears to have less potential for harmful interaction with statins than ciclosporin.

Case studies and self-assessment questions

Case 1:

A 55-year-old man was referred to an outpatient clinic for accelerated dementia and severe hypercholesterolemia. The accompanying son reported a significant decrease in the father's physical and mental abilities in the last 4-6 months. Two years ago, the patient underwent laryngeal malignancy surgery (laryngeal extirpation, permanent tracheostomy) followed by chemotherapy and radiotherapy. Before the operation, an extrovert was dominant, active at home and work. The change in mental and physical condition is gradual, initially interpreted as depressive states during oncological diagnosis and leaving work.

Objectively, in the patient bradypsychia and hypomimia is present, he responds with latency, often in one word. He does not engage spontaneously in the debate. Pale, pasty appearance, cold, dry hands. Cardiopulmonary compensated, bradycardia 56/min, blood pressure 105/70 mm Hg. His laboratory findings are in the table.

Questions:

a. Is dyslipoproteinemia present? If so, what type?
b. Can it be secondary? If so, what is the cause?
c. Based on which findings do you expect a diagnosis?
d. What treatment of the patient would you suggest?

Serum	Result	RI/cut off
Cholesterol	13.2	<5.0 mmol/L
LDL-C	8.8	<3.0 mmol/L
HDL-C	1.0	>1.0 mmol/L
TAG	4.4	<1.7 mmol/L
Glycemia	6.8	<5.59 mmol/L
TSH	395	0.27 – 4.20 mIU/L
fT4	<0.4	12.0 – 22.0 pmol/L
fT3	<0.3	3.1 – 6.8 pmol/L

Case 2:

A 30-year-old woman was referred for an examination of lipoprotein metabolism for xanthelasmas around both eyes. She feels healthy, tolerates exercise well, no risk factors for atherogenesis have been found in her personal or family history. Her total cholesterol was 4.9 mmol/L, TAG 0.70 mmol/L, HDL-C 1.4 mmol/L, postprandial glycemia 5.1 mmol/L.

Questions:

a. Does the patient have dyslipoproteinemia? If so, which one?
b. Are those laboratory findings possible in a patient with xanthelasmas?
c. Which is more significant for DLP - arcus lipoides cornae or xantelasma palpebrarum?
d. What is more significant for DLP - arcus lipoides cornae or tendon xanthomas?

SElf-assessing questions

1. Describe how dietary fats reach target cells.
2. What is the difference in the composition of chylomicron and VLDL particles?
3. What LP particles are present in the blood of a healthy person on an empty stomach?
4. What is reverse cholesterol transport?

5. What does the atherogenicity of LP particles depend on in particular?
6. What is the difference between the LDL receptor and the scavenger receptor?
7. What risk factors affect the value of optimal LDL in a prticular patient?
8. Is it true that the higher the HDL cholesterol, the lower the CV risk?
9. How are dyslipidemias divided?
10. Which secondary DLP do you know?
11. Name the criteria of the metabolic syndrome?

KEY INFORMATION

- ☑ Lipoproteins transport hydrophobic fats in the hydrophilic environment of plasma between organs and tissues.
- ☑ Chylomicrons carry fats (cholesterol, TAG, PL) ingested through food. VLDL particles transport endogenously synthesized TAG and cholesterol.
- ☑ TAGs in CM, VLDL and remnant particles are a source of energy for the body's cells and tissues.
- ☑ LDL particles are formed from VLDL after hydrolysis of TAGs in their core. In particular, they contain cholesterol, which they provide to cells equipped with LDL-R.
- ☑ LDL particles may change in both the bloodstream and subendothelial spaces. Modified LDL particles are not internalized by LDL-R, but by scavenger receptors.
- ☑ HDL particles reverse the transport of excess cholesterol - from peripheral cells to the liver.
- ☑ The size of the remnant, LDL and HDL particles is small enough (70 nm) to penetrate the subendothelial spaces.
- ☑ Atherogenesis involves the activation of the endothelium, the accumulation of lipoprotein particles in the subendothelial space, the entry of monocytes into the subendothelial spaces and their transformation into macrophages expressing scavenger receptors on the surface.
- ☑ By internalizing the lipoprotein particles, foam cells are formed from the macrophages, and by their death, extracellular cholesterol accumulates intimately, forming the nucleus of an atherosclerotic lesion.
- ☑ An important component of atherogenesis is the inflammatory reaction caused by the accumulation of pathological material in the vessel wall. Another factor that plays a significant role in the remodelling of the arterial wall is the activation and proliferation of smooth muscle cells, which includes fibrogenesis.

- ☑ Atheromas narrow the lumen of blood vessels. Rupture of the plaque (vulnerable plaques) with an onset of thrombus, occlusion of the lumen of the vessel and necrosis of the affected tissue leads to clinical manifestation, more often than narrowing alone.
- ☑ Lipid status testing is used to screen for DLP in patients at increased risk for CVD and to diagnose and monitor the treatment of primary and secondary DLP.
- ☑ Total cholesterol, triglycerides, HDL-C, LDL-C, and calculated non-HDL cholesterol (= LDL + remnant cholesterol) constitute the primary lipid panel for hyperlipidemia diagnosis and cardiovascular risk estimation.
- ☑ Additional biochemical parameters are apoB, apoAI, Lp(a), sdLDL-chol, homocysteine and examinations focused on the search for secondary DLP (liver and kidney function tests, glucose, insulin, thyroid hormones, etc.).
- ☑ LDL cholesterol is the primary target of lipid-lowering therapies. Non-HDL cholesterol or apolipoprotein B should be used as secondary therapeutic target in patients with mild-to-moderate hypertriglyceridemia, 2 – 10 mmol/L (175 – 880 mg/dL).

8

Cardiac markers

CONTENT

Cardiovascular disease (CVD) is still the leading cause of death worldwide. The epidemic of obesity and type 2 diabetes - "diabesity", which is potentiated by an unhealthy lifestyle, contributes to it in particular. CVD can be effectively influenced by preventive measures at the population level, but in particular by reducing or eliminating individual risk factors such as hyperlipoproteinemia, hypertension and smoking.

Laboratory tests are an integral part of the management of patients with both acute and chronic cardiac diseases. Cardiac biomarkers with high negative predictive value (-PV) allow selection of patients who do not require hospitalization in outpatient clinics, while biomarkers with high positive predictive value (+PV) are useful for confirming the diagnosis, risk stratification of patients and monitoring the effect of treatment. This chapter is devoted to biochemical examinations used in the diagnosis and monitoring of patients with acute coronary syndrome and heart failure, with emphasis on recent recommendations of professional societies.

8.1 Acute coronary syndrome

Acute coronary syndrome (ACS) is a term for a set of clinical symptoms that result from acute myocardial ischemia, most commonly due to coronary artery atherosclerosis. ACS is essentially a working diagnosis that includes a wide range of clinical conditions, from potentially reversible ischemia (unstable angina pectoris, NAP) to sudden cardiac death due to acute myocytes necrosis (myocardial infarction, MI). The cause of ischemia is most often a sudden closure of the coronary artery or its critical stricture caused by rupture of the atherosclerotic plaque. The subsequent formation of an intraluminal thrombus critically restricts blood flow. Necrosis of cardiac myocytes begins approximately 20 – 30 minutes after onset of ischemia and leads to a gradual release of intracellular proteins that serve as biomarkers of MI. However, complete necrosis of the ischemic area occurs within 2 – 4 hours depending on the presence of collateral blood supply, the nature of coronary artery occlusion (permanent, intermittent), the sensitivity of myocytes to ischemia, and individual oxygen and nutrient requirements.

The new universal definition of myocardial infarction (2018) distinguishes other types of myocardial necrosis in addition to spontaneous MI based on atherosclerotic changes in coronary arteries (INFO 8.1).

INFO 8.1 4. universal definition of MI (joint opinion of experts, 2018)

Type 1: Spontaneous MI - occurs as a result of rupture, ulceration, erosion or dissection of the atherosclerotic plaque in one or more coronary arteries with subsequent formation of an intraluminal thrombus. Most patients have coronary heart disease.

Type 2: MI due to ischemic imbalance (imbalance between need and supply of oxygen to the myocardium) - e.g. endothelial dysfunction, spasm or coronary artery embolization, severe arrhythmias, severe anemia, respiratory failure, hypotension and hypertension with or without left ventricular hypertrophy.

Type 3: Sudden death due to MI - with typical manifestations of myocardial ischemia and the presence of new ischemic changes on the ECG (ST elevation, BLTR) or evidence of intracoronary thrombus during angiography or autopsy. Blood to demonstrate an increase in biomarkers is usually not collected or taken too soon before the markers in the blood rise.

Type 4a: MI associated with percutaneous coronary intervention

Type 4b: MI due to coronary stent thrombosis.

Type 5: MI associated with revascularization surgery (coronary artery bypass graft, etc.)

According to the degree of severity, ACS can be divided into three categories (Table 8.1):

- **unstable angina pectoris** (NAP);
- myocardial **infarction without ST-segment elevation** on ECG (NSTEMI);
- myocardial **infarction with ST-segment elevation** on ECG (STEMI).

The distinction between categories is extremely important because it influences decisions about the type and intensity of treatment. Based on ECG changes, it is possible to identify about 1/3 of patients with ACS with persistent ST-segment elevation (STEMI) who require immediate reperfusion therapy.

TABLE 8.1 DISTINGUISHING FEATURES OF ACUTE CORONARY SYNDROMES

ACS categories	NAP	NSTEMI	STEMI
Pathophysiology	Ischemia without necrosis	Ischemia with necrosis	
	Partial or transient obstruction of coronary a.		Complete obstruction
Clinical features Presenting syndrome	Severe angina (new onset, crescendo, in rest)	Prolonged "crushing" chest pain (≥20 min), more severe and wider radiation than usual angina	
12-lead ECG	No abnormalities, transient ST-elevation, ST-depression or T-wave inversion		Persistent ST-elevation, new LBBB
Cardiac troponins on arrival and after 1-3h	Negative (2X)	Dynamic change, at least 1 positive result	
Therapeutic intervention	Non-invasive	Early-invasive	Immediate reperfusion

Cardiac troponins help to distinguish from the remaining 2/3 of ACS without ST-segment elevation those patients, who have a myocardial infarction (NSTEMI) and also require an early invasive approach to treatment. The use of more sensitive and myocardium-specific biomarkers and newer imaging methods allow to detect myocardial damage or necrosis of a very small extent (so-called minor myocardial damage).

Diagnosis and risk stratification of patients with ACS is based on:

- **clinical manifestations** of myocardial ischemia (typical retrosternal pain accompanied by anxiety, dyspnoea, sweating, nausea, weakness);
- **ECG changes** (elevation or depression of the ST-segment, inversion of the T-wave, new BLT block, formation of a pathological Q-wave);
- **dynamic changes in biomarkers** of myocardial necrosis (cardiac troponins) and
- **imaging** methods (angiographic evidence of significant coronary narrowing or evidence of a new regional disorder of myocardial kinetics).

8.2 Biochemical markers of myocardial necrosis

The consequence of ischemic necrosis of cardiac myocytes is the release of intracellular molecules into the bloodstream, which are measured as biomarkers of myocardial infarction. The best validated and most widespread biomarkers are proteins - **cardiac troponins I and T** (cTnI, cTnT) and creatine kinase MB-isoenzyme (CK-MB). There is an important difference between these markers in key features such as organ specificity and clinical efficacy (Table 8.2). As the diagnosis of acute MI is important in terms of risk stratification of patients and selection of optimal treatment, examination of cardiac markers is indicated in all patients with suspected ACS.

Cardiac troponins are the recommended first-choice biomarkers to confirm myocardial necrosis, while CK-MB mass is an acceptable alternative if cardiac troponins are not available. Prospective studies on the use of troponin in ACS have repeatedly shown that the diagnostic

efficiency of cTn determination is higher compared to CK-MB values. Besides, it predicts better the early and long-term risk of adverse cardiac events. Both markers are explained in more detail below.

TABLE 8.2 CHARACTERISTICS OF CARDIAC NECROSIS MARKERS

MARKER	SP	TEMPORAL PROFILE			CLINICAL UTILITY	
		Time to detection	Time to peak	Duration of elevation	Advantage	Disadvantage
cTnI cTnT	++++ ++++	3 – 6 h 3 – 6h	24 h 24 h	5 – 10 d 5 – 14 d	Superior SN and SP. Current biomarker of choice for detection of myocardial injury, risk stratification and therapy selection. Long "diagnostic window".	Not an early marker; serial testing needed when 1st result is normal. Reduced ability to discriminate re-infarction (serial testing needed).
CK-MB	+++	3 – 4 h	24 h	24 – 36 h	Detection of repeated infarction. Alternative if cTn assays are not available.	Low SP in presence of muscle injury. Not an early marker, necrosis.
Myoglobin	+	1 – 3 h	6 – 7 h	12 – 24 h	High SN and negative PV.	Low SP in presence of skeletal muscle injury and renal failure

Cardiac troponins are the recommended first-choice biomarkers to confirm myocardial necrosis, while CK-MB mass is an acceptable alternative if cardiac troponins are not available. Prospective studies on the use of troponin in ACS have repeatedly shown that the diagnostic efficiency of cTn determination is higher compared to CK-MB values. Besides, it predicts better the early and long-term risk of adverse cardiac events. Both markers are explained in more detail below.

Many traditional enzymes used in the diagnosis of acute MI in the past, such as total creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LD) activity are not currently used in this indication due to their low specificity.

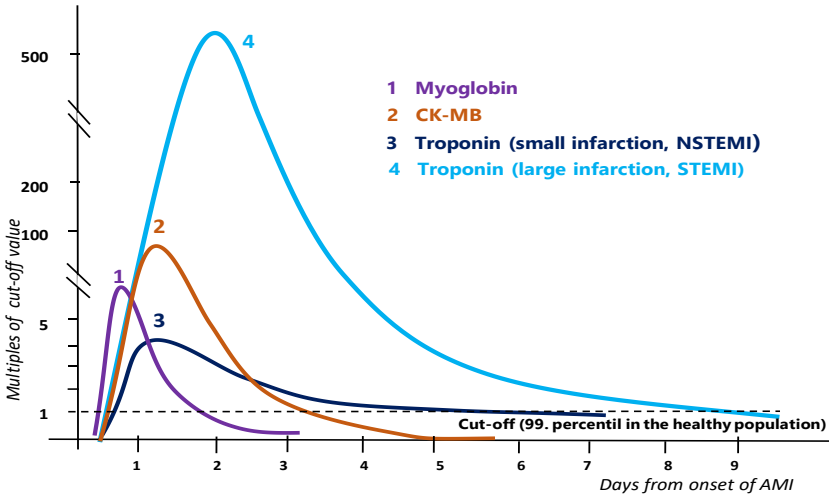


FIGURE 8.1 Temporal profile of cardiac markers after acute MI

Biomarker concentrations are plotted as multiples of the cut-off value for AMI, i.e. any measurement exceeding the 99th percentile of a normal reference population (URL = upper reference limit)

The time course of biomarker values after acute myocardial infarction is very similar (Figure 8.1). Within 2 – 3 hours, all markers in the serum are negative, then rise rapidly to a maximum between 18 and 24 hours and then return to normal at a rate that depends on the biological half-life of each biomarker. This time course of plasma biomarkers may be altered in patients treated with thrombolytic agents. The characteristic biphasic course of cardiac troponins or the rapid rise and fall of CK-MB (maximum after 10 – 18 hours) is due to the release of markers from the necrotic area of the myocardium after successful reperfusion.

Cardiac troponins

Troponins are contractile proteins present exclusively in striated muscle fibres as part of the tropomyosin complex, where they provide calcium-mediated regulation of muscle contraction (Figure 8.2). Troponins of three types (I, T and C) bind structurally to actin fibres. Troponin T and I occur in cardiac myocytes in the form of organ-specific isoforms (cardiac troponin, cTnT or cTn I), which are encoded by different genes than their muscle isoforms.

Quantitative immunochemical methods for the determination of cardiac troponins use specific antibodies invented against cardiac isoforms. Due to their high sensitivity and almost absolute tissue specificity, cardiac troponins are essential pillars of diagnosis and risk stratification of patients with ACS.

The diagnosis of MI requires the presence of typical dynamics of changes in cTn (i.e., an increase or decrease in cTn concentration) depending on the timing of collection, along with further evidence of myocardial ischemia, such as:

- Clinical manifestations of ischemia (pain with typical characteristics);
- ECG abnormality;
- Abnormal findings in imaging methods (echocardiography, isotope imaging methods, angiography, etc.).

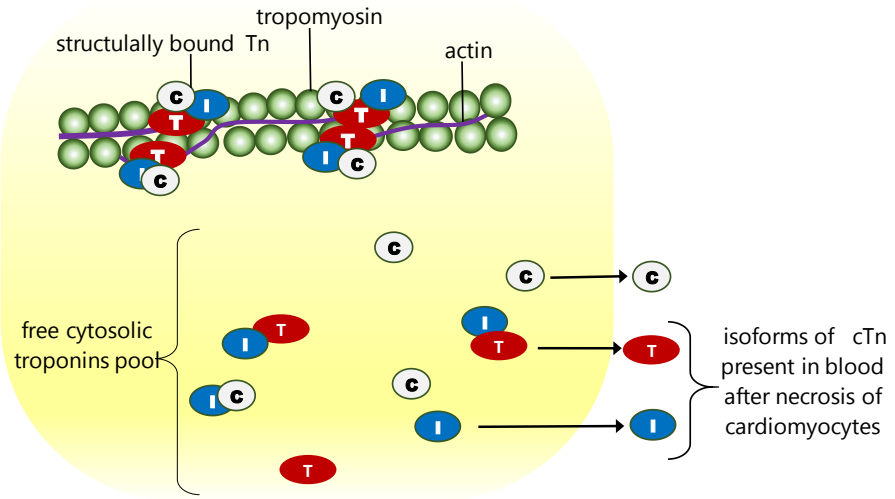


FIGURE 8.2 Troponin is a marker of cardiac damage
In human heart the cTnT and cTnT are largely insoluble, but 3 – 5% exists as a soluble cytoplasmic pool. This soluble fraction probably accounts for the early rapid release of Tn into the circulation following myocardial damage and the slower release of bound fraction accounts for the prolonged plateau of troponin release.

Current highly sensitive methods for the determination of cardiac troponins (hs-cTn) allow the detection of myocardial necrosis 100 times lower, compared to traditional methods. As a result, there has been a 20% increase in the incidence of NSTEMI and a corresponding decrease in the incidence of unstable AP in the last two decades. Detection of minimal myocardial damage often makes it difficult to interpret the patient's clinical condition. Elevated cTn levels indicate heart damage but do not indicate the cause of the damage. Interpretation of cardiac biomarkers in the context of the clinical situation is a necessary condition for the correct diagnosis of ACS. Table 8.3 summarizes the possible and known causes of the increase in cTn.

The contribution of cTn in the diagnosis of acute MI depends on the use of appropriate decision-limits (cut-off values) and on the timing of blood collection. At least two blood samples are required to confirm the dynamics of cTn changes. Increased values of cTn, t. j. values higher than the 99th percentile of values in a healthy population can be detected in the blood 2 to 3 hours after the onset of acute MI. A typical picture of an increase or decrease in cTn values makes it possible to distinguish an acute increase due to MI from a chronic increase in cTn from other causes (Table 8.3). A negative result in a blood sample taken too soon (1 – 2 hours) after the onset of pain does not rule out acute MI. A negative cTn value 6 hours after the last occurrence of clinical manifestations precludes acute MI (but not unstable AP).

TABLE 8.3 POSSIBLE CAUSES OF ELEVATED CARDIAC TROPONINS

Acute MI type 1	NSTEMI, STEMI
Acute MI type 2	Cardiogenic, hypovolemic, septic shock, acute and chronic respiratory insufficiency, arrhythmias, severe anemia, severe hypertension, aortic dissection, myocardial hypertrophy
Acute MI type 4-5	Cardiosurgical procedures
Demand ischemia	Critically ill patients (respiratory failure, sepsis), arrhythmias, aortic dissection
Non-ischemic damage of myocardium	Injuries and surgery on the chest and heart, rhabdomyolysis, cardiotoxicity of drugs, infiltrative and inflammatory diseases of the myocardium
Multifactorial causes	Heart failure, pulmonary embolization, severe pulmonary hypertension, extreme physical activity
Chronic elevation	CKD, DM, chronic HF

The cTn value 24 hours after the onset of MI can be used to estimate infarct size. Normalization of values occurs in most cases within 7 days. This persistent increase in cTn values caused by their continued release from necrotic cells allows the later diagnosis of so-called subacute MI, e.g. in diabetic patients who have a lower perception of pain due to diabetic neuropathy and may present on the 3rd – 4th day after onset of necrosis. As the diagnosis of acute MI is important in terms of risk stratification of patients and selection of optimal treatment, examination of cardiac markers is indicated in all patients with suspected ACS. The best validated and most widespread biomarkers are proteins - cardiac troponins I and T (cTnI, cTnT) and MB creatine kinase isoenzyme (CK-MB).

Cardiac troponins are the recommended first-choice biomarkers to confirm myocardial necrosis, while CK-MB mass is an acceptable alternative if cardiac troponins are not available. Prospective studies on the use of troponin in ACS have repeatedly shown that the diagnostic efficiency of cTn determination is higher compared to CK-MB values. Besides, it predicts better the early and long-term risk of adverse cardiac events.

Many traditional enzymes used in the diagnosis of acute MI in the past, such as total creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LD) activity are not currently used in this indication due to their low specificity.

Decision algorithms

In the past, time intervals of 0-6-12 hours were used to monitor cTn dynamics and their relative percentage change compared to the first measured value or multiples of the cut-off value was evaluated. The pressure for early differential diagnosis of patients with acute chest pain, especially in the intake wards, and the effort to shorten the time to reperfusion therapy led to the introduction of algorithms where the time of the second cTn determination was shifted to 3 to 1 hour from the initial examination (Figure 8.3). In particular, the emergency department uses a 0/1-hour examination algorithm, validated for both hsTnT and hsTnI, which allows patients without elevation of the ST-segment on the ECG to exclude or confirm acute MI with high diagnostic efficiency (Figure 8.4).

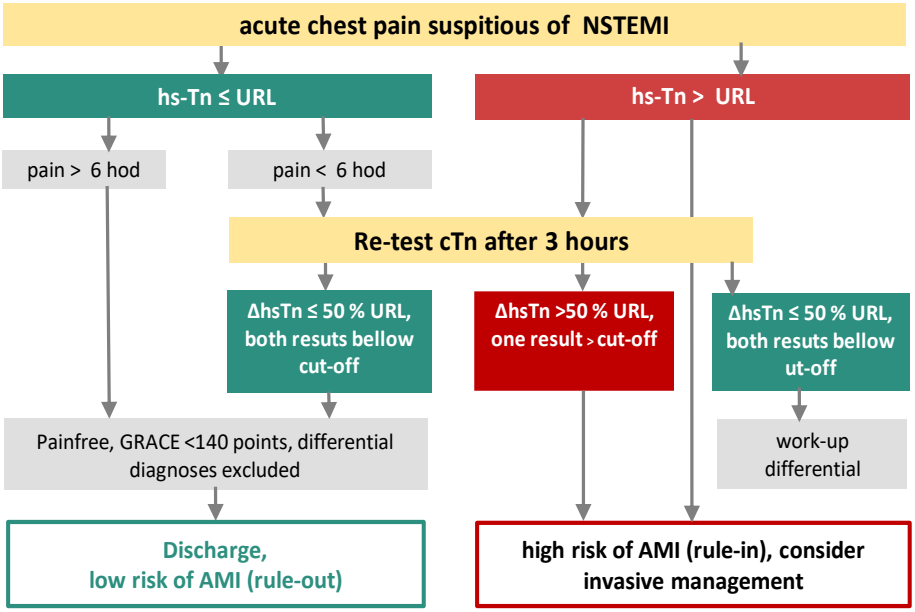


FIGURE 8.3 ESC 3-hour algorithm for troponin utilization in patients with chest pain and suspected STEMI. ESC - European Society of Cardiology, ULN - upper limit of the reference value (99th percentile in healthy), GRACE = Global Registry of Acute Coronary Events, Δ - the change depends on the method used.

It should be emphasized that these algorithms cannot be used alone, but always in conjunction with the patient's clinical and ECG findings. They are not suitable for patients who have a short interval from the onset of clinical manifestations (up to 2 to 3 hours), critically ill patients and patients with elevated cardiac troponins for reasons other than acute type 1 myocardial infarction (elderly patients, advanced renal insufficiency, etc.). For additional information read also INFO 8.2).

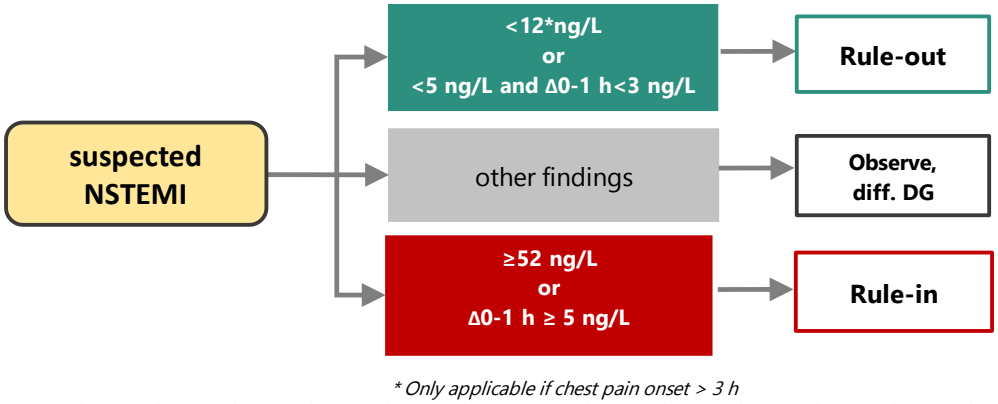


FIGURE 8.4. **Example of 0/1hour algorithm using hsTnT for rule-out or rule-in AMI:** Absolute values and changes in hsTnT concentration were derived from clinical studies. Values for rule-out exclude those who do not have AMI (NPH >99%), but they need differential diagnosis of chest pain. Values for rule-in with a 75 – 80% probability (PPH) confirm acute MI, which requires angiography (SP 98%). It can be used in patients with pain lasting more than 3 hours. 25 – 30% of patients require further observation and examination. According to: Roffi M et al. Eur Heart J, 2016; 37: 267-315

INFO 8.2 Cardiac troponins - FAQ

Can a false-positive cTn result be expected?
Analytical false-positives may occur in the presence of potentially interfering factors such as heterophilic antibodies or rheumatoid factor. Medical false-positives in the absence of ACS may occur with many underlying diseases (Table 8.3). The term "false positivity" is misleading in this case because the cTn values reflect the point necrosis of cardiomyocytes and, even in the absence of clinical signs of myocardial ischemia, correlate with a worse prognosis of the patient. Serial cTn testing is necessary to distinguish an acute coronary event, characterized by a typical rising and falling pattern, from other more chronic conditions.

Can a false-negative cTn result be expected?
A negative result of cTn can be expected, if the interval from the onset of pain is too short (up to 2-3 hours) due to the time dependence of troponin release from necrotic cardiomyocytes. Repeated testing of cTn is important up to 6 hours after infarction. The very rare presence of circulating anti-troponin antibodies can cause analytical false negativity. Although a negative cTn result excludes acute MI, it does not rule out the presence of ischemic heart disease.

What changes in cTn values are considered significant?
There is no full consensus on this issue yet. At low concentrations (close to the cut-off for AIM), changes in cTn ≥50% from baseline are considered significant. Two consecutive results should differ by 50 – 80% (so-called critical difference) to ensure that the change in concentration is not just due to the sum of analytical and biological variability. The changes of the initial values used in the 0/1h algorithm are relatively small - by 3 ng/L and 5 mg/L. It is therefore uncertain whether all laboratory analytical systems can distinguish them with sufficient accuracy.

What is the difference between cTnI and cTnT?
Both cTnI and cTnT are equally effective in the diagnosis and stratification of patients with ACS. As there is only one producer of a diagnostic assay for cTnT and several manufacturers of cTnI, there is greater variability between the cTnI assays antibodies and calibrators used. Both troponins differ in the dynamics of release and removal, including renal clearance. There is a different clearance pattern from the circulation and the duration of cTnT elevation is longer (2 weeks) than cTnI (1 week). Conditions with reduced GFR increase cTnT more often than cTnI.

It should be emphasized that these algorithms cannot be used alone, but always in conjunction with the patient's clinical and ECG findings. They are not suitable for patients who have a short

interval from the onset of clinical manifestations (up to 2 to 3 hours), critically ill patients and patients with elevated cardiac troponins for reasons other than type 1 acute MI (elderly patients, advanced renal insufficiency, etc.). For additional information read also INFO 8.2).

Creatine kinase and CK-MB isoenzyme

Creatine kinase (CK), the cytosolic enzyme, has three major isoenzymes, MM, MB and BB, consisting of two type B or M polypeptide chains. High levels of CK are found in skeletal muscle and heart cells. Plasma CK activity is more than 95% CK-MM isoenzyme. In muscle, more than 98% of CK occurs in the form of CK-MB, less than 2% as CK-MB. The content of CK-MB in skeletal muscle may increase to 5 – 15% in some patients with muscle diseases and also in trained endurance athletes. Cardiac muscle cells contain about 70 – 80% creatine kinase in the form of CK-MM and 20 – 30% in the form of the isoenzyme CK-MB. It can be stated that the heart is the only organ that contains more than 5% CM-MB. The last brain-derived CK-BB isoenzyme rarely appears in the blood and is currently of no diagnostic value.

Determination of total CK activity has low specificity in the diagnosis of myocardial necrosis. Elevated CK values occur after injuries, operations, intramuscular injections, prolonged physical exertion, hypo- and hyperthyroidism and many other pathological conditions. Immunochemical determination of the mass CK-MB concentration (CK-MB mass), which is able to detect even partially degraded and enzymatically inactive molecules, can be used as an alternative diagnostic method if the determination of cardiac troponins is not available. According to ESC recommendations (2015), CK-MB may be of additional importance for the time estimation of recurrence of myocardial infarction.

8.3 Heart failure

Heart failure (HF) is a complex clinical syndrome that manifests with typical symptoms and objective signs of insufficient heart function as a pump. A structural or functional abnormality of the myocardium lead to reduced cardiac output and an inappropriate increase in ventricular filling pressure, resulting in an insufficient supply of oxygen to vital organs and peripheral tissues. Heart failure is primarily a condition of older people, and thus the widely recognised ageing of the population also contributes to its increasing incidence. The term **'congestive heart failure**' (CHF) is reserved for patients with breathlessness and abnormal sodium and water retention resulting in oedema.

HF can result from any cardiac disease that compromises ventricular systolic or diastolic function or both. The most common causes of HF are coronary artery disease, hypertension, or their combination. Other causes include tachycardia-induced or dilated cardiomyopathy, valvular diseases, infiltrative diseases, toxins and chemotherapy induced cardiomyopathies, and chronic obstructive pulmonary disease. It should be emphasised that many of these causes may be completely reversible given appropriate and timely treatment/intervention.

Neurohormonal activation (sympathetic nervous system, RAAS, natriuretic peptides) plays a pivotal role in the development as well as the progression of HF. In the acute phase, neurohormonal activation seems to be beneficial in terms of maintaining adequate cardiac

output and peripheral perfusion. Sustained neurohormonal activation, however, eventually results in dilation and ventricular remodelling, which contribute to disease progression in the failing myocardium. which eventually leads to further neurohormonal activation.

The European Society of Cardiology has recently issued a uniform and practical definition of HF. Heart failure can be classified based on several criteria:

- Time course - **acute** or **chronic** HF. Acute HF can be an acute decompensation of previously stable chronic HF (about 60%) or the first manifestation of HF (about 40%).
- **Left ventricular ejection fraction** (LVEF):
- HF with preserved LVEF - defined as LVEF >50%
- HF with reduced LVEF - defined as LVEF <40%
- HF with mid-range LVEF - defined as LVEF between 40 – 49%
- Localization – **left-sided** HF (stasis in the pulmonary veins leads to pulmonary oedema) or **right-sided** HF (venostasis causes peripheral oedema and/or hepatomegaly).

The severity of HF is assessed using the **different classification systems**, based on symptoms and functional physical. **Dyspnea** is the most common sign of HF, but it is not specific only for acute HF.

Diagnosis of heart failure

Diagnosis of heart failure is often difficult, as clinical symptoms are highly variable and non-specific (Table 8.4). Targeted treatment of the patient and the clinical course of the patient depend on early and accurate diagnosis. Risk stratification and treatment selection is based on knowledge of the etiology of HF, the hemodynamic profile of patients (INFO 8.3) and the severity of their condition.

TABLE 8.4 ESC DEFINITION OF HEART FAILURE

Heart failure is a clinical syndrome with the following features:	
Symptom typical of HF	Breathlessness at rest or on exercise, fatigue, tiredness, ankle swelling, orthopnea
Signs typical of HF	Tachycardia, tachypnea, pulmonary rales, pleural effusion, raised jugular venous pressure, peripheral oedema, hepatosplenomegaly
Objective evidence of structural or functional abnormality of the heart at rest	Cardiomegaly, third heart sound, cardiac murmurs, abnormality on ECG, raised natriuretic peptide concentration

Diagnosis of HF in patients without acute manifestation is based on clinical examination (typical or suspicious findings in the anamnesis and physical examination), ECG, or chest X-ray. Any positive finding is an indication for an examination of natriuretic peptides, which are recommended biomarkers of HF. The concentration of natriuretic peptides bellows the cut-off value excludes HF. Increased concentration of natriuretic peptides is an indication for echocardiographic examination, which provides information about morphology and function of cardiac compartments and allows the measurement of filling volumes and pressures, including LVEF (Figure 8.5).

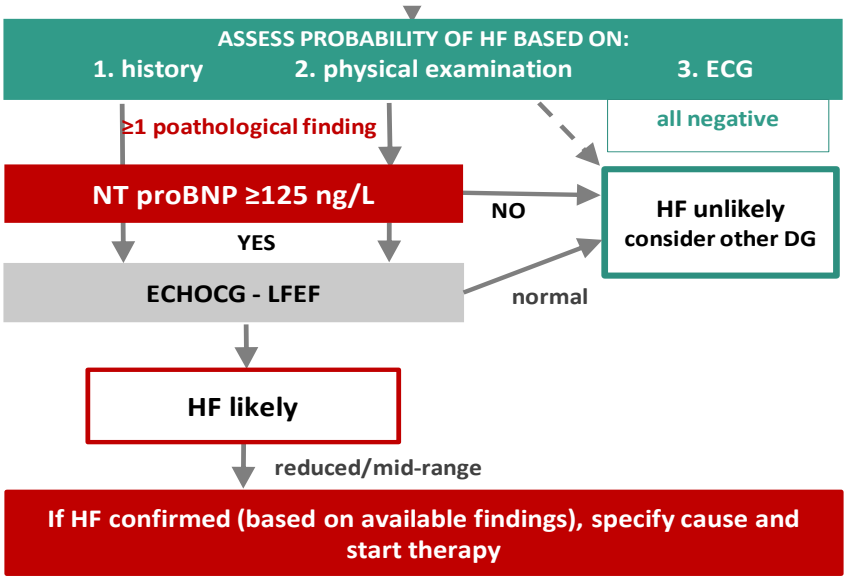


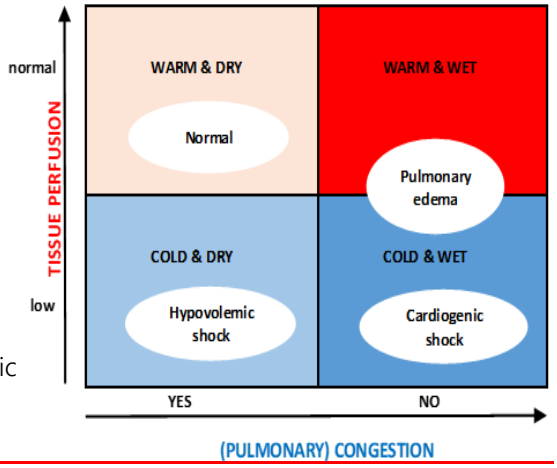
FIGURE 8.5 Diagnostic algorithm in suspected heart failure (Adopted from ESC, 2016 (www.escardio.org)

INFO 8.3 Hemodynamic profile in acute HF

Patients with acute HF have an altered hemodynamic profile manifested by signs of congestion ("wet") and hypoperfusion ("cold").

Signs of hypoperfusion (low cardiac output): Low blood pressure, narrow pulse pressure, pulsus alternans, tachycardia, cool extremities, confusion, agitation, drowsiness, oliguria, low serum sodium.

Signs of congestion (fluid overload): Severe dyspnea, ↑jugular venous pressure, gallop rhythm, rales, pulmonary edema (chest X-ray), ascites, hepatic distension.



8.4 Laboratory tests in heart failure

Natriuretic peptides

Natriuretic peptides (NPs) belong to a group of peptides with a different genetic origin but a similar structure (e.g. ANP - atrial NP, BNP - brain NP, CNP - C-type NP), which affect several physiological functions. ANP and BNP are synthesized in the cardiac atria and ventricles, their release is activated by increased tension of the walls of those cardiac compartments, mostly during volume overload of the heart. The physiological effects of ANP and BNP include arterial vasodilation, increased natriuresis, increased glomerular filtration and diuresis, inhibition of the renin-angiotensin-aldosterone system, and antiproliferative effects on the myocardium. During

HF, these compensatory effects of natriuretic peptides protect the body from volume overload, hypertension and marked vasoconstriction.

The biomarker used in the diagnosis and monitoring of HF is BNP, which is formed in the cardiac atria and, to a lesser extent, in the ventricles as a precursor molecule - proBNP. Equimolar amounts of hormonally active BNP and an inactive N-terminal fragment, NT-proBNP, are formed from proBNP by enzymatic cleavage and are secreted into the circulation (Figure 8.6).

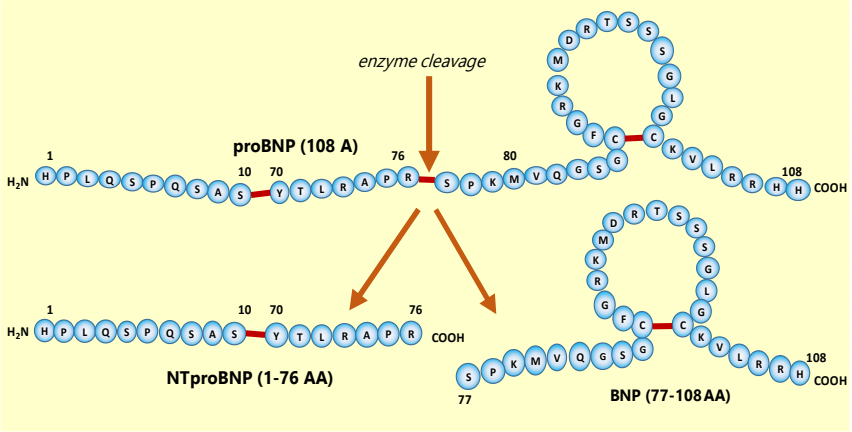


FIGURE 8.6 Release of BNP and NT-proBNP

BNP and NT-proBNP are quantitative markers of cardiac stress that are released into blood after cleavage of the precursor protein proBNP.

Natriuretic peptides are degraded in two ways: by binding to the so-called clearance receptor (NPR-C) or by hydrolysis to inactive peptides by the enzyme neprilysin (neutral endopeptidase). BNP and NT-proBNP levels in the blood are elevated in the blood of patients with HF and their levels correlate well with each other. Because their absolute concentrations are different, different cut-off values for BNP and NT-proBNP are used. The better stability in serum sample and wider measuring range make NT-proBNP more suitable molecule for laboratory determination (Table 8.5).

TABLE 8.5 PROPERTIES OF B-TYPE NATRIURETIC PEPTIDES

Characteristic	BNP	NT-proBNP
Biologically active	yes	no
Prohormone fragment	C-terminal (proBNP 77–108) 32 amino acids	N-terminal (proBNP 1–76) 76 amino acids
Half-life (min)	20	60 – 120
Sample stability (room temperature)	4 hours	>2 days
Sample type	whole blood, plasma	plasma or serum

NT-proBNP in diagnostics and monitoring

Determination of natriuretic peptides is an integral part of diagnostic procedures in HF. They are beneficial in the diagnosis of HF with unclear symptoms, risk stratification in patients with clinically ongoing HF, and the management of treatment. NT-proBNP is a sensitive and specific biomarker for the confirmation or exclusion of **acute heart failure** in the differential diagnosis of patients with acute dyspnea, especially in conditions of emergency admission (Figure 8.7).

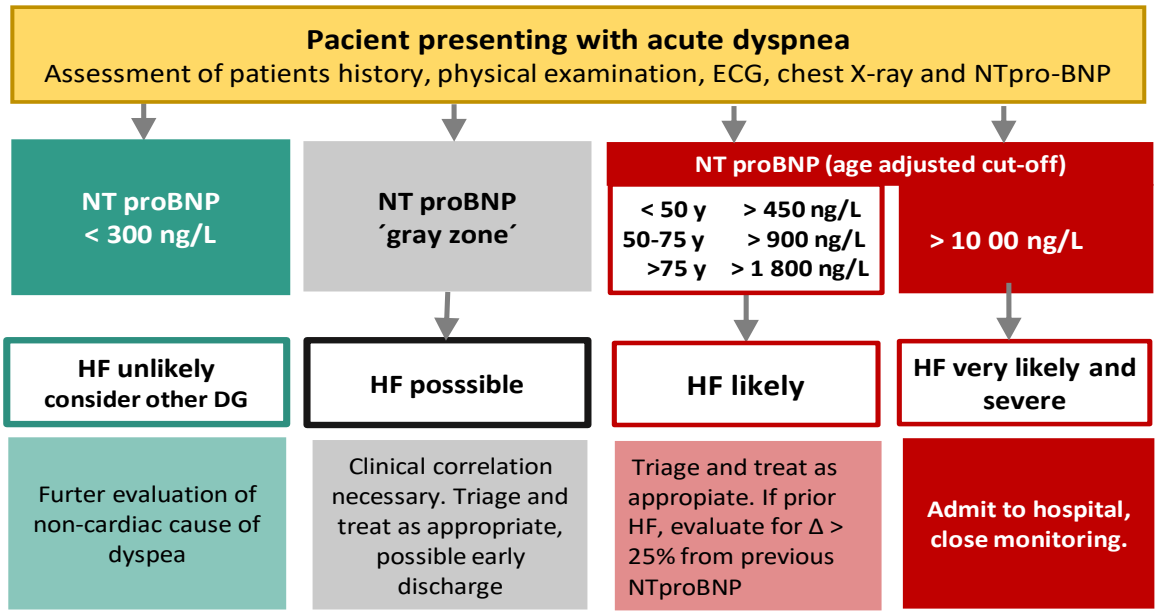


FIGURE 8.7 NT-proBNP in the evaluation and triage of emergency patients with an acute dyspnea

According to a clinically validated algorithm (International NT-proBNP Consensus Panel), one low value of natriuretic peptides for all age categories (cut-off for NT-pro BNP 300 ng/L) excludes the presence of acute HF. To confirm the diagnosis of acute HF, age-adjusted values are recommended, which have 90% sensitivity and 84% specificity. In about 20% of patients, NT-proBNP values occur in the so-called grey zone, which does not allow to distinguish between acute dyspnoea of cardiac and extra-cardiac origin.

Decision-making based on natriuretic peptide levels has also limitations. The concentration of BNP and NT-proBNP may increase in many other cardiovascular and non-cardiac diseases without presence of HF. Individuals with reduced GFR have elevated NP levels not only due to decreased renal clearance of peptides but also due to hypervolemia resulting from failing renal function and the presence of numerous cardiovascular pathologies in chronic kidney disease.

Natriuretic peptides also inform the prognosis of heart failure. The baseline value in patients hospitalized with acute HF is of diagnostic significance, a second sample in approximately two weeks will identify patients with a better prognosis (decrease in NP by 30% or more) and serve as a reference value in outpatient treatment management. Patients with a small decrease in the level of NP or even an increase in it have a much higher mortality and a high risk of repeated acute decompensation and thus repeated hospitalization. Natriuretic peptides respond by decreasing to successful HF treatment and allow monitoring of pharmacological treatment. For additional information about natriuretic peptides as diagnostic marker see also INFO 8.4.

A new class of drugs in the treatment of HF - **neprilysin inhibitors** (neutral endopeptidase degrading BNP), for example, LCZ696 in the PARADIGM-HF study, leads to significantly higher serum BNP values. NT-proBNP is not a substrate for neprilysin, so its level does not increase. In addition to the several new molecules that have been investigated in clinical and preclinical studies as potential diagnostic or prognostic markers of HF (e.g. soluble ST2-receptor, galectin-3, FSTL1).

INFO 8.4 Natriuretic peptides – FAQ

How important is NT-proBNP in the grey zone'?
NT-proBNP values between the rule-out and rule-in cut-off values for HF in the ED are referred to as intermediate or grey zone values (see Figure 7.4). Gray zone patients usually have mild HF with fairly good short-term outcome. Nevertheless, a grey zone NT-proBNP value should not be ignored because it is associated with worse outcomes compared with NT-proBNP values below the rule-out cut-off.

What is the optimal timing of NT-proBNP measurement?
Timing of the initial NT-proBNP measurement in ED patients with suspected HF is critical because delayed measurement has been reported to be associated with delays in treatment and an increase in hospital mortality. Subsequent serial measurement at 4, 12 and 24 hours from admission is useful in confirming the diagnosis of acute HF.

Can NT-proBNP be used to guide therapy?
Natriuretic peptide levels are commonly reduced by treatment with diuretics, ACE inhibitors, angiotensin receptor blockers, aldosterone antagonists and cardiac resynchronization therapy. Also, changes of NT-proBNP over months are predictive of outcome of HF. This suggests that NT-proBNP could be useful to guide therapy in selected cases. However, there is no consensus among experts (mixed results of small controlled trials) and tailoring therapy to achieve a target NT-proBNP level is not warranted at this time.

Is there a value for combined measurement of cTn and NT-proBNP in HF?
Both NT-proBNP and cTn can identify patients with HF at increased risk for an adverse outcome. Elevated cTn is not uncommon in HF and provides prognostic information in patients hospitalized with acute decompensated heart failure. The combination of NT-proBNP and cTn is even more powerful, and, in ED patients with acute HF, the highest mortality rate was observed when both markers were elevated. The therapeutic approach to such patients has not been established at this time.

Other laboratory tests

Full blood count: Anemia and high lymphocyte percentage are strong risk factors and prognostic markers of poor survival.

Serum electrolytes (including calcium and magnesium): Baseline electrolytes - sodium and potassium should be obtained in all patients. Frequent finding in patient with HF is decreased sodium (<135 mmol/L) and altered potassium (due to treatment of HF).

Serum creatinine and urea: Reflects tissue perfusion, fluid status, rules out renal disease. May be found normal to elevated.

Glycemia: Screening for diabetes mellitus as a comorbid condition. Diabetes mellitus has been associated with a 3- to 5-fold increase in the risk of developing HF.

LFT: Reflects abdominal congestion.

Thyroid function tests (especially TSH): Screening for hypo- or hyperthyroidism. Both can be a primary or contributory cause of heart failure (see Chapter 10).

Blood lipids: Screening for dyslipoproteinemias/metabolic syndrome. Elevated in dyslipidemia, decreased in end-stage HF, especially in the presence of cardiac cachexia.

Serum ferritin and **transferrin saturation** for evaluation of cardiomyopathy due to iron overload cardiomyopathy/hemochromatosis (see Chapter 13).

Case studies and self-assessment questions

Case report 8.1

A 70-year-old woman, obese, with signs of metabolic syndrome - increased fasting blood glucose, treated for hypertension and mixed hyperlipidemia, reports retrosternal pain in the last 2 weeks, several times at rest. The last occurrence of pain 10 hours ago, radiated to both sides of the lower jaw, lasted up to 10 minutes. ECG: sinus rhythm, no signs of ischemia. The cTnT values at the initial CPO and after 3, 6 and 12 hours are as follows: 25 - 35 - 26 - 29 ng/L (cut-off 14 ng/L). Further laboratory tests are listed in the table. Angiography revealed 2 sub-occlusive stenoses of the right coronary artery.

Serum	Result	RI
CRP	33	<5 mg/L
eGFR	0.75	>1.5 ml/s/1,73m^2
D-dimers	2.2	< 0.5 μg × 10^9/L
WBC	9.9 × 10^9	4.4 – 11.3 × 10^9/L
NT-pro BNP	400	<125 ng/L excludes chronic HF <300 ng/L excludes acute HF

Questions:

a. Is acute coronary syndrome present? If so, what form?
b. Which findings support your diagnosis?
c. How would you rate the increased NT-proBNP values?

Case report 8.2

A 54-year-old man with a 15-year history of T2DM was admitted to hospital with pneumonia and suspected sepsis and was treated with a combination of ATB. A cardiologist was called on day 3 because the patient complained of chest pain and weakness. The pain was pleuritic, worsening with movement and deep breathing, and subsided at rest.
ECG: Q waves in leads II, III and aVF, new BTR and slight increase of ST-segment in V4-V6. ECHOKG showed a marked impairment of the mobility of the lower posterior and lateral walls of the heart. Urgent catheterization confirmed occlusion of the right coronary artery, which was treated with angioplasty and stent placement. Laboratory findings of the patients are in the table.

Serum	Result	RI
cTnT	178	<14 μg/L
CRP	151	<5 mg/L
eGFR	1.25	>1.5 ml/s/1.73m^2
D-dimers	3.6	<0.5 μg/mL FEU
WBC	14.6 × 10^9	4.4 – 11.3 × 10^9/L
NT-pro BNP	980	<125 ng/L excludes chronic HF <300 ng/L excludes acute HF

Questions:

a. Are the criteria for AIM present?
b. How would you explain the absence of typical retrosternal pain?
c. Is it necessary to repeat the examination in cardiac troponins?

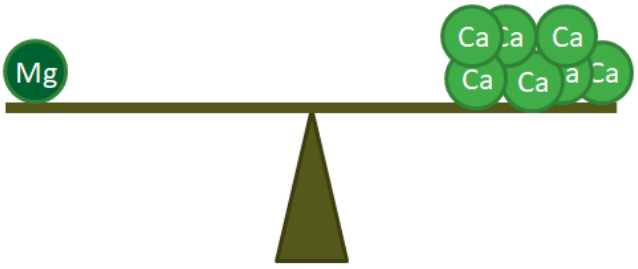
Self-assessing questions

1. What are the diagnostic criteria for acute myocardial infarction?
2. Which laboratory parameter is recommended as a marker of first choice in the diagnosis of myocardial infarction and why?
3. Name examples of elevated cardiac markers in absence of acute coronary syndrome.
4. How has the introduction of ultrasensitive troponin (hs-cTn) methods affected the diagnosis of various forms of ACS?
5. What is the clinical significance of the NT-proBNP test?

KEY INFORMATION

- ☑ Diagnosis and risk stratification of patients with ACS is based on dynamic changes of cardiac troponins in conjunction with other evidence of myocardial ischemia (typical retrosternal pain, ECG changes, evidence of coronary stenosis/thrombosis using imaging methods).
- ☑ In acute MI, biochemical markers of myocardial necrosis increase 2 – 6 hours after the onset of clinical symptoms, peak after 18 – 24 hours and normalize within 7 – 10 days.
- ☑ Examination of hs-cTn makes it possible to exclude patients with chest pain who do not have ACS (low levels of hs-cTn) and to diagnose acute MI in patients without typical ECG changes (NSTEMI) who require early invasive treatment.
- ☑ CK-MB isoenzyme examination (CK-MB mass) can be used as an alternative diagnostic method if cardiac troponin determination is not available.
- ☑ A structural or functional abnormality of the myocardium that causes heart failure leads to reduced cardiac output and insufficient oxygen supply to vital organs and peripheral tissues.
- ☑ Natriuretic peptides ANP and BNP cause vasodilation, increase natriuresis and diuresis, thereby protecting the body from volume overload, hypertension and excessive vasoconstriction during HF.
- ☑ Examination of natriuretic peptides (in practice especially NT-proBNP) is part of the diagnosis of HF in patients, especially if they have typical clinical signs and manifestations, or pathological findings detected by other diagnostic methods (e.g. ECG, imaging methods).
- ☑ In addition to the diagnosis of acute and chronic HF, natriuretic peptides are also used as prognostic biomarkers and also to monitor treatment.

9



Disorders of calcium-phosphate and magnesium balance

CONTENT

Disorders of calcium, phosphorus, and magnesium metabolism cause many serious clinical complications, including arrhythmias, seizures, and respiratory failure. The calcium-phosphate (Ca-P) balance is maintained by the activity of four mutually cooperating systems. The endocrine system affects the intestinal absorption of calcium and phosphorus, their exchange with bone supply and renal excretion to maintain their physiological concentration in the blood despite fluctuations in intake and changing needs of the body. Bone contains about 99% of the body's total calcium (Ca) reserves, 85% of its phosphorus (P) reserves, and more than 60% of the body's magnesium (Mg) reserves.

This chapter provides a basic overview:

- interactions between hormones regulating mainly Ca-P metabolism,
- causes of Ca-P balance disorders,
- causes of magnesium balance disorders with emphasis on their laboratory diagnosis.

9.1 Basic physiology

Calcium is an essential element available only from food, which provides a wide range of biological functions. In the body, Ca is the fifth most abundant element in the body (∽1 kg) and its distribution in the body is uneven (Figure 9.1):

1. 99% (∽ 990 g) is bound in bones and teeth mainly in the form of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and phosphate complexes, where Ca provides skeletal strength and is a dynamic reservoir for maintaining intra- and extracellular Ca;
2. 1% (∽ 10 g) Ca is found predominantly in the ECT, as the intracellular calcium concentration is very low and usually amounts to only 1/10 000 of the ECT concentration. Intracellular calcium is a key signal (second messenger) in many important cellular processes (e.g. hormone secretion, cell division, change in cell shape and motility).

The serum concentration of total calcium (S-Ca) is maintained in the range of 2.10 – 2.70 mmol/L and consists of three different forms of Ca:

- 50% is free, ionized (Ca^{2+}), which is the only biologically active form, tightly regulated by hormones;
- 50% bound, of which:
  - 40% is bound to proteins, mainly albumin,
  - 10% are complexes of Ca with citrate, sulphate or phosphate.

Keeping the concentration of **extracellular Ca^{2+}** within a narrow physiological range is necessary to maintain normal neuromuscular excitability and muscle contraction. It also has important functions in the activation of coagulation factors and complement.

The resulting serum Ca (and P) concentration depends on the balance between intake and intestinal absorption, bone activity, and renal filtration and reabsorption (Figure 9.1). Unlike other electrolytes, bone plays an important role in maintaining Ca-P balance. Massive bone supply can absorb excess Ca (mineralization) or replace serum Ca deficiency (demineralization). Changes in bone Ca supply are slow and can be neglected in acutely ill hospitalized patients, but should be taken into account in long-term outpatient care.

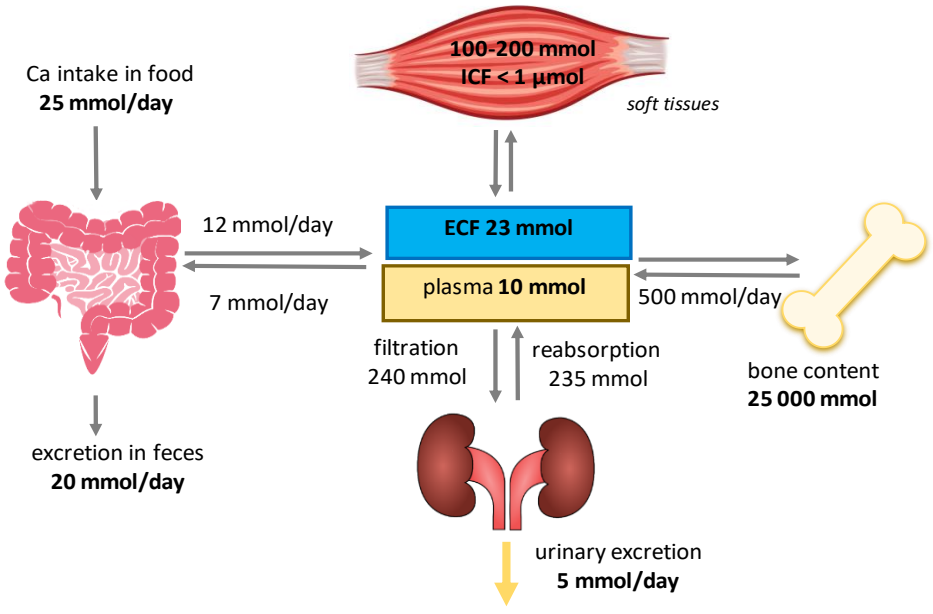


FIGURE 9.1
Approximate daily turnover of calcium in the body

The main hormonal regulators of Ca-P balance are (Table 9.1 and Figure 9.2):

- parathyroid hormone (PTH),
- calcitriol (1,25-dihydroxyvitamin D - 1,25 $(OH)_2D$),
- fibroblast growth factor (FGF23),
- calcitonin, the effect of which is small under physiological circumstances

TABLE 9.1 EFFECTS OF MAJOR HORMONES REGULATING CA-P BALANCE

Hormone	S-Ca	S-P	Bone	Intestine	Kidney
PTH	↑	↓	↑osteoblastic activity -resorption	*indirect:* ↑ 1-hydroxylation of Vit D	↑ Ca reabsorption ↑ P excretion ↑ hydroxylation
Vitamin D	↑	↑	Indirect: stimulation of osteoblasts	↑ absorption of Ca + P	No direct effect
Calcitonin	↓	↓	↓osteoclastic activity	No direct effect	↑ Ca + P excretion
FGF23	↓	↓	No direct effect	*indirect:* ↓ absorption Ca due to ↓ Vit D	↑ P + Ca excretion

PTH is a polypeptide hormone (84 AK) that is synthesized and secreted by the chief cells of the parathyroid glands. These cells contain transmembrane calcium-sensing receptors (CaSR), which response to the concentration of ionized calcium in the blood. An increase in Ca^{2+} suppresses PTH production via the CaSR, while a decrease in Ca^{2+} stimulates PTH secretion. In addition to hypocalcemia, PTH production is also stimulated by low serum magnesium, adrenergic agonists and dopamine. Sufficient levels of calcitriol block the formation of PTH at the gene level. PTH increases the concentration of Ca in the blood in three ways:

1. Minimizes urinary excretion of Ca by increasing its reabsorption in the renal tubules while reducing tubular phosphate reabsorption and increasing phosphaturia;
2. Stimulates the conversion of 25-hydroxyvitamin D (calcidiol) to 1,25-dihydroxyvitamin D (calcitriol) by stimulating 1α hydroxylase activity in the kidney;
3. Together with calcitriol mobilizes Ca and P from the bones.

In the study of hypercalcemia in malignancies, a PTH-like peptide - PTHrP (parathyroid hormone-related peptide) - was discovered. PTHrP is produced by various cells in the body and activates PTH receptors. It plays a role in smooth muscle relaxation, cell proliferation and differentiation, but in a healthy individual, it minimally affects calcium homeostasis.

Calcitriol is a vitamin D-derived hormone that is synthesized predominantly in the skin from the precursor of 7-hydroxycholesterol by a non-enzymatic reaction dependent on UV radiation. Vitamin D may be ingested in small amounts in food of animal (cholecalciferol) or plant (ergocalciferol) origin. It is converted to a metabolically active hormone, calcitriol (1,25-dihydroxyvitamin D), by two enzymatic hydroxylation's in the liver and subsequently in the kidneys. Calcitriol promotes the synthesis of calcium-binding protein, which is essential for the absorption of Ca and P in small intestinal cells. Together with PTH, calcitriol stimulates osteoclastic activity, which results in the release of Ca and P from the bones. In the kidney, calcitriol regulates its own homeostasis by simultaneously stimulating 24-hydroxylase activity and suppressing 1α-hydroxylase activity. Vitamin D deficiency occurs when there is insufficient exposure to sunlight, its low intake in the diet or most often a combination of both factors.

FGF23 is synthesized in osteoblasts with increasing serum phosphorus concentration. FGF23 is the major phosphaturic hormone. Its main target organ is the kidney, where it increases the fractional excretion of phosphates and leads to a reduction in phosphatemia. FGF23 also reduces the synthesis of both PTH and calcitriol by suppressing 1α-hydroxylase activity. The effect of FGF23 in the kidney requires its transmembrane protein Klotho, which acts as a co-receptor and increases the affinity of FGF23 for its receptors.

Calcitonin is a peptide hormone secreted by thyroid C cells in the case of elevated levels of ionized calcium. The effect of calcitonin on calcium level is opposite compared to PTH; it reduces osteoclastic activity, slows Ca release from bone, and slightly inhibits renal Ca and P reabsorption. Under physiological circumstances, the effect of calcitonin on maintaining Ca-P balance is small. The concentration of calcitonin in the blood is very low to undetectable in most people; its elevations occur in severe hypercalcemia and in most cases of medullary thyroid carcinoma (∽30% of patients have normal calcitonin).

Hyperthyroidism can cause increased osteoresorption with secondary osteoporosis. Hypercalcemia is very rare in severe hyperthyroidism because the concomitant decrease in PTH secretion leads to increased urinary Ca losses. Estrogens, prolactin and growth hormone may increase calcitriol production and increase Ca absorption during pregnancy, lactation and growth.

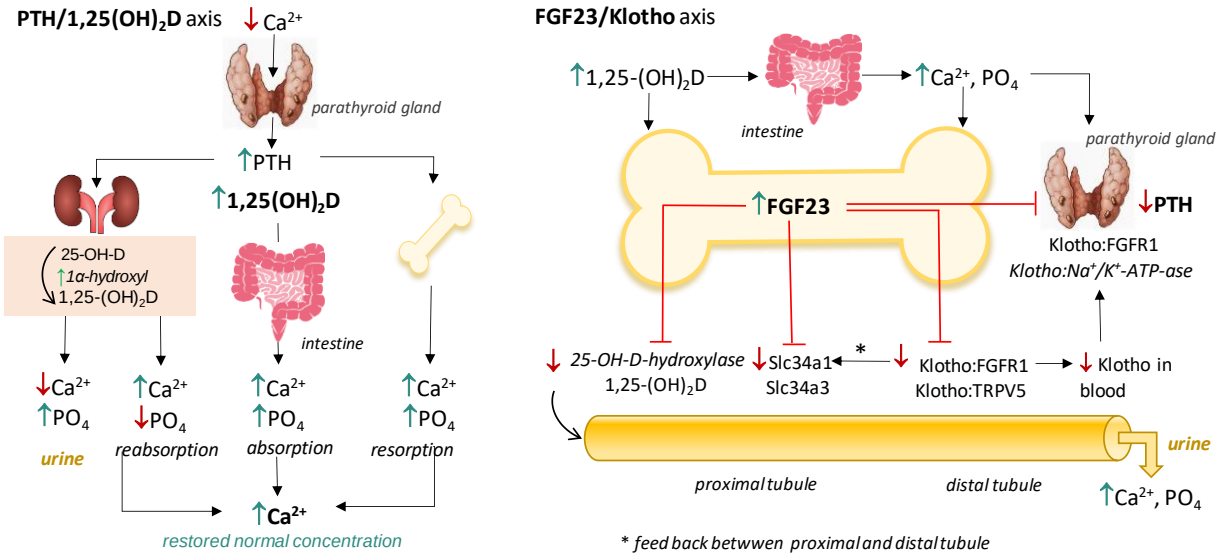


FIGURE 9.2 Hormonal regulation of Ca-P homeostasis *(adopted from J Clin Invest 2008; 118(12): 3820–3828)*

The PTH/1,25(OH)2D axis. Decrease in blood Ca^{2+} stimulates secretion of PTH, which in the kidneys reduces urinary Ca excretion, stimulates 1α hydroxylase activity and increases the fractional excretion of phosphates (PO4); in bone, increases Ca and P deposition. Increased formation of 1,25 (OH)2D increases intestinal Ca uptake, followed by suppresses PTH secretion.

The FGF23/Klotho axis. Receptor for FGF23 in the kidney is Klotho: FGFR1 complex in the distal tubule; FGF23 reduces the renal reabsorption of both P and Ca in proximal tubules (through feedback between the distal and proximal tubules) and acts as a counterweight to the hypercalcemic and hyperphosphatemic effects, by inhibiting the formation of PTH in parathyroid and 1,25(OH)2D in the kidney.

Serum calcium levels are also affected by **other factors**:

a. The **concentration of proteins**, especially albumin (Alb), changes the total serum calcium (S-Ca), but not the concentration of ionized calcium. Calcium corrected for normal albumin

concentration can be calculated. If the serum albumin (S-Alb) concentration is <40 g/L, then: $Calcium_{corr}$ (mmol/L) = S-Ca + 0.02 (40 - S-Alb).
If S-Alb >45 g/L, then: $Calcium_{corr}$ (mmol/L) = S-Ca - 0.02 (S-Alb - 45).

b. **Changes in pH** affect the concentration of ionized calcium because H^+ competes with Ca^{2+} for binding sites on proteins. As the H^+ concentration decreases during alkalosis, binding to albumin decreases and the serum Ca^{2+} concentration decreases proportionally. In contrast, in acidosis, the Ca^{2+} level is increased by a similar mechanism and at the same time, the release of Ca from the bones into the ECT is increased due to the buffering effect. Increased renal filtration leads to loss of Ca in the urine. Chronic acidosis can cause bone demineralization and osteomalation. In disorders of acid-base balance, it is indicated direct measurement of ionizing calcium in serum.

c. Diuretics decrease Ca reabsorption in renal tubules (loop diuretics - furosemide) or increase (thiazide diuretics), which in this case may contribute to hypercalcemia.

9.2 Disorders of calcium balance

Hypercalcemia

Hypercalcemia (serum calcium >2.7 mmol/L, ionized >1.36 mmol/L) is a consequence of following mechanisms, which often occur simultaneously: increased intestinal Ca absorption, increased bone resorption, decreased urinary Ca excretion. Mild hypercalcemia is asymptomatic or manifests with non-specific symptoms (INFO 9.1). Severe hypercalcemia >3.5 mmol/L) is a life-threatening acute situation. The most common causes of hypercalcemia are (Table 9.2):

- primary hyperparathyroidism,
- malignant tumours,
- granulomatous diseases,
- milk-alkali syndrome.

INFO 9.1 Symptoms and signs of hypercalcemia

Mild hypercalcemia is usually asymptomatic or manifests only in non-specific symptoms. Patients report proximal muscle weakness, fatigue, loss of appetite, depression, pain abdomen and constipation.
Gastrointestinal symptoms: Hypercalcemia stimulates gastrin secretion and may contribute to the formation of peptic ulcers. Severe hypercalcemia may cause acute pancreatitis, probably due to inappropriate trypsinogen activation in the pancreatic parenchyma.
Renal disorders in hypercalcemia are common and includes in particular:

- nephrolithiasis - a consequence of chronic hypercalcemia,
- nephrogenic diabetes insipidus due to tubule resistance to the effects of ADH - manifests itself
- polyuria, polydipsia and may lead to volume depletion,
- renal failure - the primary cause is hypovolemia combined with calcium-induced vasoconstriction, which reduces renal blood flow. With prolonged hypercalcemia, calcification and ischemia result in irreversible renal damage.

Cardiac signs: A shortened QT interval on the ECG in hypercalcemia is a relatively benign finding. Atropine-responsive bradycardia and cardiac arrest may also occur in hypercalcemia.

TABLE 9.2 CAUSES OF HYPERCALCEMIA

Mechanism	Example
Parathyroid gland disease	Overproduction of PTH: primary and tertiary HPT, MEN syndromes
Tumours	Bone lytic lesion: myeloma, metastatic cancer PTHrP: carcinoma of lungs, oesophagus, head and neck, kidney, ovary, bladder Ectopic production of calcitriol: lymphomas
Vitamin D excess	Endogenous calcitriol production: sarcoidosis, tuberculosis Increased intestinal calcium absorption: vitamin D overdosing
Bone diseases	Increased bone turnover: immobilization, thyrotoxicosis, hypocorticism, Paget's disease, overdose of vit. A/retinoic acid Reduced bone mineralization: aluminium intoxication (during dialysis)
Medicines	Decreased renal calcium excretion: thiazide diuretics increased set point for inhibition of PTH secretion by calcium: lithium
Rare	CaSR gene mutations: familial hypocalciuric hypercalcemia
Artificial	Increased S-Alb concentration: excessive venous stasis during blood collection, severe dehydration

Hyperparathyroidism

Primary hyperparathyroidism (HPT) represents increased autonomic production of PTH by benign parathyroid adenomas (>85%), their hyperplasia (15%) or rarely carcinoma. The tumour does not respond to typical feedback stimuli that reduce PTH secretion, such as elevated serum Ca and vitamin D levels. Serum PTH and Ca levels are elevated and P levels decreased. Despite increased Ca reabsorption in the renal tubules, slightly increased urinary Ca excretion is often present leading to the formation of urinary stones.

Secondary hyperparathyroidism is caused by increased PTH secretion in response to low ionized calcium. Secondary HPT occurs relatively early in chronic kidney disease (CKD), even before patients need dialysis. Another cause is vitamin D deficiency and malabsorption syndrome. Chronic secondary HPT can lead to nodal hypertrophy of the parathyroid glands that do not respond to stimuli that reduce PTH secretion (INFO 9.2).

INFO 9.2 Secondary hyperparathyroidism and kidney

Secondary HPT occurs in patients with CKD by the following mechanism:

1. Decreased GF causes an increase in S-P, which is compensated by FGF23 secretion.
2. The damaged kidney does not synthesize enough calcitriol (impaired 1α-hydroxylation + depressant effect of FGF23). 1,25- $(OH)_2D$ normally inhibits PTH secretion.
3. Lack of calcitriol reduces the level of ionized calcium and increases S-P.
4. Decreased Ca^{2+} levels and hyperphosphatemia stimulate PTH secretion.
5. Increased PTH reduces phosphate reabsorption in the proximal tubule, normalization of S-P is elevated PTH levels; the greater the decrease in GFR, the greater the increase in PTH concentration.
6. This regulatory cycle can be interrupted by reduced dietary P intake or medication P-binding drugs, or so-called calcimimetics that stimulate CaSR in the parathyroid glands.

However, in the advanced stages of CKD, this compensation system ceases to be effective. Kidneys are not able to excrete phosphates when GFR is reduced regardless of PTH concentration.

Secondary HPT can progress to tertiary hyperparathyroidism, in which PTH secretion becomes autonomous, similar to primary HPT. Laboratory manifestation of this transition is hypercalcemia. Tertiary HPT occurs mostly in patients with CKD or after kidney transplantation (Table 9.3).

TABLE 9.3 LABORATORY FINDING IN DIFFERENT TYPES OF HYPERPARATHYROIDISM

Hyperparathyroidism	S-Ca	S-P	Therapy
Primary	↑	↓	surgery
Secondary	↓-N	↑ in CKD ↓ other causes	Low P intake, active VITD, calcimedins
Tertiary	↑	N-↑	surgery

Malignant hypercalcemia

Hypercalcemia accompanying malignancy is caused by three main mechanisms:

1. **PTHrP** secretion by tumour cells accounts for about 80% of cases and occurs mainly in squamous cell carcinomas. By binding to PTH receptors in bone and kidney, PTHrP increases bone resorption and renal Ca reabsorption.
2. The remaining almost 20% of cases of hypercalcemia are due to increased **osteolytic activity** at the site of bone metastases, which are common in malignant tumours of the breast, lung, multiple myeloma and lymphomas. Activation and secretion of cytokines stimulating osteoclasts, such as TNF-α and IL-1 and IL-6 in osteolytic lesions result in hypercalcemia.
3. Rarely, hypercalcemia may occur due to ectopic secretion of **vitamin D** caused by unregulated 1α-hydroxylation of 25-OH-vitamin D outside the kidney, which has been described especially in certain types of lymphomas.

Typical laboratory findings in malignant hypercalcemia include:

- increased concentration of total calcium,
- low to normal PTH level,
- high concentration of PTHrP (specific method of determination!),
- increased activity of ALP or its bone isoenzyme (B-ALP, bone-ALP) supports the diagnosis of hypercalcemia in primary bone tumours or osteoplastic metastases.

Other causes of hypercalcemia

Milk-alkali syndrome: It represents a combination of hypercalcemia, alkalosis and renal insufficiency, which occurs with high milk intake and alkali (e.g. $NaHCO_3$ or $CaCO_3$) used as antacids in people with gastroduodenal ulcers or in the treatment of osteoporosis. High Ca intake and reduced renal excretion is involved in the pathogenesis of that type of hypercalcemia.

Chronic granulomatous diseases: Hypercalcemia is most common in sarcoidosis, but may also occur in tuberculosis, Wegener's granulomatosis, rarely in Crohn's disease and histoplasmosis. It is caused by excessive formation of calcitriol in granulomatous tissue by activated macrophages.

Laboratory tests in hypercalcemia

A combination of a thorough personal history, physical examination and laboratory tests identifies the etiology of hypercalcemia in 99% of cases (Table 9.4).

TABLE 9.4 LABORATOTRY FINDINGS IN DIFFERENT TYPES OF HYPERCALCEMIA

Diagnosis	S-P	S-PTH	S-Calcidiol	U-Ca, FE-Ca
Primary hyperparathyroidism	↓	↑↑	variable	↓/N
Malignancy	variable	↓/N	↓/N	↑
Vitamin D overdosing	↑/N	↓/N	↑↑	↓/N
Granulomatous diseases	↑/N	↓/N	N (↑calcitriol)	↑
Milk-alkali syndrome	↑	↓/N	N	N
Thiazides	↑	↓/N	N	↓

From laboratory tests, the following may be useful:

S-Ca: including calculation of albumin-corrected calcium.

Ionized calcium (Ca^{2+}): confirms the diagnosis in unclear cases. Some patients with hyperparathyroidism have total Ca at normal, only Ca^{2+} is elevated.

S-P: in addition to secondary HPT, is reduced or normal in most cases of hypercalcemia.

PTH: Current diagnostic methods determine the biologically active form (PTH 1-84); in patients with renal insufficiency, other fragments (especially PTH 7-84) may accumulate in circulation and interfere with some assay methods. PTH 1-84 levels in patients with GF < 0.5 mL/s should be maintained within 2 – 9 times the reference interval for healthy subjects (KDIGO, 2009). Lower PTH values in these patients are associated with adynamic bone disease, while higher values indicate secondary or tertiary hyperparathyroidism with increased bone turnover.

Calcidiol (25-OH-vitamin D): the marker of vitamin D stores allows the diagnosis of deficiency and overdose. Determination of the level of active hormone calcitriol is justified only in selected situations, e.g. on confirmation of ectopic vitamin production in **granulomatous diseases.**

ALP: The isolated increase (without GMT) reflects increased bone activity in most cases.

Serum protein electrophoresis: is a simple test allowing the detection of monoclonal gammopathy, e.g. multiple myeloma, with pathological bone resorption.

FE-Ca: is a screening test to distinguish familial hypocalciuric hypercalcemia (FHH) from HPT; a value >0.01 indicates HPT, a value <0.01 is at >80% FHH.

PTHrP: not a routinely available test, its specificity for confirming the malignant origin of hypercalcemia is not absolute (∽20% of patients with malignant hypercalcemia have normal PTHrP), it is also present in healthy subjects (3%) and in primary hyperparathyroidism (6%).

While waiting for laboratory results, it is useful to check the list of drugs used, look for vitamin D and calcium in pharmaceutical and nutritional supplements, thiazide diuretics and lithium.

Increased Ca intake is often accompanied by metabolic alkalosis. The presence of PTHrP in cancer can only be assumed because the availability of specific laboratory methods for its determination is low (Figure 9.3).

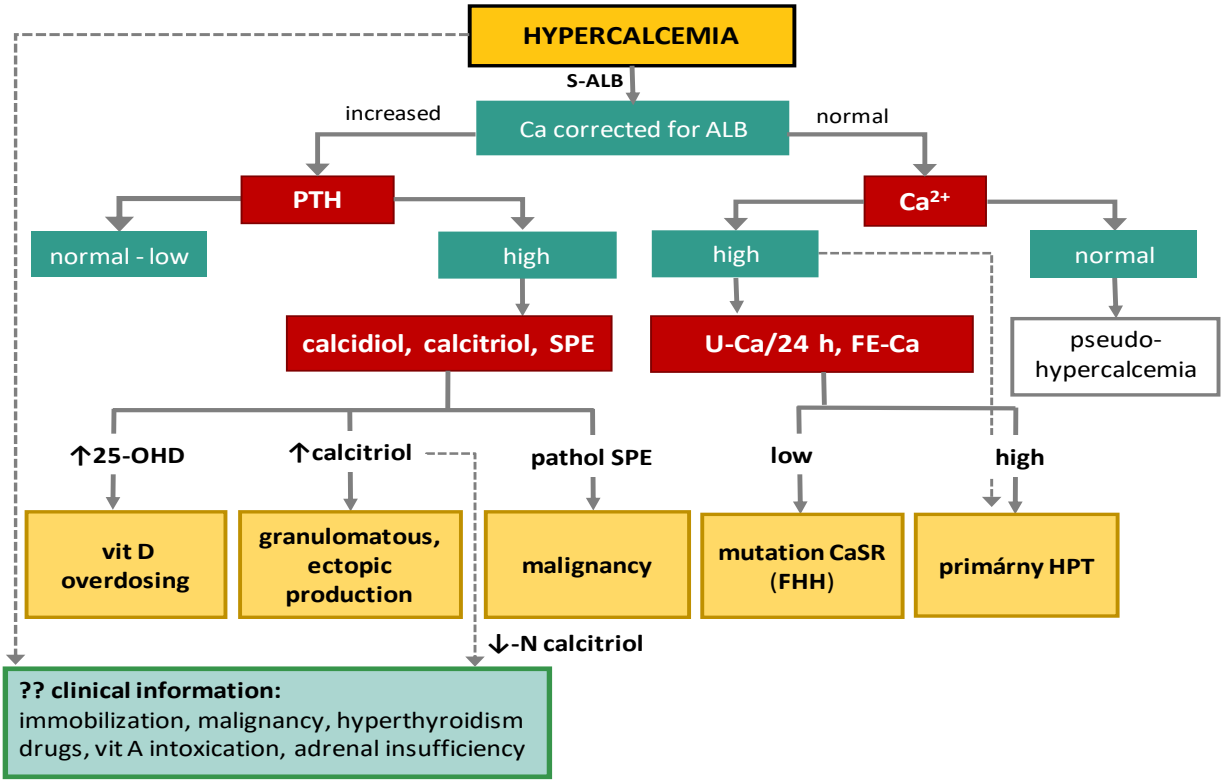


FIGURE 9.3 Simplified diagnostic algorithm in hypercalcemia

Hypocalcemia

Hypocalcemia, serum calcium level below the lower limit of the reference interval (<2.10 mmol/L), is a common finding in hospitalized patients, especially in the critically ill ones. Clinical manifestations of hypocalcemia are briefly described in INFO. 9.3.

INFO 9.3 Symptoms and signs of hypocalcemia

The main manifestation of hypocalcemia is increased neuromuscular excitability, which can range from numbness around the mouth and limbs to severe cramps and tetanus. The classic signs of latent tetany are the Chvostek´s symptom (tapping the facial nerve causes the facial muscles to contract) and the Trousseau symptom (inflating the pressure gauge cuff causes carpal spasm). Tetany can cause laryngospasm or bronchospasm with subsequent respiratory distress.

Cardiovascular symptoms include bradycardia, heart failure, and hypotension due to loss of vascular tension. An extended QT interval and T-wave inversion can be found on the ECG. Hypocalcemia causes resistance to digoxin.

Skin symptoms: Petechie due to damaged capillaries are a common sign of hypocalcemia.
Depression, memory loss and hallucinations are possible psychological manifestations of hypocalcemia.

In the first step, hypocalcemia due to hypoalbuminemia or due to contamination of the sample with anticoagulant additives (EDTA, citrate, oxalate) should be ruled out. Hypocalcemia (Table 9.5) is most frequently caused by one or more concomitant causes:

- reduced intestinal Ca absorption,
- increased bone mineralization,
- reduced bone resorption,
- increased urinary Ca excretion,
- redistribution or storage of Ca in tissues.

TABLE 9.5 CAUSES OF HYPOCALCEMIA

Mechanism	*Mechanism* and example
Hypoparathyroidism	*Increased renal loss + decreased bone resorption:* damage of parathyroid glands (during surgery or autoimmune), Mg deficiency, *Resistance of target organ to PTH:* pseudo-hypoparathyroidism
Hypoalbuminemia	Critically ill patients, nephrotic syndrome
Vitamin D deficiency	*Low 1-hydroxylation of calcidiol*: CKD *lack of sunlight*: various populations
Low intestinal absorption	Malabsorption syndrome Low calcium intake
Medicines	*Increased vitamin D metabolism in the liver*: phenytoin, theophylline, rifampicin, isoniazid
Tissue deposition	tumour lysis syndrome acute pancreatitis hungry bone syndrome
Artificial	*blood Ca chelation*: contamination or admixture of EDTA/citrate in blood – measurement of Ca in plasma, blood transfusion, haemodialysis and plasmapheresis

Vitamin D deficiency

A common cause of **hypocalcemia** is vitamin D deficiency, which results in low intestinal Ca resorption. The prevalence of vitamin D deficiency among healthy adults is almost 30%, among seniors in social facilities up to 40 – 100%.

The main causes of hypocalcemia due to vitamin D deficiency include:

- *nutritional deficiency* resulting from a lack of vitamin D in the diet, insufficient exposure to sunlight or a combination of both;
- *malabsorption of vitamin D* and Ca due to various GIT diseases including celiac disease, chronic pancreatitis and biliary obstruction, gastric or intestinal surgery;
- *kidney disease:* loss of α1-hydroxylase activity in the damaged kidney results in reduced conversion of 25-OH-D to calcitriol. Hyperphosphatemia also blocks the synthesis of the active hormone calcitriol. Hormonal and electrolyte imbalance predisposes patients with CKD to bone mineralization disorders (osteomalacia, osteoporosis), which is called renal bone disease (formerly renal osteodystrophy).

Hypoparathyroidism

Decreased PTH secretion is often caused by surgery or radiation in the neck area, which damages the parathyroid gland (usually temporarily). The major mechanism leading to

hypocalcemia in primary hypoparathyroidism is renal calcium loss. PTH release is a magnesium-dependent process, severe hypomagnesemia (<0.4 mmol/L) may suppress parathyroid reactivity to existing hypocalcemia and reduce PTH secretion (secondary functional hypoparathyroidism). Paradoxically, high concentrations of Mg, which acts as a Ca^{2+} antagonist on CaSR, also suppresses PTH secretion.

Redistribution and storage of calcium in tissues

Acute tissue Ca deposition occurs **in acute hyperphosphatemia**, as in tumour lysis syndrome and rhabdomyolysis. In **acute pancreatitis**, calcium binds to free fatty acids produced by increased lipase activity. **Hungry bone syndrome** is a specific case of bone deposition in patients with chronic hyperparathyroidism, which has left massive bone demineralization. After surgical removal of the parathyroid glands, the bone massively mineralizes the osteoid and draws tens of grams of Ca from the ECF at high speed.

The effect of **citrate** on calcium is manifested after blood transfusions, continuous hemodialysis treatment or plasmapheresis. Citrate in blood preparations and dialysis solutions chelates Ca in plasma, reduces the concentration of Ca^{2+} in particular, while total Ca may remain unchanged. Predisposing factors are advanced kidney and liver diseases that reduce citrate metabolism. Nephrotic syndrome is regularly associated with hypocalcemia (total and ionized Ca), mostly due to the presence of **hypoalbuminemia**, as well as the loss of vitamin D and its binding protein in the urine. Acute **respiratory alkalosis**, due to an increase in pH, causes an increase in the binding of calcium to albumin, which in turn leads to a decrease in its serum concentration.

Hypocalcemia in critically ill patients

The etiology of hypocalcemia in patients with severe disease is **multifactorial**. It includes disorders of PTH secretion and activity, vitamin D deficiency, drug side effects, citrate transfusions or the effect of circulating catecholamines. Differential diagnosis of hypocalcemia in this group of patients is complicated by problems in the interpretation of total Ca concentration in hypoalbuminemia and acid-base imbalances. Ca^{2+} determination is needed to assess the situation. According to some authors, hypocalcemia in critically ill patients represents an adaptive, beneficial response of the organism, which prevents the increase of intracellular Ca and subsequent damage to cells and tissues.

Laboratory tests in hypocalcemia

The following biochemical tests may be useful in the diagnosis and differentiation of hypocalcemia (Figure 9.4):

S-Ca: including calculation of the value corrected for normal serum albumin, where a value of corrected Ca <2.1 mmol/L confirms hypocalcemia.

Creatinine and GFR estimation: allow the diagnosis of CKD, which is a common cause of hypocalcemia.

Ionized calcium (Ca^{2+}): confirms the diagnosis in controversial cases and especially in patients with hypoalbuminemia.

PTH: Low PTH values are found in hypocalcemia due to Mg deficiency or, paradoxically, in hypermagnesemia and idiopathic hypocalcemia. Increased PTH values associated with hypocalcemia may signal vitamin D deficiency, CKD or pseudohypoparathyroidism.

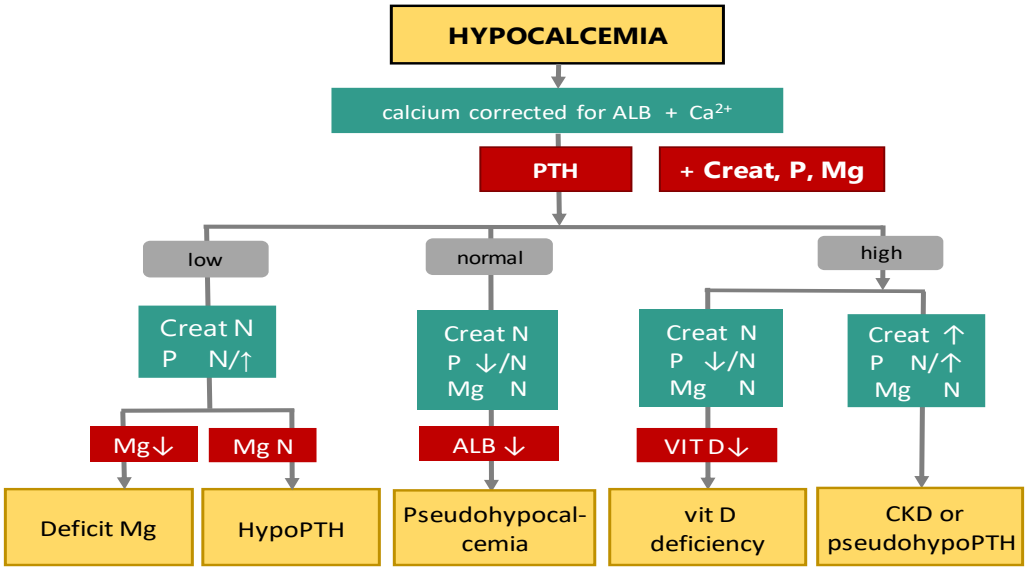


FIGURE 9.4 Simplified diagnostic algorithm in hypocalcemia

S-Mg: Mg deficiency causes functional hypoparathyroidism due to a reduced CaSR response to Ca levels.

S-P: Elevated serum P level associated with hypocalcemia is a typical marker of cell necrosis (e.g. tumour lysis syndrome, rhabdomyolysis) or precipitation of Ca-P in soft tissues.

Calcidiol (25-OH vitamin D): low values (<20 pg/L or <50 nmol/L) confirm vitamin D deficiency.

1,25-(OH) 2D (calcitriol): the test is justified in some cases in patients with advanced-stage CKD and suspected congenital genetic diseases with low levels of 1,25- (OH) 2D, such as vitamin D-dependent rickets type 1 (1α-hydroxylase gene mutation) or hypophosphatemic rickets (mutations increasing the level of FGF23).

9.3 Disorders of phosphate balance

Physiological functions of phosphates

Most phosphorus in the body exists in the form of phosphates. About 85% of phosphates are found in bones, where hydroxyapatite is an important component. In soft tissues, phosphates are found in the intracellular compartment bound in numerous organic compounds, including nucleic acids and phospholipids of cell membranes. Phosphates in the form of energy rich compounds (e.g. ATP, ADP) are part of the energy metabolism of cells (oxidative phosphorylation) and 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes plays a key role in the release of O_2 in tissues. Only a minor part of intracellular phosphorus occurs in the form of inorganic phosphates, which at physiological pH occur mainly in the form of hydrogen phosphates $(HPO_4)^{2-}$ and dihydrogen phosphates $(H_2PO_4)^-$. In this form, phosphates represent

an important intracellular and urinary buffer, which is essential as an H^+ acceptor during its excretion by the kidneys.

Only about 1% of phosphorus is found in the ECF (Figure 9.5). In serum/plasma, P exists predominantly in the form of inorganic phosphates (>80%), which are determined by biochemical examination. The physiological serum concentration of phosphorus (S-P) in adults varies in the range of 0.75 – 1.45 mmol/L, in children in the period of growth the values are 30 – 50% higher.

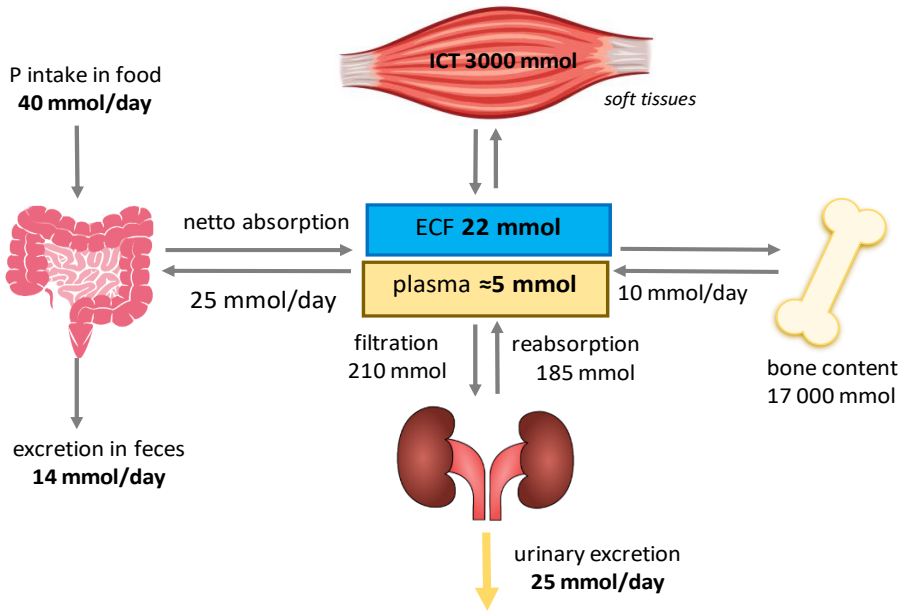


FIGURE 9.5
Approximate daily turnover of phosphorus in the body

Hyperphosphatemia

Increased serum P concentration is usually due to increased intake, decreased renal excretion, and redistribution from ICF to ECF. Acute and chronic renal failure is the most common cause of hyperphosphatemia, and some degree of renal dysfunction is often combined with other possible causes of hyperphosphatemia (Table 9.6).

TABLE 9.6 CAUSES OF HYPERPHOSPHATEMIA

Mechanism	Example
High intake	P containing laxatives and enemas, I.V. therapy, vitamin D overdosing, bisphosphonates
Low renal excretion	acute/chronic kidney failure (GFR decrease bellow 0.5 mL/s is followed by increase in S-P)
High tubular reabsorption	hypoparathyroidism (see hypocalcemia) pseudo-hypoparathyroidism hyperthyroidism
Release from cells (necrosis or shift)	Tumour lysis syndrome, rhabdomyolysis, burns, intravascular hemolysis, catabolic conditions DKA - low glycolysis and decreased intracellular P utilisation
Mixed	Acromegaly (↑activity of α1-hydroxylase, ↑ production of calcitriol)
Pseudo hyperphosphatemia	hypergammaglobulinemia *(multiple myeloma)*, hemolysis, severe lipemia, delayed separation of serum from coagulum

Clinical manifestations of hyperphosphatemia are usually caused by secondary hypocalcemia, which is a consequence of Ca precipitation with phosphorus. They belong here:

- paresthesia, muscle cramps, tetany,
- calcifications of soft tissues, including smooth muscles in blood vessels,
- renal mineral and bone disease (renal osteodystrophy) - a disorder of bone mineralization caused by hormonal and electrolyte imbalance in CKD (INFO 9.4).

INFO 9.4 Phosphorus and kidney

Hyperphosphatemia is a typical finding in advanced stages of CKD. As GFR decreases, bone mineral homeostasis progressively worsens and changes in PTH, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and FGF23 levels occur. Bone disorder in CKD can take the form of low-turnover adynamic disease to osteitis fibrosa cystica due to increased bone turnover (diagnosis is biopsy). Impaired mineral metabolism also affects extra-osseous tissues (calcifications in blood vessels and soft tissues), so the term renal mineral and bone disease (CKD-MBD) is used to express mineral, bone, hormonal and cardiovascular abnormalities in patients with CKD.

According to the recommendations of KDIGO (Kidney Disease: Improving Global Outcomes), serum concentrations of Ca, P, PTH and 25-hydroxyvitamin D, are regularly monitored in patients with CKD. If PTH levels progressively rise and persist above the upper limit of RI, treatment is initiated to maintain serum Ca and P levels at the reference interval in patients in the pre-dialysis period (stages 3-5).

Hypophosphatemia

Hypophosphatemia is less common compared to hyperphosphatemia, but has more serious clinical consequences (Table 9.7). **Reduced dietary P intake** or reduced intestinal absorption rarely results in hypophosphatemia because the proximal renal tubules can reabsorb almost 100% of the filtered phosphate and compensate for extracellular loss or decreased intake.

TABLE 9.7 CAUSES OF HYPOPHOSPHATEMIA

Mechanism	Example
Low intake	Long-term starvation, malabsorption, diarrhoea Vitamin D deficiency Phosphate binders (Ca-acetate, sevelamer) Antacids containing Ca, Mg
Increased bone mineralization/low bone resorption	Primary or secondary hyperparathyroidism Hungry bone syndrome Vitamin D deficiency/ peripheral organs resistance
Urinary losses of phosphates	Hyperparathyroidism, diuretic therapy, Tubulopathies *inherited* (Fanconi syndrome, hereditary fructose intolerance, vitamin D resistant rickets) or *acqiured* (myeloma, T2DM, Wilson disease), kidney transplantation (↑FGF23)
Shift to cells	Treatment of DKA or HHS Refeeding syndrome Acute RAL - salicylates overdosing, artificial ventilation,
Mixed	Chronic alcohol abuse (low intake + vitamin D deficiency)

Numerous congenital defects of tubular function cause hypophosphatemia by a mechanism of insufficient phosphate reabsorption, along with their **urinary losses**. Increased P **shift to the cells** may cause hypophosphatemia in individuals with reduced intracellular P stores who pass from catabolic to anabolic phase of the disease. Examples are patients with DKA treated with insulin (loss of P during osmotic diuresis before treatment) or with refeeding syndrome.

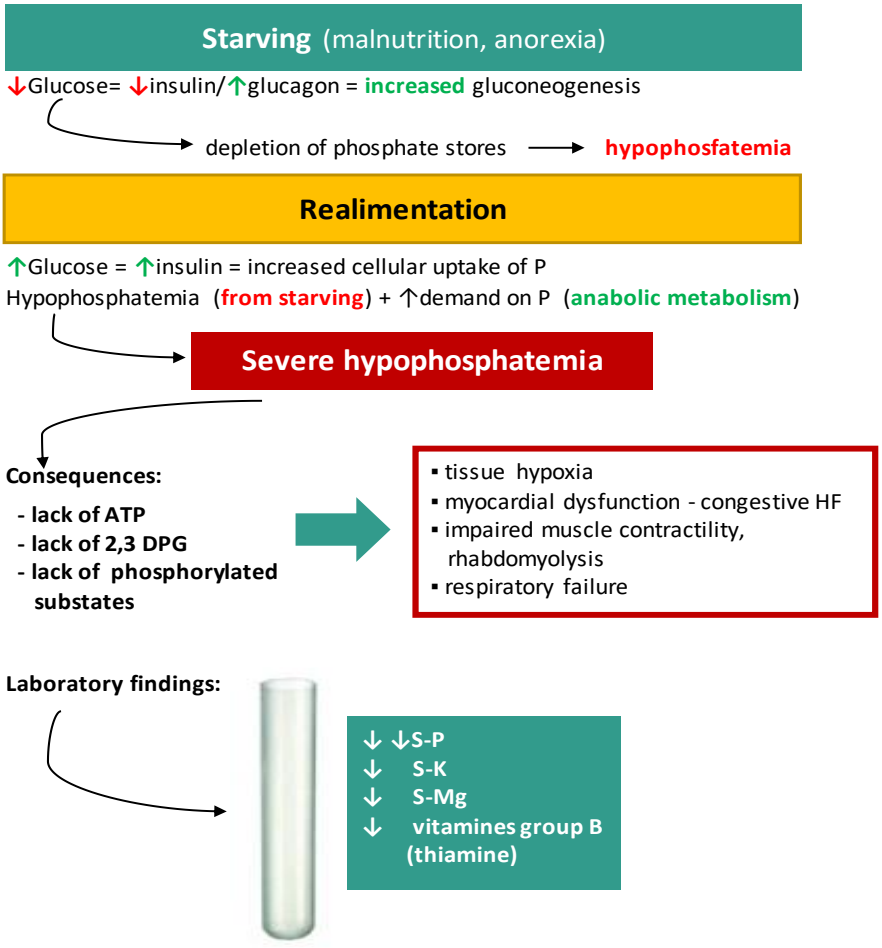


FIGURE 9.6 Refeeding syndrome

Refeeding syndrome is a term for clinical manifestations caused by electrolyte and water balance disorders, which usually occur 3 – 4 days after starting re-alimentation in patients with previous malnutrition or fasting (Figure 9.6). Typical laboratory findings are hypophosphatemia, hypokalemia, and hypomagnesemia, which arise as a result of depletion and rapid transfer of these ions from the ECF to the cells. The causative factor is the administration of carbohydrates with subsequent release of insulin, which intensively promotes anabolic processes in cells, where severe starvation or malnutrition has caused severe ionic and vitamin deficiency (B vitamins are cofactors of many enzymes).

Intracellular phosphate deficiency has adverse consequences for the function of all organ systems, as it is essential for the integrity of cell membranes, for energy recovery (ATP), is part of the enzyme cascades involved in intracellular signalling, is the most important urinary buffer and affects hemoglobin binding with oxygen (2,3-DPG). Serum P <0.3 mmol/L is associated with generalized cell dysfunction, which may be the cause of cardiorespiratory failure.

Laboratory diagnostics of hypophosphatemia

A detailed personal history and physical examination of the patient help to detect hypophosphatemia and find the indications needed to reveal the cause. Clinical signs in many patients are absent or non-specific, usually appear at serum phosphate level below 0.5–0.4 mmol/L, and are caused by the following mechanisms:

- **Increased bone degradation**, which results in bone demineralization, in children leads to rickets, bone changes and bone pain;
- **Decreased intracellular ATP** supply, which causes disorders of muscle contractility (generalized muscle weakness, paresthesia, swallowing disorders, dysarthria, ileus, respiratory and heart failure), metabolic encephalopathy (disorders of consciousness, coma), increased rigidity of the Era (tendency to hemolysis) and granulocyte dysfunction (phagocytosis and chemotaxis);
- **Reduced content of 2,3-DPG in RBC**, which increases the affinity of hemoglobin for oxygen and leads to its reduced release in tissues with subsequent ischemia.

Laboratory testing includes, in addition to the basic metabolic panel (Ca, P, glycemia, creatinine, eGFR), also PTH, vitamin D and fractional excretion o - FE-PO_4 (Table 9.8).

TABLE 9.8 LABORATOTRY FINDINGS IN DIFFERENT TYPES OF HYPOPHOSPHATEMIA

Diagnosis	S-Ca	S-PTH	ALP	VIT D	FE-PO4	U-Ca
Hyperparathyroidism	↑	↑↑	N-↑	N	↑	Variable[a]
Renal hypophosphatemia	↑	N-↑	↑	N	↑	↓
Fanconi syndrome[b]	N-↓	N-↑	N-↑	N	↑	↑
Malabsorption, malnutrition	N-↓	N-↑	N-↑	↓	↓	↓
Refeeding syndrome	↓	N	N	↓	↓	↓

[a] depends on severity of hyperparathyroidism, [b] urinary losses of other ions, glucose and amino acids

9.4 Magnesium balance disorders

Basic physiology

The human body contains about 1 000 mmol of magnesium (Mg); 60% of this is bound in bone to hydroxyapatite crystals, almost 40% is in muscle and soft tissue cells and only about 1% is in the extracellular fluid (Figure 9.7). Mg is the second most important intracellular cation after potassium, found mainly in mitochondria (60%), where it is necessary for ATP activation. Mg is a cofactor of more than 300 enzymes, which catalyze mainly reactions using ATP (e.g. adenylate cyclase, Na^+/ K^+ ATPase, glycolysis reactions, oxidative phosphorylation, nucleotide metabolism, proteosynthesis). It acts as a natural calcium channel blocker, that inhibits Ca influx into cells, and is involved in the regulation of smooth muscle tension. Mg also modulates parathyroid function through increased upregulation of key cellular receptors (CaSR, vitamin D receptor - VDR and FGF23/Klotho system).

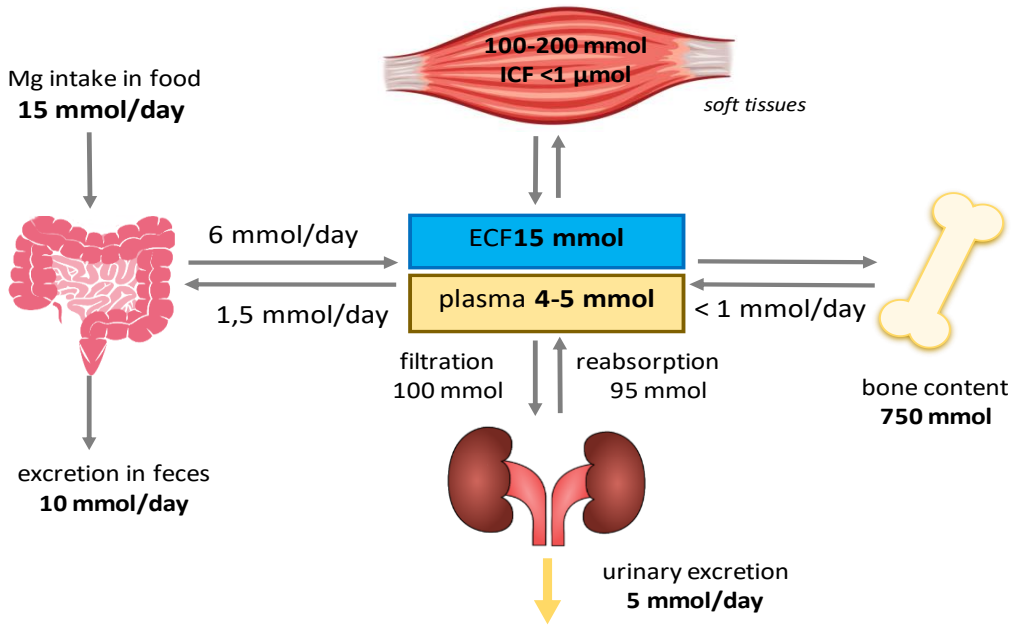


FIGURE 9.7
Approximate magnesium turnover in the body

Magnesium occurs in serum in three different forms: protein-bound (30%), free ionized (60%) or bound in complexes with anions, e.g. phosphate or citrate (10%). The physiological concentration of total magnesium in serum (S-Mg) is kept in a narrow range of 0.7–1.1 mmol/L by the interaction of three organ systems - intestine, kidneys and bones. Since only 1% of Mg is found in the ECF, serum magnesium level is a poor indicator of the body's total Mg stores. Maintaining magnesium balance differs from other ions in the following respects:

1. **Bone**, as the main reservoir of magnesium, does not exchange Mg quickly with ECF, so in the case of a negative Mg balance, the normalization of its ECF level takes several weeks. Hormones such as glucagon, adrenaline and PTH partially mobilize the release of Mg from bones and other tissues.
2. In **the small intestine**, the absorption of calcium and magnesium influences each other. High intake of Ca reduces the absorption of Mg and low intake of Mg may increase the absorption of Ca. PTH and glucocorticoids increase the intestinal absorption of Mg, which physiologically represents about 40% of the intake.
3. More than 95% of Mg is reabsorbed in the **renal tubules** (FE-Mg is 0.03 – 0.05 or 3 – 5%). Renal reabsorption of Mg is only minimally affected by hormones; PTH increases it slightly. Factors that affect the processing of Mg by renal tubules include:
   a. hypervolemia (ECF expansion) increases the excretion of Ca, Na and Mg; hypermagnesemia and hypercalcemia and hyperaldosteronism have the same effect;
   b. the decrease in GF increases the compensatory FE-Mg to maintain the normal for serum Mg concentration as long as possible;
4. **Chronic MAC** results in urinary Mg loss, while chronic MAL has the opposite effect.

Hypomagnesemia

Hypomagnesemia is defined as a serum magnesium concentration below the low limit of reference range (<0.7 mmol/L) and generally indicates an intracellular Mg deficiency. It occurs

in 10 – 20% of all hospitalized patients, in approximately 50% of intensive care unit patients, and almost 70% of individuals with chronic alcohol intake. It can be caused by decreased intake, increased renal and gastrointestinal losses and redistribution of Mg from ECF to ICF (Table 9.9).

TABLE 9.9 FREQUENT CAUSES OF HYPOMAGNESEMIA

Mechanism	Example
Low intake and absorption	Low Mg content in food, malabsorption, starvation, parenteral nutrition, alcoholism
Gastrointestinal losses	diarrhoea, laxative abuse, vomiting, nasogastric suction, fistulas, resection of intestine, PPI
Renal losses - tubular disorders - drugs	Hyperaldosteronism, Gitelmann and Bartter syndromes, RTA, other inherited tubulopathies, diuretics (loop, osmotic, long-term use of thiazides) aminoglycosides, amphotericin B, anticancer drugs (cisplatin, cetuximab) immunosuppressive drugs (tacrolimus, cyclosporine)
Transcellular shift - uptake by cells	treatment of DKA and HHS refeeding syndrome hungry bone syndrome acute pancreatitis (binding of Mg in necrotic fat tissue)
Mixed	pregnancy - high demand of the body + hypervolemia)

The clinical signs of magnesium deficiency are non-specific and usually correlate with the co-existing hypokalemia and hypocalcemia. The critical hypomagnesemia is <0.5 mmol/L and is usually accompanied by the following findings:

- neuromuscular: muscle weakness, tremors, muscle spasms to tetany, convulsions, swallowing disorders;
- cardiovascular: atrial and ventricular arrhythmias, atrial fibrillation, ECG changes (prolonged PR interval and broad QRS complexes);
- metabolic: hypomagnesemia can be combined with other ionic disorders, especially hypocalcemia and hypokalemia, therefore it is appropriate to determine all three ions together.

Calcium competes with magnesium for uptake in the Henle loop, the increased Ca filter charge impairs Mg reabsorption. Besides, hypomagnesemia causes a decreased response of CaSR and decreased PTH secretion (functional hypoparathyroidism), peripheral resistance to both PTH and vitamin D, leading to hypocalcemia. Hypokalemia is common in patients with hypomagnesemia, mainly due to the common underlying causes of both ionic disorders. However, there is evidence that hypomagnesemia may increase renal potassium loss (INFO 9.5).

Laboratory tests in hypomagnesemia

Laboratory tests for hypomagnesemia S-Mg: Low concentration confirms magnesium deficiency, normal concentration, however, does not rule out a deficit. S-Alb, CB: Hypoalbuminemic conditions are accompanied by low concentrations of S-Mg, with a reduced protein-bound fraction. FE-Mg: A fractional excretion >3% in an individual with normal renal function signals probable renal losses of Mg, while FE-Mg values <1% indicate extrarenal

causes of hypomagnesemia. S-K, S-Ca: The finding of unexplained hypocalcemia or refractory hypokalemia in a patient with suspected Mg losses (personal history) may also indicate magnesium deficiency in a patient with normal S-Mg.

INFO 9.5 Further diagnostic steps in hypomagnesemia

- Look for medication and alcoholism.
- Stool microbiological and immunological tests for celiac disease may explain the cause of diarrhoea.
- Increased inflammatory markers in serum (CRP) and feces (calprotectin) signal inflammatory bowel disease.
- Low levels of serum iron or vitamins B12, D and folate may signal malabsorption syndrome.
- Elevated INR indicates a possible vitamin K deficiency.
- Increased serum lipase or amylase activity are markers of possible acute pancreatitis. USG or CT scan of the abdomen reveals signs of chronic pancreatitis.
- Primary hyperaldosteronism may cause increased urinary Mg and K loss.
- Low or unmeasurable PTH levels in the presence of hypocalcemia indicate hypoparathyroidism.
- Low S-P combined with hypomagnesemia and hypocalcemia may indicate a history of hungry bone syndrome
- Genetic testing confirms the presence of inherited tubular defects associated with Mg urine loss.

Hypermagnesemia

Serum Mg levels above the upper limit of normal (>1.1 mmol/L) is less common than hypomagnesemia. It arises, for example, as a result of increased intake, decreased renal excretion and release of Mg from cells, which often combine (Table 9.10).

TABLE 9.10 CAUSES OF HYPERMAGNESEMIA

Mechanism	Example
High intake	oral, parenteral (therapy of preeclampsia, Mg containing antacids and laxatives)
Low renal excretion	Acute /chronic kidney failure in oliguric phase hypoaldosteronisms, Addison´s disease hypothyroidism therapy with lithium
Transcellular shift – release from cells	Cell necrosis (e.g. rhabdomyolysis, severe burns, tumour lysis sy.) diabetic ketoacidosis hypoxic damage of cells (sepsis, shock, etc.)

Clinical symptoms of hypermagnesemia are usually not present at S-Mg up to 2 mmol/L, but may occur earlier with concomitant hypercalcemia, hyperkalemia or uremia:

- **Neuromuscular** - increased S-Mg antagonizes the effects of calcium in the body, blocks neuromuscular conduction and leads to the weakening or disappearance of deep tendon reflexes (>3 mmol/L), facial twitching and muscle weakness. Clinical symptoms and signs of severe hypermagnesemia (>5 mmol/L) include respiratory depression, respiratory failure, and coma;

- **Cardiovascular** - slow conduction of stimuli in the heart and on the sympathetic ganglia results in bradycardia up to cardiac arrest (>5 mmol/L), vasodilation leads to hypotension;
- **Hypocalcemia** is the result of decreased PTH secretion or target organ resistance to the effects of PTH;
- **Coagulation disorders** in hypermagnesemia results from Mg interference with platelet adhesion and thrombin production.

Laboratory findings in hypermagnesemia

S-Mg: Elevated serum concentrations are a rarely isolated laboratory finding, hyperkalemia and hypercalcemia often occur simultaneously.

S-Creatinine and estimated GFR allows the assessment of renal function; S-Mg increases with decreasing GFR below 0.5 mL/s.

ABR may reveal respiratory acidosis present due to respiratory depression.

9.5 Biochemical bone markers

The basic functions of bones in the body include providing mechanical support for muscles and organs of the body, creating space for the bone marrow, supplying calcium and phosphorus (including influencing their metabolism) and participating in the regulation of ABR as a source of HCO_3^- and HPO_3^{2-}. Bone tissue is subject to constant renewal or remodelling throughout life depending on changing conditions (e.g. load, damage repair). Bone metabolism involves several cell types (osteoblasts, osteoclasts, osteocytes) as well as extracellular bone mass, which consists of proteins (type I collagen and non-collagen proteins) and the bone mineral hydroxyapatite.

The process of continuous bone remodeling (Figure 9.8) consists of removing old bone by osteoclasts and replacing it with new bone mass (osteoid) created by osteoblasts, which is then passively mineralized. The part of the bone in which the remodeling cycle takes place is called the bone multicellular unit (BMU). About one million remodeling units are currently active in an adult. Many hormones (PTH, oestrogens, androgens, growth hormone via IGF-1, IGF-2, cortisol, insulin) as well as local regulatory factors (osteoprotegerin, RANKL, sclerostin, etc.) are involved in the remodeling, the calculation and description of the effect of which is beyond the scope of this textbook.

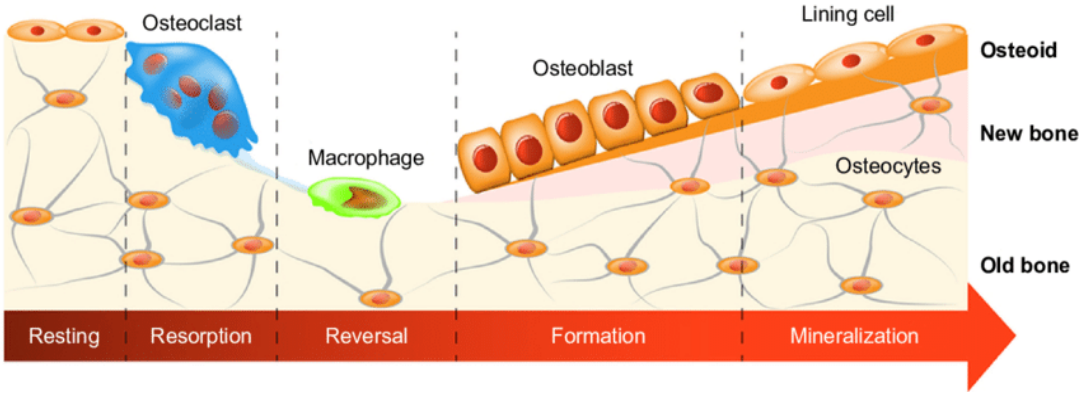


FIGURE 9.8 Scheme of the bone remodeling process
source: https://www.researchgate.net/publication/325824066

Assessment of bone resorption and neoplasia activity is enabled by bone markers - biochemical products of bone metabolism, which can be measured in blood or urine (Table 9.11), whereby:

- **bone formation** markers reflect bone matrix protein synthesis or osteoblast enzymatic activity,
- **bone resorption** markers inform about the intensity of bone protein degradation or osteoclast activity,
- markers of **osteocyte activity** are not yet used routinely, only in research (e.g. RANKL, sclerostin, FGF23).

TABLE 9.11 THE MOST FREQUENTLY USED BONE MARKERS

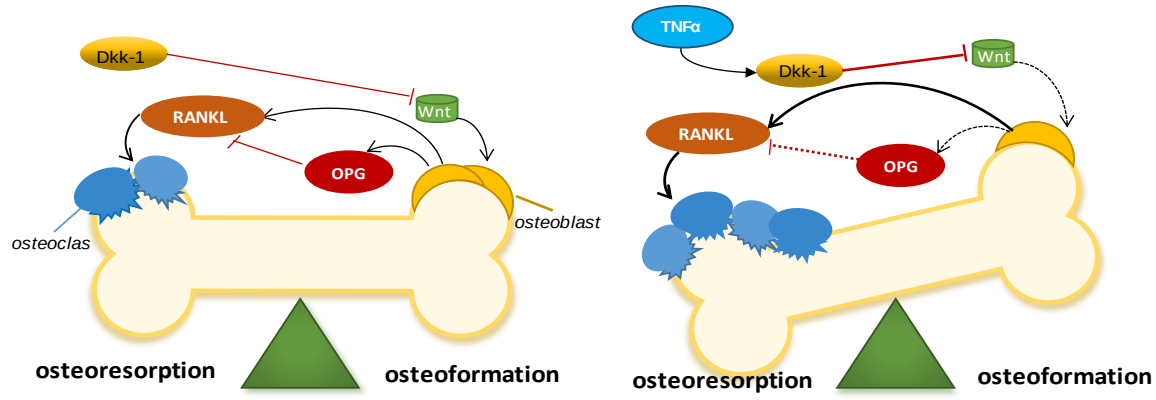
Bone formation markers	Bone resorption markers
P1NP – N-terminal procollagen type 1 propeptide P1CP – C-terminal procollagen type 1 propeptide BALP – bone isoenzyme ALP Osteocalcin – marker of bone turnover	CTX – C-terminal cross-linking telopeptide of collagen type 1 NTX – N-terminal cross-linking telopeptide of collagen type 1 U-pyrridinoline

The basic indications for the examination of laboratory bone markers in practice include:

- differential diagnosis of secondary metabolic bone diseases,
- monitoring the effect of treatment,
- prediction of fractures,
- monitoring of patients after ending or interruption of long-term antiresorptive therapy - determination of the length of the pause in treatment (drug holiday).

At least one marker of osteosynthesis (P1NP - procollagen type 1 N-terminal propeptide) and osteoresorption (CTX - cross-linking telopeptide of collagen type 1) is recommended by international professional societies. When interpreting laboratory bone markers, the physician considers the balance between resorption and neoplasia bone turnover, but also the absolute rate of bone turnover (increased, normal-ranged, and decreased), always in the context of the patient's current clinical condition and treatment.

The balance between bone resorption and formation of bone may be a physiological condition in adults, but may also occur in a situation with reduced or increased bone turnover (Figure 9.9). Bone turnover is higher during pregnancy, lactation, weight loss, and immobilization. Lower bone turnover has been found during the period of involution, in old age and during treatment with bisphosphonates. Advantages of determining bone markers include availability, non-invasive collection, and faster response to treatment than a change in bone mineral density (BMD). The disadvantages are high biological variability and significant circadian rhythm, influence age and gender, interference with collagen turnover in other tissues (skin, cartilage).



Balance between osteoresorption and osteoformation: bone turnover can be normal, high, or low

Osteoresorption dominates over osteoformation-bone loss: immobilization, estrogen deficiency, corticoids, osteolytic metastases etc.

FIGURE 9.9 Schematic representation of balanced and unbalanced bone remodelling
RANKL – receptor activator of nuclear factor kappa-B ligand, OPG – osteoprotegerin, Wnt – a group of signal transduction pathways necessary for cells proliferation, Dkk-1 – inhibitor Wnt

Case studies and self-assessment questions

Case report 9.1

A 60-year-old woman is examined by a specialist for several weeks of muscle weakness of the upper and lower limbs, fatigue, thirst and polyuria (urination even at night). She returns repeatedly for the last two days. Her GP ruled out diabetes mellitus 2 weeks ago. Physical examination revealed a blood pressure of 90/60 mm Hg and a lower skin turgor. Current biochemical findings in the blood are in the table.

Serum	Result	RI
Na^+	155	135 – 145 mmol/L
K^+	3.6	3.6 – 5.3 mmol/L
Urea	15	2.2 – 8.0 mmol/L
Creatinine	119	44 – 90 µmol/L
Ca	2.9	2.1 – 2.70 mmol/L
Alb	31	36 – 50 g/L

Questions:

a. What is the total calcium corrected for albumin?
b. What is the probable diagnosis?
c. What other tests could be useful?
d. What would you address first in the patient's treatment?

Case report 9.2

A 65-year-old man, a well-known chronic alcoholic, was brought in by police for generalized seizures that began an hour ago, shortly after his arrest for vagrancy. The patient reports diarrhoea and muscle cramps of the lower limbs for several days; he is allegedly not taking any medication. Positive findings on physical examination: patient lethargic but oriented, blood pressure 110/70 mm Hg, pulse 100/min, palpable rigid

edge of the liver +5 cm, increased reflexes on DK, intermittent carpopedal spasms and the present Chvostek symptom. Laboratory findings are in the table.

Serum	Result	RI
Glucose	3.9	3.3 – 5.5 mmol/L
Creatinine	115	55 – 100 µmol/L
Na^+	134	135 – 145 mmol/L
K^+	2.7	3.6 – 5.3 mmol/L
Ca	2.2	2.1 – 2.70 mmol/L
Mg	0.5	0.7 – 1.1 mmol/L
P	0.9	0.8 – 1.45 mmol/L
Alb	32	36 – 50 g/L
Hb	120	120 – 160 g/L
WBC	9.5	3.8 – 10 × 10^9/L

Questions:

a. What is the possible cause of the patient's seizures?
b. What ionic abnormalities are common in chronic alcoholism?
c. What are the possible causes of hypomagnesemia?

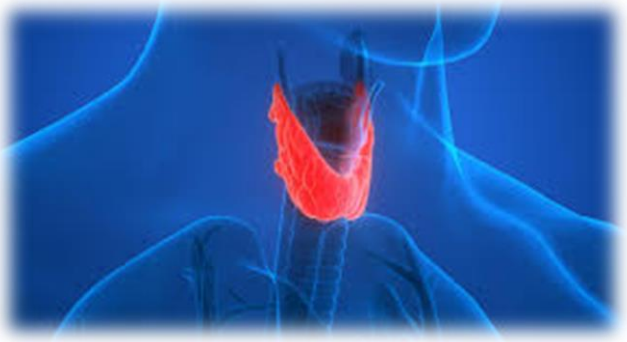
Self-assessing questions

1. Explain the effect of Ca^{2+} on PTH secretion.
2. Calculate calcium corrected for albumin in a patient with S-Alb of 58 g/L and total S-Ca 2.1 mmol/L.
3. What is the effect of PTH and calcitriol on serum Ca and P concentrations?
4. Which drugs could be useful in treatment a patient with hypercalcemia and why?
5. Why is the use of an EDTA tube not suitable for the measurement of calcium?
6. Why do we often detect hyperphosphatemia in the laboratory in a hemolyzed sample?
7. Explain the development of hypophosphatemia in a diabetic patient during DKA treatment.
8. What is the most common cause of hypermagnesemia?
9. Why do patients with hypomagnesemia often have hypocalcemia at the same time?

KEY INFORMATION

- ☑ The metabolism of calcium, phosphorus and magnesium is interconnected.
- ☑ Serum Ca and P concentrations result from the cooperation of kidney, intestine and bone, as well as the influence of regulatory hormones, especially PTH, calcitriol and FGF23 / Klotho.
- ☑ Calcium is quantitatively the most abundant mineral in the body, with 99% found in the bones.
- ☑ PTH is a key regulator of blood Ca^{2+} levels. Calcitriol is necessary for the absorption of Ca and P in the small intestine and to ensure their long-term concentration in the blood.
- ☑ Clinical manifestation of hypocalcemia is paresthesia, convulsions and osteomalacia. Causes of hypocalcemia involve reduced food intake, vitamin D deficiency, chronic kidney disease, malabsorption, some drugs (e.g. loop diuretics) and hypoparathyroidism.
- ☑ Common causes of true hypercalcemia (increased Ca^{2+}) are primary hyperparathyroidism, malignancies, some drugs (e.g. thiazide diuretics, vitamin D overdose).
- ☑ Changes in S-Alb concentration affect the concentration of total S-Ca, but do not affect ionized calcium.
- ☑ Ionized calcium is a biologically active form and changes in its concentration are the cause of clinical manifestations in the patient. It should be examined if hypocalcemia (paresthesia, convulsions, etc.) is suspected, even in patients with hyperparathyroidism (total Ca may be normal).
- ☑ Hyperphosphatemia is common in patients with CKD and can result in severe vital dysfunction to death.
- ☑ Hypomagnesemia is a manifestation of a lack of magnesium in the body. It is often associated with a deficiency of other ions and causes a disorder of neuromuscular signalling.
- ☑ Hypermagnesemia is normally rare condition, most often due to excessive enteral or parenteral Mg intake. Acute or chronic renal failure is a common cause.
- ☑ Some disorders of bone mineralization (biochemical bone diseases) including rickets, osteomalacia, renal bone disease, are associated with abnormalities in serum and urinary Ca and P levels. Others, like osteoporosis or Paget´s disease, have findings normal concentrations of serum calcium and phosphorus.

10



Disorders of thyroid function

CONTENT

Thyroid disorders make up 90% of all endocrine diseases, affecting an average of 5% of women and 1% of men in EU countries. The incidence of thyroid disorders slightly increases with age (especially hypothyroidism); prevalence over 60 years in women reaches up to 10 – 15%. There are two types of disorders in thyroid disease, which are often combined: a pathological production of hormones and morphological changes in the gland. Most functional disorders of the thyroid gland are primary, caused by the pathology of the gland itself.

The laboratory examination is always preceded by a clinical examination (history and physical findings), which determines a combination of suitable laboratory tests according to the presumed thyroid dysfunction. Increased and decreased thyroid function (hyperthyroidism, hypothyroidism) occur in both manifest and subclinical forms. In most cases, it is a lifelong illness requiring regular medical and laboratory examinations.

10.1 Basic physiology

Thyroid hormones are essential for normal cell growth, development and metabolism. The thyroid gland produces the hormones thyroxine (T4) and triiodothyronine (T3). The synthesis of T4 and T3 takes place inside follicles that contain a high concentration of thyroglobulin (TG). It serves as a donor of tyrosine residues, which are enriched in iodine by thyroid peroxidase, then condensed into T3 and T4 and, upon release from TG, are incorporated back into thyrocytes by endocytosis (Figure 10.1). The natural ratio of T4 and T3 hormones secreted into the circulation is approximately 20 to 1. The thyroid gland, as the only endocrine organ, makes hormones in supply. The released hormones in the blood bind to transport proteins (70% to thyroxine-binding globulin -TBG, 30% to prealbumin and albumin).

Only a small fraction of the total amount are biologically active free hormones (0.03% fT4 and 0.2% fT3) that enter cells and interact with their receptors. Enzyme conversion of T4 to biologically active T3 by several selenium-dependent deiodases takes place in peripheral tissues, with inactive forms of reverse rT3 and rT2 also being by-products. About 15% of T3 is produced directly by the thyroid gland and the remainder is formed from T4 at the periphery.

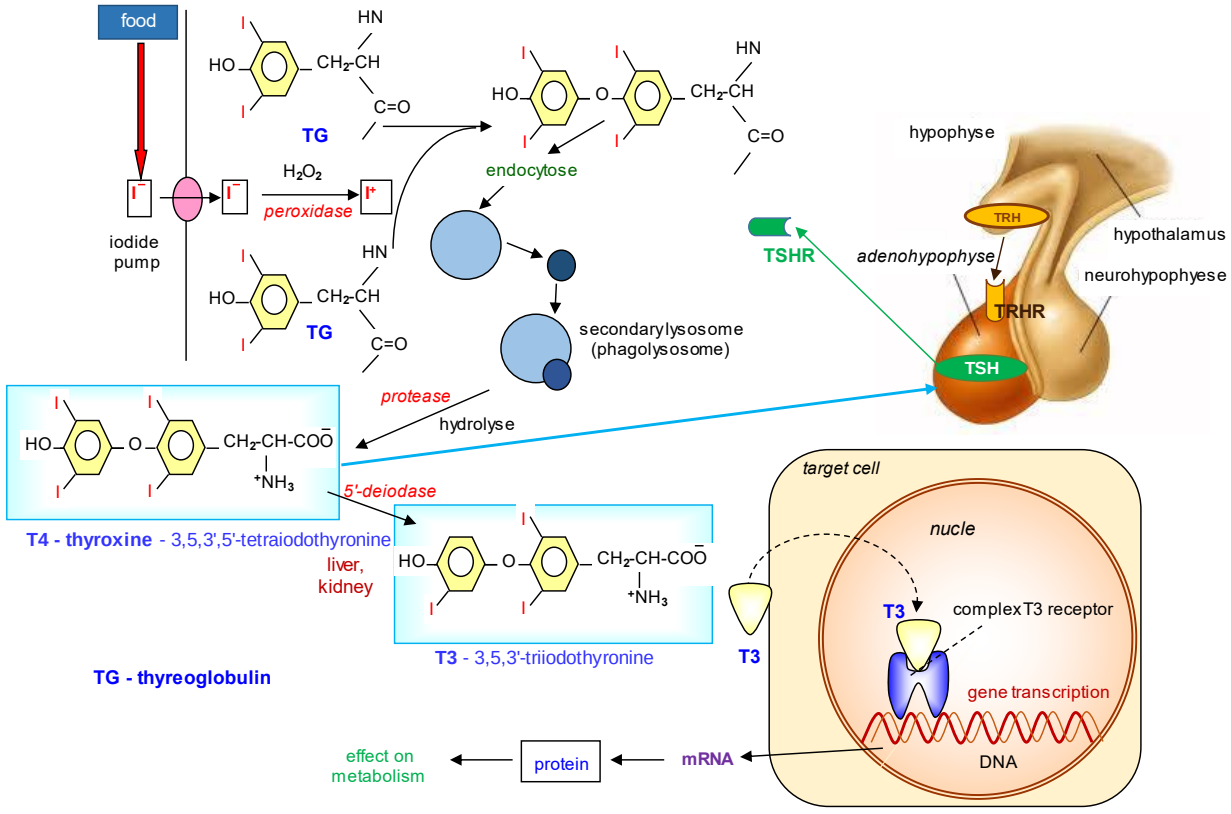


FIGURE 10.1 Biosynthesis, secretion, and mechanism of action of thyroid hormones
TSHR: thyroid stimulating hormone receptor

The synthesis of thyroid hormones is regulated by thyroid stimulating hormone (TSH), the production and secretion of which from the adenohypophysis is controlled mainly by the feedback mechanism between the level of fT4, fT3 in the blood and the hypothalamic tripeptide TRH (thyroliberin). In addition, TRH secretion is also stimulated by stress, cold, leptin, adrenergic receptors, heat, dopaminergic and opioid receptor pathways.

The biological effect of TSH also depends on post-translational glycation, so there is no correlation between TSH concentration and clinical manifestations, especially in central thyroid dysfunction. Upon binding to a specific receptor, TSH has a stimulatory effect on thyrocytes, leading to increased hormone production. There is an inverted logarithmic relationship between the concentrations of fT4, fT3 and TSH, i.e., a small decrease in the concentration of peripheral hormones causes a significant increase in the level of TSH.

10.2 Disorders of thyroid function

Disorders of thyroid function are manifested by increased or decreased production and secretion of thyroid hormones, with changes in the shape and structure of the thyroid gland (goiter, nodules, tumors) being, but not necessarily, present. Thyroid hormones affect the function of each organ through their receptors (nuclear, membrane and mitochondrial) or by interacting with other hormones. As a result, any thyroid dysfunction can have a wide range of manifestations.

Manifest diseases have expressed clinical signs of hypothyroidism or hyperthyroidism and pathological serum concentrations of fT4, fT3. In **subclinical disorders**, clinical signs are absent or present only in a minimal non-specific form (Table 10.1). TSH levels are marginally increased or decreased at normal peripheral hormone concentrations.

TABLE 10.1 CLINICAL SYMPTOM AND SIGNS OF THYROID DYSFUNCTIONS

Suspition	Hypothyroidism	Hyperthyroidism
High	Goitre, slow reflexes, bradycardia, Diastolic hypertension	Struma (possibly murmur), protrusion of eye bulbs
Moderate	Fatigue, cold intolerance, weight gain, constipation, dry and rough skin, swelling of the face, a hoarse voice	Fatigue, weight loss, muscle loss, heat intolerance, sweating, increased reflexes, gentle tremor of fingers, accelerated intestinal peristalsis, palpitations
Low- nonspecific symptoms	Hair loss, muscle and joint pain, depression, memory impairment, menorrhagia, decreased libido	Nervousness, insomnia amenorrhea/oligomenorrhoea, muscle weakness, warm and moist skin, hair loss

Primary hypothyroidism affects about 1 – 2% of women and only 0.1% of men. It is characterized by TSH value above the upper limit of the reference interval, which varies between 4 – 5 mUI/L in most laboratories, and low fT4 concentration. Some experts argue that the serum TSH distribution shifts towards higher values with age; the upper limit of normal could be as high as 6 to 8 mU/L in healthy octogenarians.

The most common cause of hypothyroidism is chronic autoimmune thyroiditis (Hashimoto's lymphocytic thyroiditis). The disease often occurs asymptomatically and manifests itself only in the stage of hypothyroidism. Untreated hypothyroidism can have serious consequences for the patient. A rare complication of undiagnosed or untreated hypothyroidism is myxedema coma. Other possible causes of primary hypothyroidism are listed in the Table 10.2.

TABLE 10.2 CAUSES OF PRIMARY DISORDERS OF THYROID FUNCTION

Hypothyroidism	Hyperthyroidism
Chronic lymphocytic thyroiditis Post-partum thyroiditis	Autoimmune: Graves-Basedow´s disease, early stage of thyreoditis
Iatrogenic: surgery, radioiodine therapy, radiotherapy of neck area, amiodaron	Benign adenoma, toxic multimodal goitre, carcinoma
Inherited	Drugs: amiodarone, sudden supply of iodine, immunomodulatory drugs, cytokines, overdosing with T4 - thyrotoxicosis factitia
Lack of iodine Excess intake of strumigens	Ectopic: struma ovarii

Subclinical hypothyroidism affects more women than men and its incidence increases up to 10% in women over 65 years of age. A typical finding is elevated TSH at normal fT4 levels, clinical manifestations are usually absent. Treatment of subclinical hypothyroidism is recommended in patients with recurrent TSH values >10 mIU/L, or at lower TSH concentrations of antibodies to thyroid peroxidase, clinical signs of hypothyroidism, atherosclerotic cardiovascular disease or pregnancy are present. The decision to supplement thyroxine depends on the clinical picture and the age of the patient. Untreated patients progress to hypothyroidism (2 – 5% per year).

Primary hyperthyroidism affects 2% of women and 0.2% of men, and manifests by low to unmeasurable TSH levels and elevated fT4 and/or fT3 levels. In the most frequent cause of hyperthyroidism, Graves' disease, the stimulating antibodies against TSH receptors lead to a toxic nodular goitre with increased production of thyroid hormones. Graves' disease is frequently complicated with ophthalmopathy, thyrotoxic crisis, atrial fibrillation, osteoporosis, or congestive heart failure. Thyroiditis accompanied by hyperthyroidism affects women after childbirth or can be of a viral in origin.

Subclinical hyperthyroidism affects about 1% of adults and its incidence increases slightly with age. The TSH value is below the reference interval at normal concentrations of fT4, fT3 and the patient has no clinical manifestations. Treatment of subclinical hypothyroidism should be considered in patients with multi-node goitre or adenoma, as well as in elderly patients at risk of arrhythmias and exacerbation of osteoporosis.

TABLE 10.3 DIFFERENCES BETWEEN PRIMARY AND SECONDARY HYPOTHYROIDISM

Feature	Primary hypothyroidism	Secondary hypothyroidism
Goiter	Usually present	Absent
Deficiency of pituitary hormones	Absent	Present (amenorrhea, infertility, anorexia, hypoglycemia, hyponatremia, weight loss, diabetes insipidus)
TSH	High	↓ in 90%, N - ↑ in 10% of patients
Thyroidal Ab	Variable	Absent
TRH stimulating test	Normal	Pathological

Central disorders of thyroid function arise from hypothalamic (secondary) or pituitary (tertiary) causes. **Central hypothyroidism** is caused by decreased TSH synthesis due to damage of pituitary gland (trauma, infections, tumours, surgery, vascular disorders) or insufficient secretion of the hypothalamic TRH. As a result, TSH secretion does not increase appropriately as T4 secretion falls. Therefore, the symptoms and the serum fT4 value must be used to make the diagnosis. TSH levels may be normal or even increased in about 10% of cases as biologically inactive forms of TSH are formed (Table 10.3).

Excessive, more or less autonomous production of TSH by pituitary tumors leads to **central hyperthyroidism**. The characteristic biochemical abnormalities in patients with hyperthyroidism caused by a TSH-secreting adenoma are normal or high serum TSH concentrations and high serum fT4 and fT3 concentrations. The diagnosis is confirmed by pituitary imaging methods and dynamic tests.

10.3 Laboratory examination of thyroid disorders

The diagnosis of thyroid disorders is based mainly on the examination of TSH and levels of free hormones fT4 and fT3 in the blood (Figures 10.2, 10.3). Assuming the proper function of the hypothalamic-pituitary axis, TSH is the most sensitive parameter that signals thyroid dysfunction. Examination of asymptomatic individuals is not indicated; therefore, the doctor will follow the clinical signs and signs of hyperthyroidism and hypothyroidism.

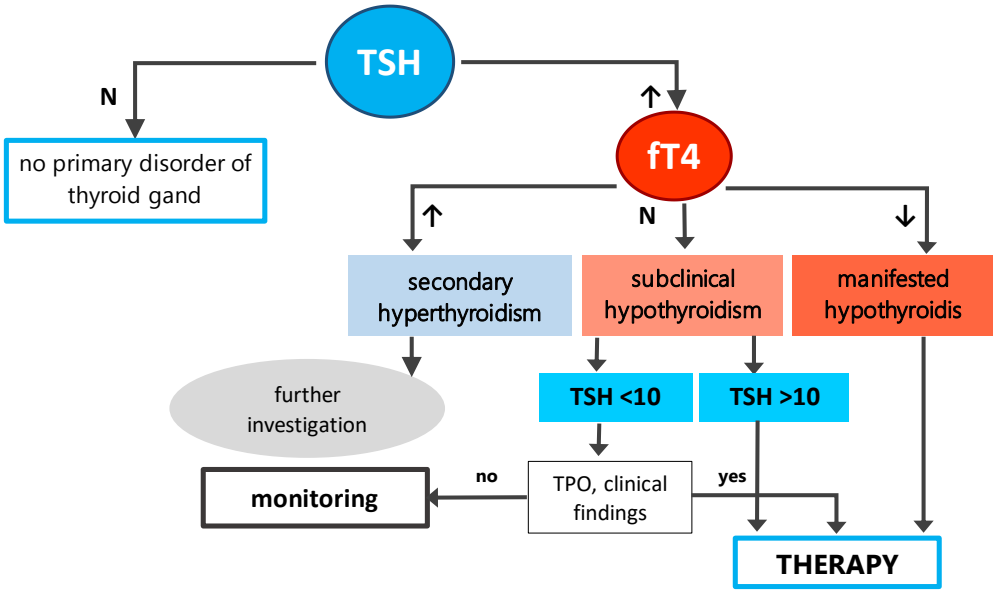


FIGURE 10.2 Diagnostic algorithm in case of suspected hypothyroidism

Screening is indicated in certain groups of patients who have an increased risk of thyroid disease or have clinical manifestations that may be caused by thyroid dysfunction, such as:

- pregnant women and women with fertility disorders;
- individuals with autoimmune disease (T1DM, Addison's disease, celiac disease);
- conditions after surgery and radiotherapy in the neck area, after radioiodine treatment;
- patients with overt coronary heart disease and/or tachyarrhythmia;

- patients with hypercholesterolemia;
- patients taking medicines that affect thyroid hormones;
- women older than 50 years with non-specific manifestations;
- children with growth disorders;
- some genetic diseases (Down's or Turner's syndrome).

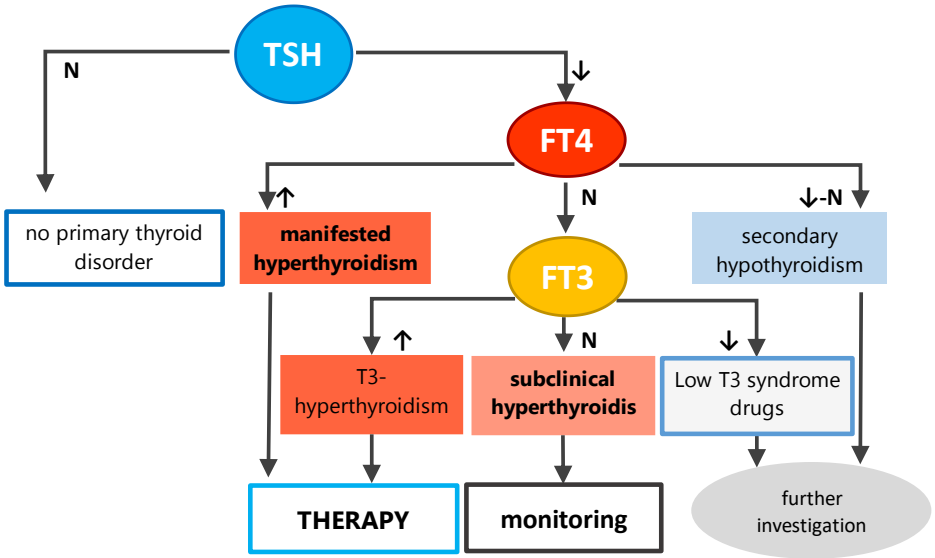


FIGURE 10.3 Diagnostic algorithm in case of suspected hyperthyroidism

The list of biochemical parameters routinely used in the diagnosis of thyroid disease is summarized in the Table 10.4. All parameters are currently determined by immunochemical assays based on the interaction of antigen with antibody. Laboratory results should be evaluated in the context of clinical findings (medical history, physical examination) and medications used.

TABLE 10.4 LABORATORY PARAMETERS IN DIAGNOSTICS OF THYROID DISORDERS

Parameter	Clinical use
TSH – thyroid-stimulating hormone	Assessing thyroid function
fT4 - free thyroxine	Assessing thyroid function
fT3 – free triiodothyronine	Assessing thyroid function
aTPO – anti-thyroid peroxidase antibodies	Autoimmune thyroiditis
TRAb (aTSHR) – anti-TSH receptors antibodies	Graves´ disease
aTG – anti-thyroglobulin antibodies	Marker of thyrocytes damage, interfere with TG assay
Calcitonin	Tumor marker of medullary carcinoma
TG - thyroglobulin	Tumor marker of differentiated carcinoma

To avoid pre-analytical effects, blood sampling should be performed in the morning, always before using a medicine containing T4 or T3. All thyroid parameters show intra-individual variability (circadian rhythm, pulsed TSH secretion) and even more pronounced inter-individual variability. These differences concentration of hormones between individuals are given mostly genetically.

Thyroid-stimulating hormone

Thyroid-stimulating hormone (TSH) is a glycoprotein composed of two subunits; the alpha subunit is common with FSH (follicle-stimulating hormone), LH (luteinizing hormone) and hCG (human chorionic gonadotropin), the beta subunit is specific for TSH and determines its immunological and biochemical properties. TSH has a diurnal rhythm with pulsed secretion, reaching a minimum between 7:00 and 13:00. The biological variability of TSH values in the same individual during the day and between days can reach up to 50 – 40%.

TSH determination is the most sensitive indicator of thyroid function. Even a small change in the concentration of thyroid hormones in the blood will cause an exponential change in the concentration of TSH. The TSH value in the reference interval (RI) excludes primary thyroid failure. The examination will also detect subclinical forms of thyroid diseases. Methods with a functional sensitivity of at least 0.02 mIU/L make it possible to reliably distinguish thyroid hyperfunction from frequent TSH suppression.

Completion of the fT4 and fT3 assays is performed automatically by biochemical laboratories based on expert guidance. Similarly, the physician may request that the test be supplemented from the original sample, which is stored in the laboratory for at least 48 hours after analysis. Isolated TSH examination is not sufficient for:

- suspicion of central forms of hypothyroidism, where TSH may be within the reference interval;
- monitoring patients at the start of treatment when TSH responds more slowly than fT4.

Free thyroxine (fT4) and triiodothyronine (fT3)

The determination of free hormones completely replaced the measurement of total T4 or T3 concentrations. Their values are not influenced by the concentration of binding proteins, which may increase due to hormones (oestrogens, androgens, cortisol) or decrease due to malnutrition or liver failure. If TSH is elevated, fT4 measurement is sufficient to confirm hypothyroidism, fT3 determination is not indicated. With reduced, even immeasurable TSH, the presence of hyperthyroidism is confirmed by increased levels of fT4 and fT3 (about 5% of hyperthyroidism).

Anti-thyroid antibodies

Thyroid autoantibodies are measured to confirm the autoimmune origin of thyroid diseases and, partly, to monitor disease activity. Antibodies to thyroid peroxidase (TPO), antibodies to TSH receptors (TRAb) and thyroglobulin (ATG) belong to most common tests. These antibodies are heterogeneous in nature, their ability to bind antigens used in immunochemical methods is variable, therefore the laboratory results obtained by different assays may differ significantly. Low SN and SP of autoantibodies cause, that autoimmune thyroiditis can occur in the absence of antibodies, and conversely, antibodies can be markedly positive in other autoimmune diseases.

Anti-TSH receptor antibodies (TRAb) are immunoglobulins with a stimulating or rarely blocking effect on thyroid function. Conventional assays do not distinguish between the two types of antibodies. They have the highest sensitivity (95%) and specificity (98%) for Graves' disease; they are also examined to monitor her treatment. Remission of disease is accompanied

by a decrease, while an increase in antibodies is a marker of relapse. In some patients, both stimulatory and blocking antibodies may occur and such a patient oscillates between thyroid hyper- and hypofunction. TRAbs cross the placenta, therefore high titer in the mother´s blood predicts hyperthyroidism in a newborn.

Anti-thyroid peroxidase antibodies (TPO) are a major marker of the autoimmune origin of thyroid disorders. When thyroid tissue is damaged, peroxidase is released from thyrocytes and activates the production of autoantibodies. TPO positivity is detected in more than 90% of patients with chronic lymphocytic thyroiditis (Hashimoto) and 75% of patients with Graves' disease. Individuals with normal thyroid function and positive antibodies have a higher risk of gradual destruction of the gland with the development of hypofunction.

Anti-thyroglobulin antibodies (ATG) are elevated in 70% of patients with chronic thyroiditis and 20–30% of patients with Graves' disease. When TG is determined as a tumour marker, ATG positivity signals possible interference in TG determination.

Tumour markers

Thyroglobulin (TG) is a glycoprotein with high content of amino acid tyrosine. It can only be synthesized by differentiated thyrocytes and is a very strong autoantigen. Destruction of thyroid tissue and leak of TG from the cells may increase its blood levels, but higher TG level may be also found in pregnancy. TG determination has no additional information in the diagnosis of thyroid function. However, it is used as a tumour marker after thyroid removal for a differentiated thyroid tumour. In individuals after removal of the thyroid gland, the TG level is undetectable, so new methods of determination should be able to detect even very low concentrations of TG. TG examination is also indicated in lung and bone metastases of unclear origin. Antibodies against TG (ATG) are also investigated together with TG, and their positivity indicates possible interference in TG measurement. **Calcitonin** is a hormone produced by para-follicular thyroid cells and is used as a marker of medullary carcinoma.

10.4 Monitoring the treatment of disease

The goal of treatment of thyroid disorders is to normalize TSH and fT4 values and to eliminate the patient's clinical (subjective and objective) difficulties. The patient's age and comorbidities may affect the target TSH value as well as the rate at which normalization is achieved. For example, elderly patients require a slower course of treatment. The biological variability of TSH values should be taken into account when monitoring patients (Figure 10.4).

Hypothyroidism

TSH levels remain elevated for 6 – 12 weeks after initiation of substitution therapy. A more suitable parameter to monitor treatment during this period is the concentration of fT4 or a combination of TSH and fT4. In some patients, TSH can be maintained in the reference range only at the cost of a slightly elevated fT4 value. After normalization of TSH in stabilized patients, it is sufficient to monitor only TSH at intervals of 6 – 12 months. When monitoring thyroxine replacement therapy, fT3 has a limited value, its concentration may be affected by

other factors, such as concomitant acute or chronic diseases. Even in patients treated with triiodothyronine preparations, the assessment of fT3 is problematic because blood levels fluctuate significantly after drug administration.

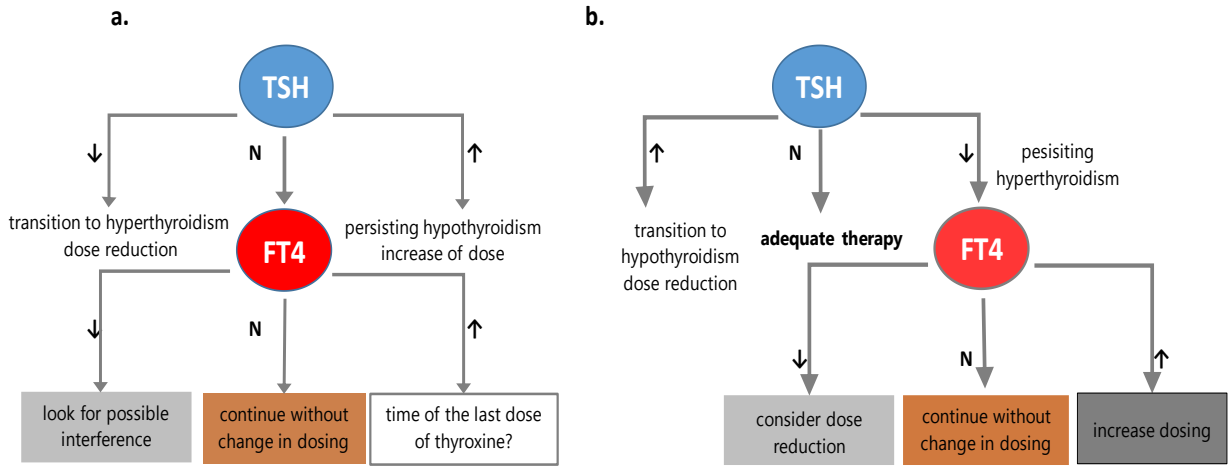


FIGURE 10.4 Monitoring scheme for patients with thyreostatic and substitution treatment

Hyperthyroidism

Suppressed TSH levels may persist for 3 – 6 months after initiation of antithyroid therapy (thyrostatics, radioiodine or subtotal thyroidectomy), depending on the previous duration of hyperthyroidism. During biochemical monitoring, the concentration of fT4 is monitored in this period in 4 – 6 week intervals. In patients with T3 hyperthyroidism, monitoring of fT3 levels is also logical. After normalization of TSH and remission of clinical difficulties, it is sufficient to monitor TSH at 2 – 3 month intervals.

An increase in TSH is a sign of thyrostatic overdose or a spontaneous transition to hypothyroidism. Patients treated with radioiodine have a higher risk of developing hypothyroidism than patients treated with surgery. During treatment with propylthiouracil, its side effects, especially agranulocytosis, should be monitored. TRAbs is also monitored in patients with Graves' disease, as they inform about the effect of treatment and their increase is a sign of relapse.

Secondary thyroid disorders

TSH is a useful indicator of thyroid function only if the hypothalamic-pituitary axis is not disrupted. Therefore, in thyroid dysfunction of central origin, monitoring of fT4 is a more appropriate indicator of substitution treatment.

10.5 Thyroid gland in pregnancy

The stimulatory effect of hCG on the thyroid gland during the first trimester of pregnancy is manifested in some women by an increase in both fT4 and fT3 and a transient decrease in TSH. Due to high oestrogen level, concentration of TBG increases during pregnancy.

The reference intervals for thyroid hormones for pregnant women are different - higher fT4, Ff3 and lower TSH. Excessive pregnancy nausea and vomiting (hyperemesis gravidarum) may be caused by transient pregnancy thyrotoxicosis. Adequate levels of thyroid hormones are essential for conception, proper foetal development and pregnancy. As thyroid disorders are relatively common in pregnancy and mostly asymptomatic, pregnancy screening for thyroid diseases using TSH (fT4) and TPO tests is recommended. In case of a positive result, the patient is sent for endocrinological examination.

Undiagnosed subclinical hypothyroidism in pregnancy can have adverse consequences (hypertension, preeclampsia, preterm birth and risk of mental disability). In women treated for hypothyroidism before pregnancy, the goal of substitution treatment during the first trimester is to reduce TSH levels below 2.5 mIU/L, which is achieved by increasing the dose of thyroxine by 30 – 50%. In the further course of pregnancy, the values of TSH and fT4 should not exceed the reference interval for the respective trimester.

Hyperthyroidism in pregnancy also increases the risk of preeclampsia, miscarriage, premature birth and low birth weight. The goal of treatment is to keep fT4 in the upper third of the reference interval with the lowest possible dose of medication to prevent foetal hypothyroidism.

10.6 Inconsistent laboratory findings and interferences

If results of thyroid hormones do not agree with clinical findings, the cause of that should be investigated. The most common cause of inconsistent findings is non-thyroid diseases. In that situation, it is appropriate to ask the laboratory for repeated analysis, or to repeat the blood collection and add another diagnostic test, for example, fT4 at mismatched TSH. The consultation of a clinician with laboratory staff helps to reveal interference in some patients, e.g. drugs, biotin or animal antibodies interfering with immunochemical assays (Table 10.5). In some cases, it is possible to measure the sample with a different analytical system in another laboratory to detect interference.

TABLE 10.5 NON-THYROID INFLUENCES AFFECTING LABORATORY FINDINGS

Parameter	Decrease	Increase
TSH	Severe acute or chronic diseases, malnutrition, stress, mental anorexia, 1st trimester of pregnancy	Recovery phase of illness, acute psychiatric disease, oestrogens, older age, blood sampling afternoon or evening (diurnal rhythm)
fT4, fT3	Lithium, iodine containing medicines, phenytoin, carbamazepine, amiodarone, severe hepatitis	Amiodarone, beta-blockers, estrogens, birth control pills
TBG	Anabolic hormones, androgens, glucocorticoids	Estrogens (p.o. birth control pills, HRT), tamoxifen, methadone
TG	Decrease from the birth to adulthood	Benign disorders of thyroid gland
Calcitonin	Menopause, physical exercise	Alcohol, lactation, haemolysis

If results of thyroid hormones do not agree with clinical findings, the cause of that should be investigated. The most common cause of inconsistent findings is non-thyroid diseases. In that situation, it is appropriate to ask the laboratory for repeated analysis, or to repeat the blood collection and add another diagnostic test, for example, fT4 at mismatched TSH. The consultation of a clinician with laboratory staff helps to reveal interference in some patients, e.g. drugs, biotin or animal antibodies interfering with immunochemical assays (Table 10.5). In some cases, it is possible to measure the sample with a different analytical system in another laboratory to detect interference.

Low T3 syndrome

Low T3 syndrome is a term for the finding of pathological values of thyroid hormones in patients with acute or chronic non-thyroid disease (NTI). It results from the stress response to the presence of a serious acute illness (e.g. surgery, infection, AMI) or a chronic condition associated with energy deprivation (e.g. starvation, advanced liver or kidney disease, cancer, anorexia nervosa). The principal laboratory finding is decreased fT3 concentration, while fT4 tends to be slightly reduced and TSH is mostly in the reference interval (Figure 10.5). In some patients, TSH is suppressed in the acute phase of a disease, while elevated TSH level is a sign of recovery from non-thyroid disease.

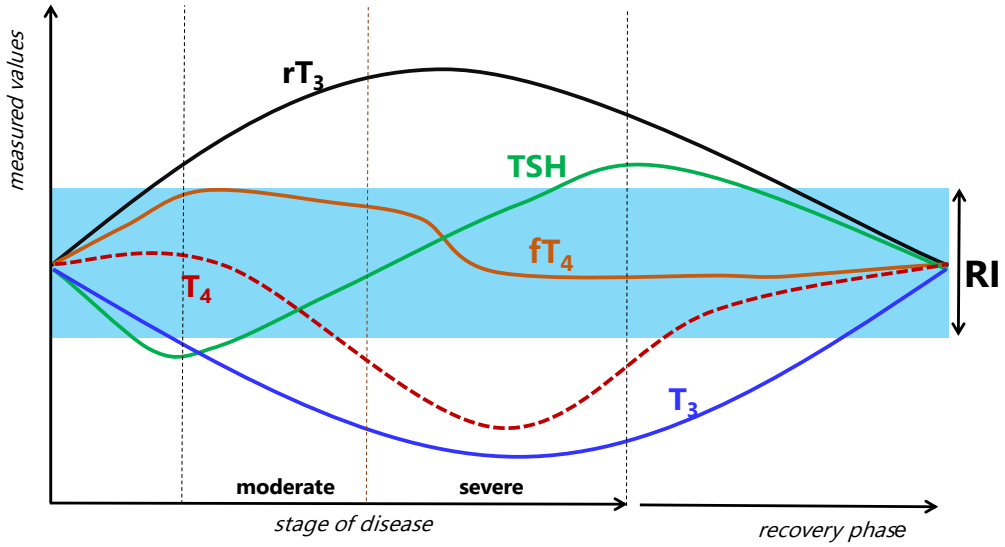


FIGURE 10.5 Low T3 syndrome - time evolution of thyroid hormone levels

Low T3 syndrome is multifactorial including transient dysfunction of the hypothalamic-pituitary-thyroid axis, decreased transport protein concentration, decreased conversion of T4 to T3 in peripheral tissues and increased production of reverse T3 (rT3). Some cytokines (TNF and others) also contribute to the development of low T3 syndrome.

The syndrome represents the adaptation process of a body to stress - the goal is to reduce the rate of metabolism and save energy resources. For this reason, it is not appropriate to examine thyroid function in patients overcoming acute illness. In hospitalized and especially elderly patients with chronic diseases, TSH levels between 0.1 and 10 mIU/L should be interpreted with caution.

Drugs

Drugs are a significant source of pre-analytical variability (Table 10.6) and can affect:

- TSH secretion;
- formation and secretion of T4 and T3;
- concentration of transport proteins;
- conversion of T4 to T3;
- bioavailability of p. o. thyroxine.

Drug effects vary with different assays for hormone measurement. Amiodarone or lithium directly cause thyroid dysfunction. The antiarrhythmic **amiodarone** has a chemical structure similar to thyroxine and a high iodine content. It reduces the peripheral conversion of T4 to T3 by inhibiting deiodases, therefore fT4 increases and fT3 decreases during amiodarone treatment. Decrease of fT3 levels leads to an increased production of TSH in pituitary cells. The common manifestation of amiodarone-induced hyperthyroidism is a sudden arrhythmia. A small proportion of patients develop hypothyroidism after a phase of destructive thyroiditis. **Lithium** and iodine preparations generally reduce fT4 concentrations. Patients taking these medicines should be monitored for thyroid function at least once a year.

TABLE 10.6 EXAMPLES OF DRUGS AFFECTING THYROID HORMONES

Mechanism	Examples
Decreased TSH secretion	dopamine, glucocorticoids, octreotide, cytokines
Increased TSH secretion	Dopamine antagonists, phytoestrogens, neuroleptics
Low synthesis and secretion of T4, T3	lithium, amiodarone, iodine, carbimazol, metimazol, propylthiouracil
Increased synthesis and binding capacity of TGB	oestrogens, tamoxifen, opiates, methadone
Decreased synthesis of TBG	androgens, anabolic hormones, glucocorticoids
Competition on protein binding sites	furosemid, salicylates, NSAID, carbamazepine, heparin
Increased liver metabolism	Proton pump inhibitors, H2-blockers, phenytoin, carbamazepine, rifampicin
Impaired conversion T4 to T3	amiodarone, β-blockers, radiocontrast agents
Impaired intestinal absorption of T4	cholestyramine, calcium, soy proteins, Al(OH)3
Immunological influence	IL-1, interferons, TNF-γ, statins

In hormone replacement therapy, several concomitant medications block the intestinal absorption of thyroxine (bile acid sequestrants, calcium, iron-containing preparations) and others increase hepatic T4 biodegradation (anti-convulsive, proton pump inhibitors or H2-blockers).

Other influences and interferences

The **concentration of binding proteins** may be increased in pregnancy or due to oestrogen-containing medicines and, conversely, decreased in impaired hepatic proteosynthesis (chronic diseases, malnutrition, anabolic use). In both situations, only the concentration of total T4 and T3 changes, while fT4 and fT3 are not affected. **Genetic variants** of albumin and prealbumin are a rare, possible cause of the increased affinity of these transporting proteins for T4 and the

increased T4 values. **Endogenous antibodies** to thyroid hormones may occur in patients with some autoimmune diseases. Autoantibodies also positively interfere with fT4 and fT3 assays. **Analytical interferences** of biotin and heterophile endogenous animal antibodies result in surprisingly high or low results using immunochemical assays (Figure 10.6). Details are explained in INFO 10.1.

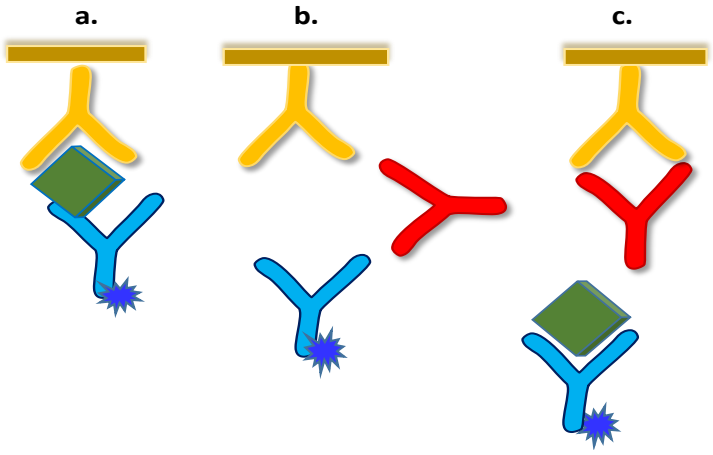


FIGURE 10.6 Negative and positive HAMA interference in immunochemical analyzes
yellow - binding Ab anchored to the solid phase; blue - labeled antibody against analyte; green - antigen under investigation; red - endogenous HAMA

Info 10.1 Analytical interference

Biotin (vitamin B7, vitamin H) is a freely available food supplement belonging to the group of B vitamins, used as part of multivitamin preparations or means for improving the condition of hair and nails. High doses of biotin are used by patients with some rare metabolic disorders, but also with multiple sclerosis. Biotin interferes with many immunochemical hormone assays that use biotinylated antibodies (e.g., TSH sandwich assays) or biotinylated antigens (competitive fT4 assays). Biotin interference can be both positive and negative. With the competitive principle used for low molecular weight analytes (fT4, fT3, cortisol), biotin gives false high results. With the sandwich principle (large molecules such as TSH, FSH), biotin interference is negative.

Analytical interference is caused by specific human anti-animal antibodies (HAAA). This includes HAMA (human anti-mouse antibodies), which interfere in particular with sandwich-based immunochemical methods (see figure) using antibodies of murine origin against the antigens to be determined.

HAMA interference in immunochemical analyzes (see Figure 11.6):

a. the normal course of the sandwich method;
b. positive interference caused by a "bridge effect" when HAMA forms a bridge between the binding and labeled antibodies (Ab) in the absence of the antigen to be determined;
c. negative interference - HAMA prevents the binding of the antigen to be determined with the binding antibody.

Case studies and self-assessment questions

Case report 10.1

A 48-year-old man hospitalized at the coronary ICU on day 3 after AMI was examined for thyroid hormones even though he had no clinical signs of thyroid disease. His current laboratory results (1), as well as the follow-up examination after recovery from the infarction 3 months later (2), are given in the table.

Serum test	1	2	RI
TSH	<0.05	2.3	0.20 – 4.5 mIU/L
fT4	11.1	18.1	12 – 25 pmol/L
fT3	1.4	4.5	3 – 7 pmol/L

Questions:

a. How would you evaluate thyroid function?
b. What other examinations would support your working diagnosis?
c. What was the cause of the pathological finding in the patient?

Case report 10.2

30-year-old woman was examined by a general practitioner for unintentional weight loss (6 kg in 3 months), she has been feeling irritated and tired, she has not tolerated warm weather in recent weeks. She denies a disease such as virosis or sore throat. On physical examination swollen palms, gentle tremor of arms in the outstretched position, and palpable small solid goitre were detected. Eye symptoms were not present. Her laboratory results are shown in the table.

Questions:

a. Which thyroid dysfunction do the laboratory test indicate?
b. What other examinations would support your working diagnosis?

Serum test	Result	RI
TSH	<0.01	0.20 – 4.5 mIU/L
fT4	22.5	12 – 25 pmol/L
fT3	14	3 – 7 pmol/L

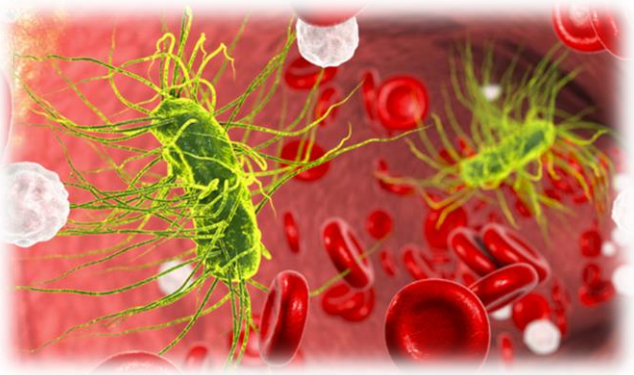
Self-assessing questions

1. Which parameter is the test of the 1st choice in thyroid screening and why?
2. Name laboratory tests that are indicative of the autoimmune origin of thyroid disease.
3. Which tests would you indicate in a patient treated with thyroxine one month after starting treatment?
4. Which parameter is examined along with thyroglobulin to monitor the patient after removal of the thyroid gland for cancer?
5. What is the typical laboratory finding in patients with severe non-thyroid disease?

KEY INFORMATION

- ☑ Routine screening for thyroid disorders in asymptomatic individuals is not recommended.
- ☑ TSH is the test of first choice to assess thyroid function. In case of a pathological TSH result and if TSH alone is not sufficient for diagnosis, an examination of other parameters (fT4, fT3, antibodies) is indicated.
- ☑ TSH testing is not sufficient in secondary thyroid diseases, non-thyroid diseases, and in the initial phase therapy.
- ☑ When interpreting inconsistent fT4 and TSH results, the TSH result is more important for diagnosis.
- ☑ Measurement of fT3 is indicated only to confirm the diagnosis of T3-hyperthyroidism and to monitor T3 replacement therapy.
- ☑ Primary thyroid disorders (hypothyroidism or hyperthyroidism) are divided into manifest and subclinical. Their treatment is guided by clinical findings and TSH levels.
- ☑ Subclinical thyroid disorders are common and are characterized by the pathological value of TSH and normal fT4.
- ☑ In hypothyroidism, the drug of first choice is thyroxine (Levothyroxine). In hyperthyroidism, there are several treatment options, e.g. thyrostatic drugs (carbimazole – methimazole, propylthiouracil), beta-blockers, radioiodine, surgical removal of the gland.
- ☑ Laboratory tests for monitoring the therapy of hypothyroidism (fT4, TSH) and hyperthyroidism (TSH, fT4, fT3 + TRAb in Graves' disease) are usually repeated after 4 – 6 weeks and initialization and every 6 months after normalization of thyroid hormone levels.
- ☑ The reference intervals for TSH, fT4 and fT3 are not universal, they vary based on the used analytical method and different RI are used for pregnant women and young children.
- ☑ Thyroglobulin testing is indicated in patients with differentiated thyroid carcinoma as a tumour marker.
- ☑ Calcitonin determination is indicated in case of suspected medullary thyroid carcinoma, in monitoring patients after treatment and in the differential diagnosis of hypercalcemia.
- ☑ Thyroid screening in pregnant women is recommended and performed in many countries around the world.

11



Markers of sepsis and inflammation

CONTENT

Infections affect people of all ages around the globe. Most individuals cope with the infection through their immune system or antimicrobial treatment. Severe forms of infection, referred to as sepsis, lead to an inadequate host response, which escalates to multi-organ failure syndrome. The global incidence of sepsis is estimated at 30 million cases per year and 25% of them die.

Diagnosis and treatment of sepsis requires interdisciplinary collaboration of health professionals. Early detection of infection, treatment or resuscitation increases patients' chances of survival. Although the diagnosis of sepsis is based primarily on clinical findings, biochemical, hematological and immunological biomarkers are an integral part of laboratory diagnosis and monitoring of sepsis.

11.1 Basic physiology

Sepsis is a heterogeneous and complex syndrome of varying aetiology, severity, and prognosis. Although sepsis has been known since ancient times, its mechanism is still not fully understood, especially due to the existence of a large number of factors that trigger and modulate the body's inappropriate response to infection. To the best of our knowledge, the body's inflammatory response to infectious insults plays a key role in its pathogenesis, triggering a cascade of the body's immune, metabolic and hormonal responses through mediators - cytokines. In most cases, the source of sepsis is a bacterial infection. Viral, fungal and parasitic infections can also cause sepsis, but the inflammatory response in these cases is less intense. The immune response to both infectious and non-infectious stimuli depends on the innate and acquired components of the immune system and is manifested by balanced pro-inflammatory (SIRS, systemic inflammatory response syndrome) and anti-inflammatory (CASR, compensatory anti-inflammatory response syndrome) forces (INFO 11.1).

INFO 11.1 SIRS, CARS a MARS

The clinical picture of SIRS is conditioned by cytokines, which are produced initially by immunocompetent cells, followed by activated endothelial cells and other cells. Membrane and cytoplasmic receptors on monocytes respond to exogenous and endogenous noxious substances (bacterial endotoxins, non-bacterial toxins, necrotic cell products) and changes in cell metabolism (oxidative stress) by the production of pro-inflammatory cytokines. The first pro-inflammatory cytokines (TNF-α, IL-1) activate the endothelium at the lesion site. Monocytes, activated endothelium, and other immunocompetent cells synthesize another pro-inflammatory cytokine, IL-6. Cytokines have a very short biological half-life and most of them act at the site of their origin. An exception is IL-6, which affects the metabolism of distant organs (e.g. CNS, liver, bone marrow).

The generalization of the inflammatory response is mediated by monocytes, which activate the endothelium outside the site of action of the primary pathogen during circulation in the bloodstream. In addition to cytokine production, activated endothelium produces other substances - vasoactive substances (vasodilators - EDRF, EDHP and vasoconstrictors - endothelin), prostaglandins (vasodilatory and antiplatelet PGI2, PGD2, vasoconstrictive and PLT aggregation supporting PGH2 and TXA2), growth factors (bFGF), tissue factor and more. Endothelial oedema develops, increasing its permeability to small protein molecules, especially albumin. Leakage of albumin into the interstitium reduces oncotic pressure, the return of water to the vessels at the venous end of the capillary is not complete - hypovolemia occurs intravascularly, extravascularly increasing albumin leads to interstitial oedema. The unregulated inflammatory response becomes self-destructive, developing malignant inflammation that can progress to multiple organ dysfunction or failure syndrome (MODS -MOFS).

The counterbalance of SIRS is the compensatory anti-inflammatory response syndrome (CARS), a time-limited response of the body, the purpose of which is to suppress unwanted damage to the body due to SIRS by suppressing the immune response without adversely affecting the elimination of the pathogen. CARS is characterized by the release of IL-4 and IL-10, which block the production of TNF-α, IL-1, IL-6 and IL-8, there is a decrease in Th1 and Th2-Ly, monocytes and activation of the hypothalamic-pituitary-adrenal axis with overproduction of glucocorticoids, which are inhibitors of the inflammatory response. Long-lasting CARS can lead to unwanted immunosuppression of the host, which helps to further multiply pathogens and reactivate latent viruses. MARS (Mixed Anti-inflammatory Response Syndrome) is a term used to describe an enhanced pro- and anti-inflammatory response in septic patients, and both hyperactivity (SIRS) and immunosuppression (CASR) can have severe destructive consequences leading to MODS, septic shock and anergic conditions.

Innate immunity (complement system, sentinel phagocytes and NK cells) is responsible for the recognition and killing of antigens, especially lipopolysaccharide components on the surface of invading pathogens (PAMP, pathogen-associated molecular patterns). The role of acquired immunity is to control the inflammatory process and its location at the site of the attack. If pro-inflammatory forces outweigh the anti-inflammatory, the local inflammatory response escalates into the systemic response of the host organism known as the **cytokine storm**. In patients with trauma, burns, ischemia-reperfusion injury after surgery, elevated levels of cytokines also occur as a result of the release of pro-inflammatory molecules from damaged mitochondria or stressed cells (DAMP). Similar genetic characteristics of bacteria and mitochondria (which are more or less encapsulated bacteria) explain why tissue damage (DAMP) and bacterial infection (PAMP) both lead to very similar body responses and very similar clinical manifestations, including the production of inflammatory formation.

The result of the inflammatory response is the interaction of SIRS and CARS. If they are in mutual balance, a pathogen is eliminated and healing occurs. If any of them predominates, the organism is damaged (MODS, MOFS). The predominance of the inflammatory reaction leads not only to the elimination of the pathogen but also to the escalation of the removal of healthy cells, with consequent damage to tissues and organs. The predominance of CARS will not allow the inflammatory reaction to eliminate pathogens which progressively damages the affected tissues and organs (Figure 11.1).

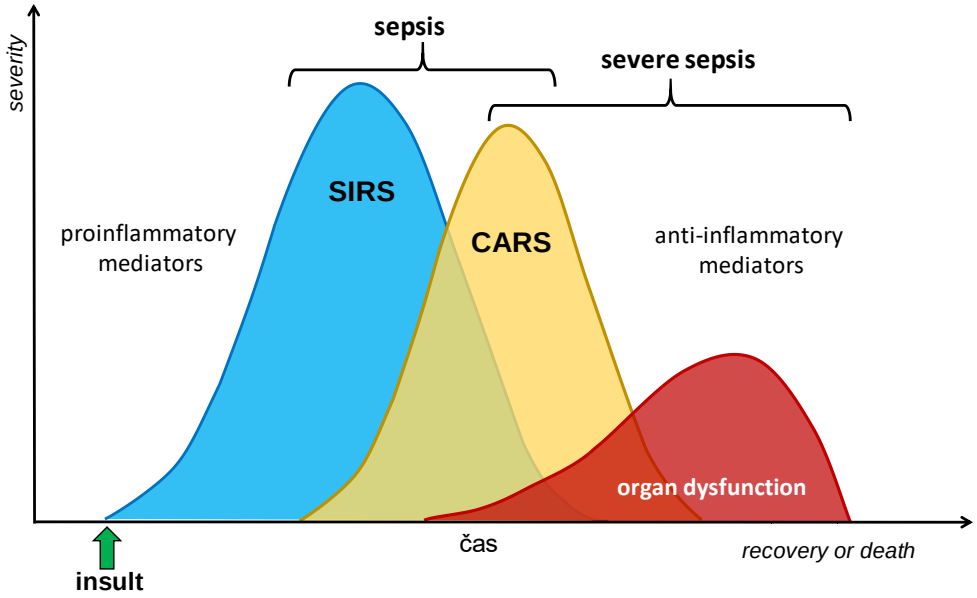


FIGURE 11.1 A model of the progression of inflammatory response to severe sepsis with organ dysfunction

11.2 Definition of sepsis

The term septic syndrome has existed in medicine since late 80s and to date three consensus definitions have emerged (Table 11.1). The first two of them are based on clinical signs of SIRS and evidence or strong suspicion of infection. The clinical criteria of SIRS have been criticized repeatedly for their low specificity, as their presence reflects a reasonable, i.e. desirable immune

system response to infectious or non-infectious insult. SIRS criteria are present in many hospitalized patients, including those who never develop infection and never incur adverse outcomes. On the other hand, clinical signs of SIRS tend to be weak in some patients, especially in diabetics, the elderly and immunosuppressed patients. Occasionally, patients with infection may progress to sepsis without to meet the SIRS criteria. Critically ill patients receive prophylactic antibiotic treatment that reduces the positivity of microbiological cultures by up to 40%. Therefore, the latest proposed definition of sepsis from 2016, known as **Sepsis-3**, defines sepsis as "a life-threatening organ dysfunction caused by an unregulated response of the host organism to infection".

Severity of organ dysfunction has been assessed with various scoring systems that quantify abnormalities according to clinical findings, laboratory data, or therapeutic interventions. The predominant score in current use is the Sequential Organ Failure Assessment (SOFA). A higher SOFA score is associated with an increased probability of mortality. The baseline SOFA score should be assumed to be zero unless the patient is known to have pre-existing (acute or chronic) organ dysfunction before the onset of infection. A change in baseline of the total SOFA score of 2 points or more represents organ dysfunction.

TABLE 11.1 COMPARISSION OF DIFFERENT SEPSIS DEFINITIONS

Consensus	**Diagnostic criteria** except **documented/suspected infection**
Sepsis-1 1991	SIRS criteria (2 and more) ▪ Temperature >38 °C or <36°C ▪ Heart rate >90/min ▪ Respiratory rate >20/min or pCO_2 <4.3 kPa (32 mmHg) ▪ WBC count >12×10^9/L or <4 ×10^9/L or >4% immature forms
Sepsis-2 2001	One or more criteria: ▪ *General symptoms*: Temperature >38°C or <36°C, heart rate >90/min, respiratory rate >30/min, impaired consciousness, visible edema, positive water balance, hyperglycemia without DM ▪ *Signs of inflammation*: Leu >12 × 10^9/L or <4.0 × 10^9/L, >10% immature forms, increased CRP or PCT ▪ *Haemodynamics*: arterial hypotension (<90 mmHg), SvO_2 >70%, cardiac index >3.5 L/min/m^2 ▪ *Organ dysfunction:* arterial hypoxemia, newly formed oliguria, creatinine increase of 44.2 μmol/L, coagulopathy (INR >1.5 or APTT >60 s), thrombocytopenia <100 × 10^9/L, ileus, hyperbilirubinemia >70 μmol/L ▪ *Tissue perfusion disorder*: lactate >3 mmol/L, decreased capillary refill or marbling.
Sepsis-3 2016	SOFA score (≥2 points) ▪ Respiration/oxygenation (↓PaO2/FiO2) ▪ CNS (↓Glasgow Coma Scale) ▪ Cardiovascular (MAP <65 mmHg or need for vasopressors) ▪ Liver (hyperbilirubinemia) ▪ Coagulation (↓PLT count) ▪ Renal function (↑S-Cr, ↓urine output) Screening qSOFA (≥2 points) ▪ Respiratory rate >22/min ▪ Systolic BP <100 mmHg ▪ Altered mental status

Screening for patients likely to have sepsis

In out-of-hospital, emergency department, or general hospital ward settings, adult patients with suspected infection can be rapidly and simply identified as being more likely to have poor outcomes typical of sepsis using the criteria of **quick SOFA** (qSOFA). The criteria monitor only three organ systems, namely blood pressure, respiratory system and state of consciousness, without any laboratory data. Deviation in two or more criteria (respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100mmHg or less) indicates a high probability of sepsis and is an indication to look for other manifestations of organ dysfunction, especially the circulatory, digestive, respiratory, endocrine, immune and neuromuscular systems.

Among the risk factors that increase the likelihood of sepsis are the following ones:
1. low (< 1 year) and high (> 75) age;
2. very frail patients;
3. disorder of the immune system (anticancer treatment, immunosuppressive treatment, splenectomy, diabetes mellitus, long - term corticosteroid treatment);
4. surgical procedures or other major invasive procedures in the past 6 weeks;
5. disruption of the skin barrier (injuries, burns);
6. intravenous catheters, unsuitable and excessive parenteral treatment;
7. pregnancy, childbirth or abortion in the previous 6 weeks.

11.3 Biochemical markers of inflammation and sepsis

Due to the non-specific properties of conventional signs of SIRS and the failure of culture methods in the early detection of infection, we use other laboratory inflammatory biomarkers to support the diagnosis of sepsis. To properly understand their value and limitations, it is necessary to understand the immunological background of a critical disease, which is mainly affected by the body's response to infection.

Laboratory markers are expected **to confirm or signal systemic inflammation** as soon as possible. They should answer the question of whether or not the patient's critical condition is caused by the infection and also **help decide on antimicrobial treatment**. Currently used inflammatory markers can support the diagnostic decision, but are not able to differentiate with 100% sensitivity and specificity between the inflammatory response to infection and non-infectious stimuli, as the complex pathomechanisms of PAMP and DAMP overlap. These markers can also be used to monitor the progression of the inflammatory condition and the effectiveness of treatment.

Microbiological identification of pathogens is essential for effective and successful treatment of sepsis. Newer methods such as PCR and mass spectrometry shorten the time of pathogen identification in blood cultures and other biological materials and are likely to replace traditional culture methods in the future.

C-reactive protein

C-reactive protein (CRP), as one of the positive reactants of the acute phase, is synthesized in hepatocytes under the influence of pro-inflammatory cytokines (especially IL-6, but also IL-1β, TNFα, adipokines). Its physiological concentration can increase up to 1 000-fold in inflammation. Although CRP production has also been described in extrahepatic cells (atherosclerotic plaques, neurons, lymphocytes, adipocytes), this synthesis has little effect on serum CRP levels. It is involved in the opsonization of pathogens, which is ensured by its binding to the C1q component of the classical complement pathway. CRP facilitates phagocytosis and plays an important role in the removal not only of pathogens but also in damaged, necrotic apoptotic cells.

Clinical utility of CRP

CRP is **the most commonly indicated marker of inflammation** that has currently replaced erythrocyte sedimentation rate. Anemia, hypergammaglobulinemia, polycythemia, pregnancy do not affect CRP level. In 90% of healthy people, the CRP level is lower than 3 mg/L, often up to 1 mg/L. During inflammation, the concentration rises above 5 mg/L. Basal CRP levels increase due to hormonal contraception, intrauterine device, smoking, obesity, age and cancer treatment (mostly up to 10 mg/L), while the use of statins and nonsteroidal anti-inflammatory drugs reduces CRP.

CRP is a biomarker with slower dynamics compared to newer markers of inflammation. It exceeds its cut-off value of 10 mg/L after approximately 6 – 12 hours of exposure to inflammatory insult, reaching a maximum after 48 hours. The biological half-life of CRP is 19 (12–24) hours, so its level approximately reflects actual production. Viral infections usually increase CRP levels less than bacterial ones. The concentration of CRP in viral diseases is usually up to 50 mg/L, in bacterial Gram-positive above 50 mg/L, in Gram-negative more than 100 mg/L. Levels above 300 mg/L are present in patients with a severe inflammatory response, including sepsis or extensive burns. If antibiotic treatment works and the inflammatory activity decreases, the CRP levels also fall. If CRP does not decrease, a change in treatment should be considered.

High-sensitive CRP

Traditional assays for determining CRP, including POCT tests, are able to detect the lowest concentrations in the range of 3 – 10 mg/L, which is sufficient for the diagnosis of inflammation. Highly sensitive methods (hsCRP) are also available, which are able to detect concentrations below 3 mg/L. Although their use does not provide new information in the assessment of patients with clinical signs of possible inflammation, new evidence has confirmed the role of hsCRP as an independent risk factor for cardiovascular disease. Elevated hsCRP level (in the range of 1–5 mg/L) is associated with low-grade endothelial inflammation and is a marker of the vulnerability of atherosclerotic lesions.

Limitations of CRP

CRP levels are elevated in most patients in intensive care units, CRP levels are unable to confirm the presence of sepsis because there is no cut-off value for sepsis. CRP is not the most appropriate parameter for the rapid diagnosis of sepsis or septic shock, but it can be used to monitor the treatment of a septic patient. In patients with hepatic dysfunction, failure or

immaturity, as well as in the elderly, the synthesis of inflammatory proteins, including CRP, is reduced and slowed.

There are exceptions where, despite severe inflammation in the body and a proven increase in IL-6 and erythrocyte sedimentation, CRP concentrations are usually not elevated (acute flare-up of SLE, scleroderma and dermatomyositis). This phenomenon is thought to be caused by the cytokine IFN-γ (interferon-gamma), which inhibits CRP production. Despite these limitations, CRP can supplement PCT in the management of patients with sepsis.

Procalcitonin

Procalcitonin (PCT) is a protein consisting of 116 amino acids and one of the most commonly studied markers of sepsis. Under physiological conditions, it is formed in thyroid C-cells as a precursor molecule of the hormone calcitonin and, to a lesser extent, in neuroendocrine cells of the lung. In the presence of infection, especially bacterial, cytokines (IL-1β) induce the expression of the CALC-1 gene and the subsequent synthesis of PCT in the liver and also in other tissues affected by inflammation, for example in the intestine, kidneys, skin, adipose tissue and the like. This inflammatory procalcitonin is excreted unchanged and independent of calcium levels (Figure 11.2). In the inflammatory response, PCT appears to function as an anti-inflammatory and immunomodulatory cytokine, therefore it is also called a **hormokine**.

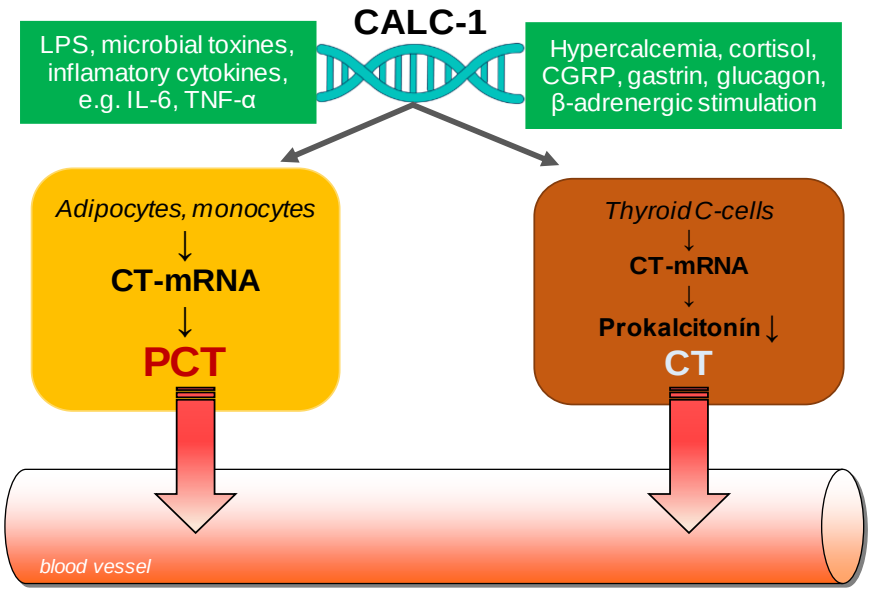


FIGURE 11.2 Production and release of calcitonin and procalcitonin during inflammation
CGRP-calcitonin gene related peptide

Diagnostic significance of PCT

Physiological concentration of PCT in healthy individuals older than 28 days is very low (up to 0.1 µg/L). PCT increases in serum 4 – 6 hours after the initiation of the inflammatory response to the bacterial infection, reaches a maximum after 12 – 24 hours and in the case of effective ATB treatment decreases daily by 50% in keeping with its biological half-life (24 – 30 hours). PCT may slightly increase in some fungal and parasitic infections. Viral and fungal infections generally do not increase significantly PCT concentration, because its synthesis is blocked by

IFN-γ. In this type of infection, helper T-lymphocytes are formed. PCT is not usually increased in common localized infections, in non-infectious inflammatory diseases of autoimmune origin.

The advantage of PCT is that it can distinguish systemic bacterial infection from SIRS of another origin with higher SN and SP than other inflammatory markers, e.g. CRP and interleukins. Elevated PCT levels in patients after surgery and in intensive care units suggest a risk of developing sepsis (high positive predictive value). Diagnostic reliability increases with PCT concentration (Figure 11.3). Local infections, e.g. bronchitis or pneumonia, do not increase PCT significantly. It does not exceed 2 µg/L, while in sepsis the median PCT concentration is in the range of 6-10 µg/L and septic shock above 40 µg/L. Interpretation of a single PCT value is problematic, evaluating the kinetics of multiple values provides much better information.

In patients with an existing or suspected infection, the physician needs to answer three basic questions:

- Is it necessary to start ATB treatment?
- Is the ATB treatment effective?
- When to stop ATB treatment?

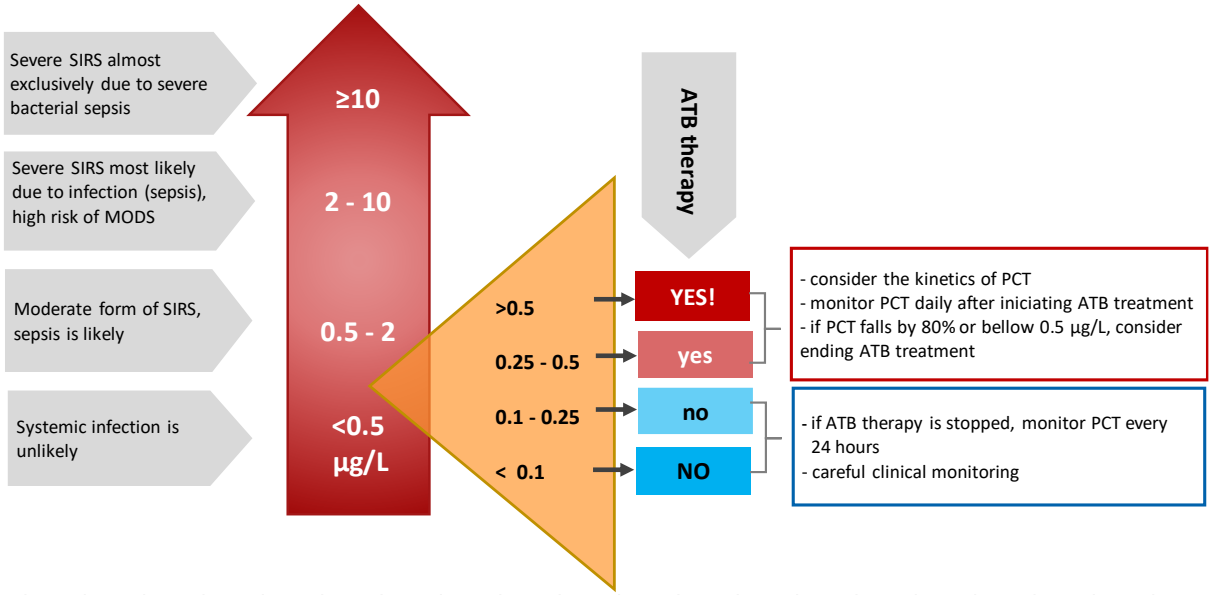


FIGURE 11.3 An example of PCT algorithm for diagnostic and monitoring of therapy of septic patients

1. **Diagnosis of infection** remains a challenge even whit PCT used as an inflammatory marker. The exact cut-off value indicating bacterial infection is not known. Most clinical studies have confirmed only 70 – 85% sensitivity and specificity. Patients with infection generally have lower PCT values (simple effect of PAMP) compared to surgical patients with infection, where DAMP and PAMP acts simultaneously. Similarly, any cell damage without infection, e.g. injury, necrosis or ischemia-reperfusion injury, causes an increase in PCT by the DAMP mechanism. In circulatory unstable patients with suspected infection, ATB treatment should be given regardless of the value of PCT or another inflammatory marker.
2. In patients treated with ATB, it is necessary to verify the **suitability and effectiveness of treatment**. Microbiological cultivation usually lasts longer (2 – 3 days), so empirical ATB therapy is used in practice, which can be ineffective in up to 30% of cases. An early change in PCT levels during the first 24 hours after initiation of ATB treatment may indicate its effectiveness. If treatment is effective, PCT begins to decline, while a further increase

in PCT usually means ineffective treatment. A decrease in PCT that is not accompanied by clinical improvement may occur when the infection is under control, but the patient needs more time to benefit from treatment. If PCT does not decrease after starting treatment, the infection is unlikely to be controlled and the source of infection and type of ATB treatment should be re-examined. In case of inappropriate susceptibility of the pathogen to ATB, confirmed microbiologically, ATB treatment should be changed regardless of the values of inflammatory markers.

3. The majority of studies have shown that PCT in the monitoring of patients reduces the **duration of ATB treatment**. The therapy is generally discontinued when PCT concentrations fall below 0.5 ng/mL or fall by more than 80% of the maximum value. A decrease in PCT in severe systemic infection does not mean the complete elimination of infection, it only indicates that the infection is no longer generalized.

However, initialization and discontinuation of antibiotic treatment is much more complex than just assessing one or more PCT values. The individualized concept ensures the right approach to the patient in everyday medical practice and consists of:

- finding of organ dysfunction,
- identification of a possible source of infection,
- monitoring clinical development and
- assessment of the value and kinetics of PCT.

Limitations: PCT proteosynthesis is, similar to CRP, reduced in case of liver failure, a common complication of sepsis. That decrease may be incorrectly evaluated as a favourable response to ATB treatment. For a similar reason, PCT has limited use in neonatal infections. The disadvantage of PCT is its lower sensitivity in the early stages of sepsis, which are the most important for the patient's prognosis.

Presepsin

Presepsin is the name for the soluble form of CD14 (sCD14-ST, soluble CD14 subtype) detectable in the blood, which reflects a systemic bacterial infection. The membrane glycoprotein mCD14 is expressed on the surface of monocytes, macrophages and polymorphonuclear neutrophils close to another TLR4 receptor (toll-like receptor 4). mCD14 serves as a binding receptor for complexes of bacterial lipopolysaccharide and its binding protein (LPSBPs) and is involved in bacterial endotoxin-initiated transmembrane signaling. Upon binding of the bacterial endotoxin to CD14, an endosome is formed that fuses with the lysosome in the cytoplasm. The protease cathepsin cleaves a constant part of the protein chain from CD14, which then enters the bloodstream, where it is detected as presepsin. Some presepsin may also be released into the circulation from the surface of activated monocytes without the involvement of phagocytosis by the action of plasma proteases.

Thus, the finding of presepsin in serum is a marker of phagocytosis of bacteria bound to the TLR4 + CD14 membrane complex (Figure 11.4). Following the binding of LPSBP to CD14, TLR4 is simultaneously activated and the intracellular signalling cascade is triggered with the production of cytokines, and thus the inflammatory response of the organism to the infectious agent.

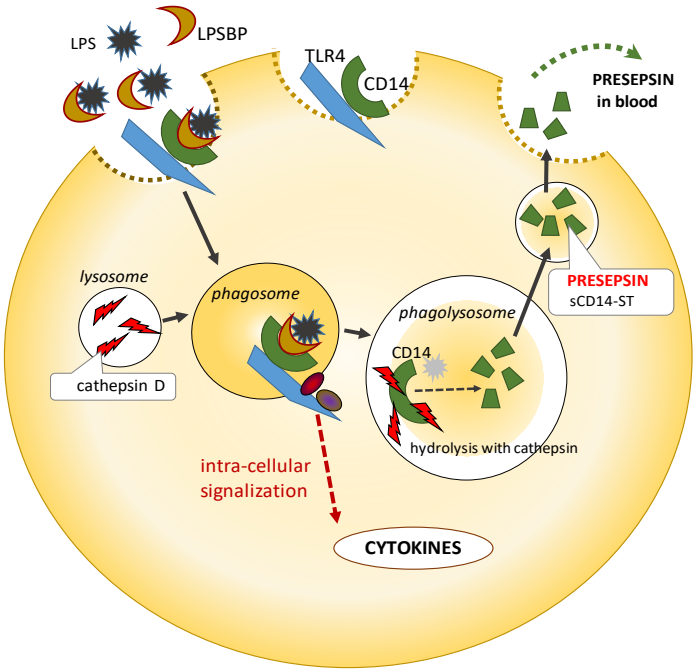


FIGURE 11.4 A simplified scheme of presepsin production

TLR – toll-like-receptor; LPS – lipopolysaccharides (bacterial endotoxin); LPSBP – LPS-binding protein; mCD14 – membranous CD14; sCD14-ST – soluble CD14 subtype

Diagnostic significance

Routine use of presepsin as an inflammatory marker is possible since 2011, after the introduction of chemiluminescent enzyme immunoassays (CLEIA). Presepsin levels increase in both localized and systemic bacterial infections. Due to the mechanism of its release, presepsin level in the blood rises earlier than other inflammatory markers. The increase in presepsin usually occurs within 2 hours after contact with bacterial endotoxins, its biological half-life is 4 - 8 hours. Values in the range of 400 – 600 pg/mL are considered cut-offs for sepsis.

The comparison of presepsin with PCT is the subject of ongoing debate; a meta-analysis of studies with presepsin showed only slightly higher sensitivity (0.84 versus 0.79) and virtually identical specificity (0.77 versus 0.78) compared to PCT. Presepsin also appears to be a promising prognostic marker. The kinetics of presepsin values may play a role in risk stratification in septic patients, e.g. in predicting the onset of multiorgan dysfunction to failure.

An important advantage of presepsin is that its synthesis is not affected by liver function, opposite to CRP and PCT. The marker can be advantageously used in cases where PCT is affected by interference with calcitonin (e.g. in some patients with small cell lung cancer or medullary thyroid cancer). As a small molecule (13 kDa), presepsin is filtered in glomeruli and subsequently reabsorbed and catabolized in tubular cells. Presepsin levels tend to be elevated in patients with decreased GFR and should be interpreted concerning renal function.

Cytokines

Cytokines are universal mediators of inflammation produced by stimulated immunocompetent and other cells, especially activated endothelium. They are early triggers and regulators of the inflammatory response, which are involved in initiating the synthesis of other, routinely used inflammatory markers (PCT, CRP). However, due to their versatility, they cannot sufficiently

distinguish between systemic and local inflammation, or its infectious and non-infectious origin.

Laboratory determination of cytokines is limited particularly by their short biological half-life. They mostly arise, act and degrade at the place of their origin. Only **interleukin 6** (IL-6), whose concentration increases very early with a maximum of 2 – 4 hours after the onset of SIRS, is sufficiently stable and accessible to laboratory diagnostics. IL-6 is currently used successfully as a marker of neonatal sepsis. It is the earliest and at the same time the only inflammatory marker identifiable in the first hours of life. Other diagnoses, in particular autoimmune diseases, rejection reactions, AIDS, alcoholic liver disease and some lymphomas, are in the process of verifying its contribution to the diagnosis and monitoring of disease dynamics.

11.4 Biomarkers of organ dysfunction

Markers of tissue hypoxia

Many routine laboratory parameters used in daily monitoring of vital organ function in critically ill patients are often part of the APACHE and SOFA scoring systems (Table 11.2). **Lactate** is the most widely used marker of hypoperfusion. Its elevated level reflects insufficient oxygen supply to tissues and pyruvate-producing glycolysis, which is converted to lactate under anaerobic conditions while regenerating the coenzyme NAD+ required for previous glycolysis reactions. Elevated lactate has several possible causes in a septic patient. The principal cause is **tissue hypoxia**, which can be generalized e.g. in cardiac failure, pulmonary oedema of cardiac or non-cardiac origin (ARDS in MODS) and pulmonary embolism. Larger local hypoperfusion, such as mesenteric artery thrombosis, often progressing to sepsis, are also a source of increased lactatemia.

TABLE 11.2 LABORATORY PARAMETER USED FOR ASSESSING ORGAN DYSFUNCTION

Organ, system	Parameter	Criterium
Kidney	S-Cr, diuresis, cystatin C albuminuria	Markers of GFR Marker of endothelial dysfunction
Liver	BIL, ammonia INR, APTT, cholinesterase	Markers of detoxication Markers of low proteosynthesis
Heart	NT-proBNP cTnT, cTnI	Marker of heart failure Early marker of myocardial damage
Pancreas	lipase, amylase	Marker of pancreas failure
Respiratory	$SatO_2$, pCO_2	Marker of lung function
Coagulation	D-dimers, PLT, APTT, fibrinogen	Markers of DIC
Peripheral tissues	lactate, a-v difference pCO_2	Marker of tissue hypoxia

In addition to tissue hypoperfusion, **liver and kidney dysfunction** or failure contribute to increased lactatemia, as they normally ensure the clearance of lactate from the blood and its

utilization in gluconeogenesis. Besides, systemic inflammation increases glucose metabolism, which exceeds the oxidative capacity of mitochondria in the affected tissues. This **mitochondrial dysfunction** leads to increased anaerobic glycolysis and lactate formation. Lactate concentration >4 mmol/L in a septic patient is a sign of shock even in the absence of hypotension as a diagnostic criterion.

Arterial-venous difference of pCO_2

Anaerobic metabolism is a typical attribute of sepsis and septic shock. The amount of produced CO_2 depends on the basal metabolism and the respiratory quotient. During anaerobic metabolism, CO_2 is formed from bicarbonate, which buffers acidic metabolites. Because CO_2 is 20 times more soluble than oxygen, it is likely to leak from ischemic tissues into venous blood. The difference measured in arterial and venous blood is considered a predictor of the capacity of the cardiovascular system to remove CO_2 produced in peripheral tissues. A value of > 6 mm Hg (0.8 kPa) during the first 24 hours in critically ill patients indicates a worse prognosis. **Endothelial dysfunction**, whether due to systemic inflammation or of another origin, is a major cause of organ failure in septic patients. In routine practice, it does not yet use any biomarker informing about endothelial activation. Another important part of vascular pathology, which has been shown to affect patient mortality, is a **coagulation system**. Tissue factor, which triggers the coagulation cascade, is released into the circulation from the primarily damaged tissue, activated immunocompetent cells, and from the activated endothelium.

Increased consumption of coagulation factors together with their reduced synthesis and inhibition of fibrinolysis and thrombocytopenia results in the clinical state of **disseminated intravascular coagulopathy** (DIC) with microthrombus formation. The diagnosis of DIC is supported by elevated D-dimers, thrombocytopenia, prolonged APTT, and fibrinogen. In the pathogenesis of DIC in septic patients, a deficiency of the natural inhibitor of thrombomodulin coagulation on the endothelial surface is also implicated. This protein binds thrombin and activates protein C, which is an inhibitor of factors Va and VIIIa. Recombinant human protein C is one of the specific drugs approved for the treatment of sepsis.

Multimarker approach to the diagnosis of sepsis

At present, there is no single isolated biomarker that is able to adequately and reliably inform about a rapidly changing situation in a septic or potentially septic patient. Due to the complex pathogenesis of sepsis, combinations of several inflammatory and metabolic markers are recommended, which has been confirmed by several clinical studies in the past decade. A combination of pro-inflammatory and anti-inflammatory biomarkers, that reflect the presence of both SIRS and CARS, is beneficial for both risk assessment and sepsis diagnosis. in the near future, the rapidly evolving field of genomics, proteomics and metabolomics may bring a completely different approach to selecting the most appropriate combination of biomarkers of inflammation and sepsis.
However, we must not forget that no marker or combination of markers will replace a thorough clinical examination of the patient, so that laboratory biomarkers remain a complementary diagnostic tool.

Case studies and self-assessment questions

Case report 11.1

A 25-year-old young man was transferred to the ICU with developing sepsis. His personal history revealed splenectomy for autoimmune lymphoproliferative disease 2 years ago. The day before after the exercise, he developed malaise, chills, and abdominal pain. No abnormalities on ECG, X-ray, blood pressure and O2 saturation were found at the first examination. In laboratory results, only mild leukocytosis was present (results in the table in column 1). After infusion of isotonic NaCl and administration of paracetamol patient was sent home. Eight hours later his condition deteriorated significantly, so he was re-admitted to the hospital (results in column 2). Following a positive rapid test for *Streptococcus pneumoniae* antigen, ATB treatment was initiated. However, the patient 's hemodynamic condition continued to deteriorated, he was translated to ICU due to hypotension (systolic BP 55 mm Hg), where despite intense fluid resuscitation he fell into septic shock with metabolic acidosis, anuric AKI, DIC (results in column 3) and rapidly spreading necrotizing skin purpura. Laboratory values peaked on day 3 after admission to the ICU (column 4).
The patient underwent intensive hemocoagulation therapy, 27 days of hemoperfusion, and intermittent dialysis until his renal function was near normal. Blood cultures confirmed infection with *S. pneumoniae* serotype 24F, which is not generally considered pathogenic.

Serum	1	2	3	4	RI
Hb	145	141	97	90	130 – 160 g/L
WBC	11.3	2.8	33	36.5	4.4 – 11.3 × 10^9/L
PLT	294	108	33	54	150 – 300 × 10^9/L
PT	69	39	31	13	70 – 100%
D-dimers	–	–	15.5	26	<0.5 µg/mL FEU
Creatinine		145	395	280	60 – 110 µmol/L
AST		22.6	75.6	92	0.60 µkat/L
ALT		7.9	29.2	31	0.80 µkat/L
CK			119	223	<3.5 µkat/L
Myoglobin			9 500	11 650	22 – 100 µg/L
Lactate			19.9	9.6	<2.0 µkat/L
CRP		85	136	249	<5 mg/L
PCT			69.2	111	<0.01 µg/L

Questions:

a. Which laboratory parameters inform about the inflammatory reaction?
b. Which laboratory findings indicate organ failure?
c. What does lactate tell you about and what type of AB disorder is likely to be present in the patient?
d. What is the probable cause of acute renal failure?
e. Which factor contributed to the patient's sepsis?

Self-assessing questions

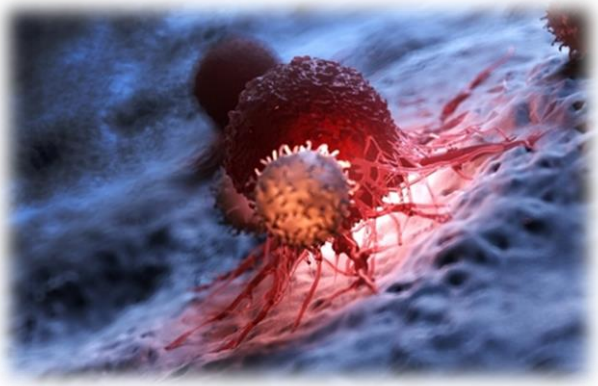
1. Name some risk factors that increase the likelihood of sepsis!
2. What organ functions are assessed within the qSOFA scoring system?
3. What is the clinical significance of the use of inflammatory markers?

4. Which inflammatory marker is the best indicator of localized and systemic bacterial infections?
5. What factors cause false negatives to CRP?
6. Which inflammatory marker would you choose to confirm and monitor adrenal infection and immature neonates and why?
7. Which laboratory parameters are suitable for monitoring myocardial function by tissue perfusion?

KEY INFORMATION

- ☑ Sepsis is a life-threatening organ dysfunction caused by an unregulated response of the host organism to infection. In most cases, the infection is of bacterial origin.
- ☑ Clinical criteria SIRS have low specificity and high false positivity.
- ☑ In particular, the risk factors for developing a septic condition are too high or too low age, immune disorder or immunosuppressive therapy, chronic diseases, invasive surgery, long-term or inappropriate antimicrobial treatment and intravenous catheters.
- ☑ Biochemical inflammatory markers are used to support the diagnosis of infection, to monitor it and monitoring the effectiveness of anti-infective treatment.
- ☑ C-reactive protein is inflammatory marker routinely used to localize the infection, and to quide antibiotic therapy. It is not suitable marker of sepsis.
- ☑ PCT is a validated marker especially of bacterial sepsis in adults and older children than 4 weeks.
- ☑ PCT can distinguish systemic bacterial infection from SIRS of another origin with higher SN and SP than other inflammatory markers.
- ☑ IL-6 is a suitable marker of neonatal infections.
- ☑ All inflammatory markers have limitations, their levels may be affected by liver function, immunosuppressive or immuno-modulating treatments.
- ☑ In addition to inflammatory markers, the function of organs that fail in sepsis is monitored in septic patients using laboratory parameters.

12



Biochemical markers of oncological diseases

CONTENT

Malignant diseases are characterized by uncontrolled growth and division of cells, which leads to a pathological increase in tissue mass or tumor formation. Cancer is the second most common cause of mortality in EU countries and other developed countries. The clinical signs and symptoms of cancer are local and systemic. Local manifestations are derived from the presence of a tumor at a particular site. For example, a tumor destroys normal tissue, spreads to surrounding tissues, causes obstruction of canals, or presses on nerve structures. Systemic manifestations of the tumor include, for example, cachexia, fever, and "paraneoplastic" syndromes, which can be endocrine as well as cutaneous, neurological, haematological, rheumatic, and others.

The presence of a tumor in the body can manifest itself in pathological laboratory findings or lead to the formation of certain typical molecules - tumor markers, which are used in the management of patients with cancer. This chapter deals with the role of biochemical examinations in the screening, diagnosis and monitoring of cancer patients with an emphasis on tumor markers.

12.1 Basic (patho)physiology

Tumorigenesis is the process by which normal cells are transformed into tumor cells. Malignancy is a multi-stage process influenced by many genetic and epigenetic factors that act for a long time, usually 5-20 years, before the appearance of invasive malignant cells. The gradual accumulation of cellular changes (mutations in proto-oncogenes and tumor suppressor genes) results in the development and growth of a tumor. Examples of unregulated cell division are persistent activation of signalling pathways by growth factors, interference at critical points in the cell division cycle, deregulation of transcription factors, inhibition of cell apoptosis, increased tumor angiogenesis, and tumor cell invasiveness. Tumor tissue consists of several subpopulations of cells with different tumorigenic potential. Table 12.1. summarizes typical properties of tumor cells.

TABLE 12.1 FEATURES OF TUMOR MARKERS

Feature	Example
Independence from factors and signals regulating cell growth	self-sufficiency in growth factors, ability to avoid growth inhibitors
Unlimited cell division	cyclin gene mutations
Increased angiogenesis	production of angiogenic growth factors (VEGF, aFGF, bFGF, TGF-α)
Impaired apoptosis - cellular immortality	lack of inducers or excess inhibitors of apoptosis
Formation of metastases	degradation of extracellular mass by metalloproteinases

VEGF – vascular endothelial growth factor, aFGF/ bFGF – acid/ basic fibroblast growth factor, TGF-α – transforming growth factor alpha

The explanation of tumorigenesis and tumor heterogeneity currently combines the theory of clonal evolution and the tumor stem cell (CSC) model. Based on the **clonal theory,** genetic changes leading to malignant disease initially occur in only one or a few cells, but in a subsequent multi-step process, mutations in genes that regulate cell proliferation, differentiation, and apoptosis accumulate in the cells. Although some types of tumors may be congenital (familial occurrence of malignant diseases), the vast majority of tumors are caused by somatic cell mutations. These arise from disorders in nucleic acid replication that are caused by long-acting external factors, e.g. carcinogens, oncoviruses, radiation, etc. According to another theory, **tumor stem cells**, which are able to self-renew and differentiate, give rise to the tumor mass. CSCs may be derived from normal stem cells of the relevant tissue or arise from the oncogenic transformation of undifferentiated progenitor cells. Cells with CSC properties are responsible for resistance to classical antitumor therapy and tumor dormancy.

12.2 Tumor markers

The term tumor marker (TM) encompasses a wide range of **molecules that indicate the presence of a tumor** or provide information about its predicted future behavior, in particular its progression or response to treatment. Traditional tumor markers are biochemical

substances, mostly proteins, produced by the tumor itself, or even by normal cells of the organism in response to its presence. Their increased concentration can be detected in the blood and other body fluids if a tumor of a certain type is present.

Scientific and technological progress in recent years, especially in genomics and proteomics, has significantly expanded knowledge about the molecular basis of tumor progression and the response to anticancer therapy. It has also helped to identify **new biomarkers**, including genes, oncogenes, DNA and RNA fragments, receptors and other substances that can be used to detect early stages of the disease, to predict treatment effectiveness or to prognostically estimate disease relapse after treatment (Table 12.2).

TABLE 12.2 CLASSIFICATION OF TUMOR MARKERS

Category	Examples
Oncofetal proteins	AFP, CEA
Cancer associated antigens	CA 125, CA 15-3, CA 19-9, CA 72-4, SCCA
Hormones and their metabolites	Insulin, calcitonin, β-HCG, HIAA, chromogranin A
Hormonal receptors	Oestrogen and progesterone receptors
Proteins	Monoclonal immunoglobulins, S-100, thyroglobulin
Enzymes	PSA, NSE, bone ALP, thymidine kinase
Ultrastructural components	Cytokeratin: CYFRA 21-1, vimentin
Genes, oncogenes	HER2/neu, ALK, BRCA1, BRCA2, BRC-ABL, KRAS
Nucleic acids	mRNA, miRNA, circulating cell-free DNA

The ideal tumor marker should be produced only by tumor cells and meet the following criteria:

- is specific for one type of tumor (has no false positivity);
- does not increase in healthy individuals (high specificity);
- can be detected in the early stages of the disease (it has no false negativity);
- enables screening and diagnosis of malignancy;
- correlates closely with tumor size;
- allows to assess the effectiveness of treatment;
- enables early detection of disease relapse.

Unfortunately, such an ideal marker does not exist in real life. Most markers can be produced also by normal cells, so the distinction between malignant and benign cancer is often only quantitative. Their sensitivity and specificity is only average depending on the used cut-off value, which serves as a decision criterion. The concentration of TM in the blood is affected by many factors, some of which are listed in Table 12.3

TABLE 12.3 FACTORS AFFECTING CONCENTRATION OF TUMOR MARKERS

Tumor associated	Marker associated
Number of producing cells (tumor size)	Mechanism of secretion
Histological type - degree of differentiation	Elimination from the organism
Growth rate	Degradation rate - biological T1/2
Vascularization	Damage of tissues normally producing TM
Necrosis of tumor cells	Dilution of serum - analytical factor

Use of tumor markers

Despite all the limitations, TMs have become an integral part of the management of patients with various specific forms of malignancies. In clinical practice, physicians follow the framework professional recommendations issued by several international professional societies, e.g. EGTM (European Group on Tumor Markers), ASCO (American Society of Clinical Oncology) or AACC (American Association of Clinical Biochemistry), as well as specific algorithms for the treatment and monitoring of specific tumor types and use appropriate tumor markers based on them (examples are in Table 12.4).

TABLE 12.4 AN OVERVIEW OF MOST FREQUENTLY USED TUMOR MARKERS

Tissue	Histological type	Tumor marker
Breast	adenocarcinoma	CA 15-3, CEA
Ovary	serous mucinous germinal granules	CA 125 CA 19-9 (CEA, CA 72-4) AFP, βHCG inhibin
Cervix uteri	epidermoid	SSCA or CYFRA 21-1
Endometrium	adenocarcinoma	CA 125, CA 19-9
Placenta	trophoblast	βHCG
Prostate	adenocarcinoma	PSA, free PSA, PHI
Bladder	adenocarcinoma	TPA
Testes	seminoma non-seminoma	βHCG βHCG, AFP
Oesophagus	epidermoidal adenocarcinoma	SCCA or CYFRA 21-1 CA 19-9 (CEA)
Stomach	adenocarcinoma	CA 72-4, CA 19-9/CEA
Liver	hepatocarcinoma metastatic	AFP/CEA CEA, AF, CA 19-9, CA 15-3,
Bile ducts	adenocarcinoma	CA 19-9, CEA
Pancreas	adenocarcinoma neuroendocrine	CA 19-9, CEA chromogranin A, 5-HIAA, insulin, glucagon, gastrin
Intestine	carcinoid	5-HIAA, chromogranin A (CEA, CA 19-9)
Colon	adenocarcinoma	CEA (CA 19-9)
Rectum	epidermoidal	SCCA/CYFRA 21-1
Adrenal medulla	pheochromocytoma	metanephrines, VMA
Lung	adenocarcinoma	CEA

	epidermoid (NSCLC) SCLC	SCCA or CYFRA 21-1 NSE, pro-GRP
Thyroid gland	adenocarcinoma medullary	calcitonin, CEA thyroglobulin
Nervous system	neuroblastoma	NSE, homovanilic acid, catecholamines
Skin	melanoma	S 100

Based on published recommendations, the main clinical use of tumor markers in:

- prediction and monitoring of the therapeutic response to anticancer treatment,
- staging of the disease,
- early detection of relapse,
- assessment of the prognosis of the disease.

Only a limited number of TMs are used to screen or diagnose malignancies. In addition to traditional biochemical tumor markers, newer biomarkers, such as circulating tumor nucleic acids, are entering routine practice (INFO 12.1).

INFO 12.1 Liquid biopsy

Despite many advances in diagnostics and multimodal treatment (surgery, radiotherapy, chemotherapy), cancer still remains one of the most important public health challenges worldwide because of the associated morbidity and mortality. "Liquid biopsy" has been developed to detect cancer at an early stage based on minimally invasive and serial body fluid tests with the advantage of following tumor evolution in real time. Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating cell-free noncoding RNAs (cfRNAs) and circulating exosomes represent the major components of liquid biopsy analysis

In healthy individuals, apoptotic cells are the main source of ccf-DNA. They release small, uniform DNA fragments with an average length of ∽185 bp. In contrast, DNA fragments derived from malignant cells have a variable size (70-200 bp; most of them are shorter fragments). Tumor cells can actively release DNA fragments in an effort to affect the transformation of susceptible cells at distant sites during metastasis. In addition, there is increased apoptosis and necrosis of tumor and surrounding healthy cells and decreased degradation of ccf-DNA by DNase and phagocytosis.

Ccf-DNA is a promising diagnostic biomarker, especially for examining the expression of some key tumor-associated genes, which may be altered for genetic reasons but also due to epigenetic modifications (e.g., overmethylation of tumor suppressor genes). Other uses for ccfDNA are: determination prognosis, early detection of recurrence of cancer, prediction of response to anticancer treatment and monitoring of its effectiveness, a biomarker of secondary resistance to treatment due to mutation.

Screening

Screening in general aims to detect early stages of disease in asymptomatic individuals. The low sensitivity and specificity of oncomarkers, especially when used alone without other diagnostic methods or in a population with a low prevalence of the respective cancer, is the reason for their relatively low positive predictive value and unsuitability for population screening. The following tumor markers are an exception:

- **Prostate-specific antigen** (PSA) is used to screen for prostate cancer along with digital prostate cancer testing in men over 45 – 50 years of age. However, opinions on its contribution are ambiguous. Some large clinical studies have shown insignificant differences in patient survival and quality of life over a 10-year follow-up between the groups of patients screened and the control group without screening. **Total PSA** measured in serum includes the predominant complexed form bound to α1chymotrypsin and unbound **free PSA** (fPSA), which occurs in multiple isoforms. At PSA concentrations of 4 –10 μg/L, the percentage of fPSA is a better indicator of cancer than total PSA. One of the isoforms of fPSA is used to calculate the **prostate health index** (PHI): ([-2]proPSA/fPSA) × $PSA^{1/2}$. PHI is used to differentiate between malignant and benign prostate disease in men over 50 years of age with normal digital prostate examination and slightly elevated PSA.
- **Fecal occult blood test** using immunochemical tests to detect human haemoglobin is the recommended population screening for colorectal cancer in asymptomatic individuals over 40–50 years in many countries.
- **α-fetoprotein** (AFP) is a marker for screening for hepatocellular carcinoma in an increased risk population of patients with liver cirrhosis due to chronic viral hepatitis B and C, hereditary hemochromatosis or biliary cirrhosis.
- **Calcitonin** is used to screen for medullary thyroid carcinoma in relatives of patients with this type of tumor (along with RET proto-oncogene mutations).

Identification of patients at increased risk of malignancy

In the last decade, scientists have identified a large number of genes predisposing individuals to the emergence of so-called hereditary tumor syndromes (Table 12.5). Genetic testing can be performed in families at high risk of cancer. A positive result for the presence of a mutation causing a known disease means, that the individual has an increased risk of developing malignancy, but does not guarantee that he/she will succumb to this disease. On the other hand, a negative result in a family with a known mutation means that the tested subject is as likely to develop cancer as the general population of the same age and sex.

TABLE 12.5 EXAMPLES OF HEREDITARY NEOPLASIA SYNDROMES

Syndrome	Predisposing gene
Hereditary breast and ovary carcinoma	BRCA1, BRCA2
Familiar adenomatous polyposis	APC
Hereditary non-polypus colorectal carcinoma	MLH1, MSH2, MSH6, PMS1
Multi-endocrine neoplasia syndrome type 1	MEN1
Multi-endocrine neoplasia syndrome type 2	RET
Retinoblastoma	RB
Familiar melanoma	CDK2, CDKN2A

The potential benefit of testing for congenital susceptibility to certain malignancies provides to the individual:

- a more accurate risk assessment with the possibility of strict regular monitoring (e.g. mammography, colonoscopy), which will allow early detection of the tumor;

- prophylaxis of the disease through more radical procedures (e.g. mastectomy, ovarian removal, colectomy).

Diagnosis

Diagnosis applies to patients with specific manifestations, which may or may not be caused by cancer. Although elevated TM levels may indicate the presence of a tumor, it alone is not sufficient to make a diagnosis. Imaging and biopsy methods are of particular diagnostic importance. In rare cases, they allow

TM distinguishing between benign and malignant disease, for example:

- CA125 and HE4 and the ROMA index calculated from them allows the differentiation of tumor and non-tumor mass detected by ultrasound examination in the small pelvis in premenopausal and postmenopausal women;
- An increase in AFP >200 μg/L together with a typical ultrasound finding of the liver confirms the diagnosis of hepatocellular carcinoma without the need for biopsy evidence.

Estimation prognosis

After removal of the primary tumor, the key issues in the management of the cancer patient are the aggressiveness of the tumor and the need for further anticancer treatment. Traditionally, the doctor decides based on histological criteria (tumor size, stage - grading, condition of local lymph nodes) and clinical criteria (age, comorbidities). Forecasting the TM value is that its concentration indirectly reflects the extent of the cancer. The main use of TM is to monitor patients after surgery or anticancer treatment and to detect the return or relapse of the disease. Examples of TMs that have prognostic value in certain cancers:

- CEA in colorectal cancer,
- βhCG and AFP in germinal testicular tumors,
- CYFRA 21-1, CEA in non-small cell lung cancer (NSCLC),
- NSE and pro-GRP in small cell lung cancer (SCLC),
- thyroglobulin after thyroid ablation (increase in its serum concentration indicates possible relapse of the disease).

Prediction of treatment effectiveness

Predictive TMs are specific biomolecules (proteins or genes) that signal the sensitivity or resistance of a tumor to a specific anticancer treatment and allow better identification of patients who will respond positively to treatment. Currently, several predictive TMs are used in clinical practice (Table 12.6), which are mandatory before initialization of specific treatment.

In addition to assessing the effectiveness of treatment, newer predictive markers also allow adjustment of the optimal dose and prevention of toxic side effects, thus contributing to the personalized treatment of cancer patients. A classic example is the examination of **HER2/neu** (human epidermal growth factor receptor 2) expression in breast cancer patients before treatment with Herceptin (anti-HER2/neu). Chronic myelocytic leukemia (CML) is another example of a disease, in which evidence of the **BCR-ABL oncogenic fusion protein** in the peripheral blood by FISH method (fluorescence in-situ hybridization) is necessary to make a diagnosis. During treatment with tyrosine kinase inhibitors (e.g. imatinib), peripheral blood

BCR-ABL levels are then monitored regularly by quantitative real-time PCR to determine individual response to treatment.

TABLE 12.6 BIOMARKERS USED FOR PREDICTION EFFECTIVNESS OF THERAPY

Tumor	Marker	Therapy
Breast ca	Oestrogen and progesterone receptors HER2	hormonal (tamoxifene, aromatase inhibitors) herceptin (anti-HER2)
Colorectal ca	KRAS	anti-EGFR (gefitinib, erlotinib, afatinib)
CML	fusion gene BCR-ABL	tyrosine kinase inhibitors (imatinib)
NSCLC	EGFR, EMOL4-ALK	EGFR tyrosine kinase inhibitors anti-ALK (crizotinib, ceritinib)
Melanoma	BRAF	anti-BRAF

Interpretive difficulties in evaluating tumor markers

The biochemical laboratory receives three types of requirements for the examination of tumor markers in patients:

1. with a diagnosed malignant disease who are regularly monitored by TM;
2. investigated for a serious suspicion of the presence of malignancy;
3. with non-specific clinical signs that could be caused by cancer.

First-group examinations are warranted and usually monitor treatment response or detect relapse after treatment. In patients of the second group, physicians should consider the benefits they expect from the results of TMs they intend to order. As already mentioned, most TMs have no sufficient sensitivity or specificity, especially in the early stages of cancer, to be diagnostic tests. A negative result does not rule out the presence of malignancy and, conversely, an increased concentration of TM does not always indicate its presence of TM (Table 12.7).

TABLE 12.7 EXAMPLES OF NON-SPECIFIC ELEVATION OF TUMOR MARKERS

Marker	Benign conditions
AFP	viral hepatitis, liver failure, inflammatory bowel disease, pregnancy
β HCG	testicular failure, marijuana smoking, pregnancy
CEA	smoking, inflammatory diseases of the intestine, liver, pancreas and stomach, cirrhosis
CA 125	irritation of the peritoneum, endometriosis, menstruation, inflammatory diseases in the pelvis, hepatitis, pregnancy
PSA	prostatitis, benign prostatic hyperplasia
β2-microglobulin	kidney and liver failure, inflammatory diseases
CYFRA 21-1	kidney and liver failure
NSE	hemolysis, cerebral hemorrhage

The specificity of some markers can be increased by serial examination and determining the rate at which the concentration of a given TM varies in a particular patient. The predictive value of a positive or negative result significantly depends on the population in which the TM is being investigated. An essential requirement in disease monitoring using TM is the use of **the same method and one laboratory**. TM values during monitoring are compared more often with the patient's baseline values before treatment than with reference intervals.

The **half-life** (T1/2) and the **minimum value** of the tumor marker reached during treatment (min [TM]) are taken into account when assessing the effect of treatment. Half-life represents the time required to reach half the TM concentration of the value at the beginning of treatment. This parameter reflects the rate at which tumor size decreases. Its value depends on the type of anticancer treatment and its effectiveness. In the initial phase of effective systemic treatment, a paradoxical increase in TM may occur, which is a manifestation of massive cell lysis or hepatocyte regeneration.

The **half-life** (T1/2) and the **minimum value** of the tumor marker reached during treatment (min [TM]) are taken into account when assessing the effect of treatment. Half-life represents the time required to reach half the TM concentration of the value at the beginning of treatment. This parameter reflects the rate at which tumor size decreases. Its value depends on the type of anticancer treatment and its effectiveness. In the initial phase of effective systemic treatment, a paradoxical increase in TM may occur, which is a manifestation of massive cell lysis or hepatocyte regeneration.

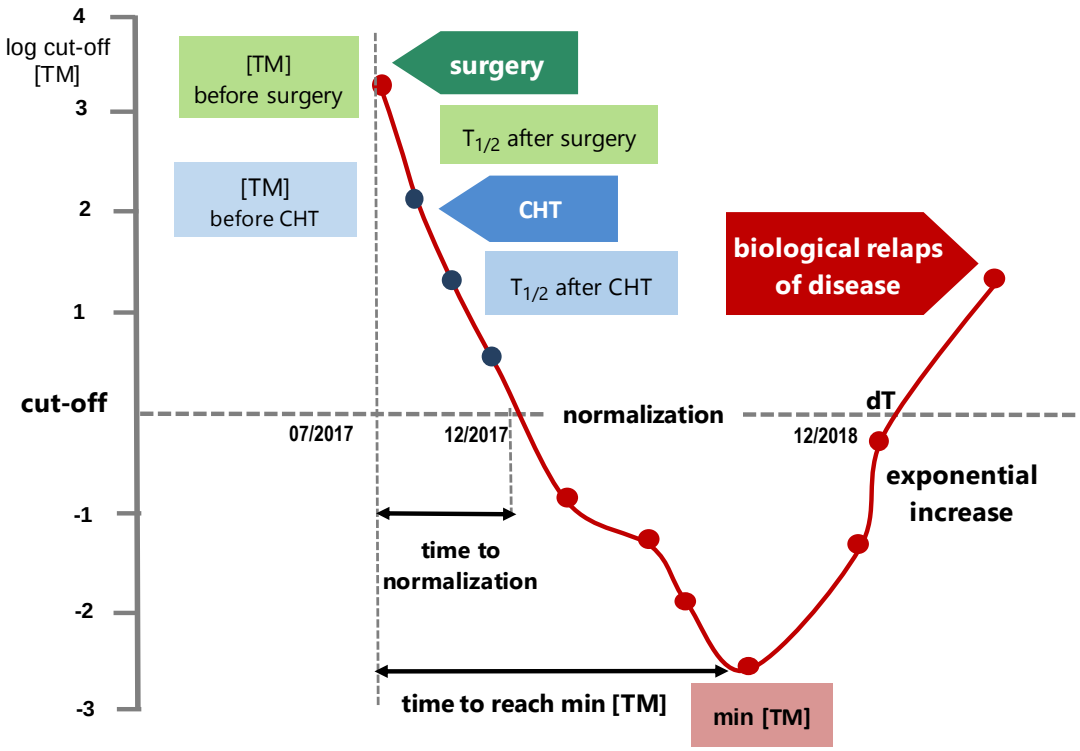


FIGURE 12.1 Example of tumor marker kinetics

Minimum TM values after treatment are a good indicator of residual disease. The persistence of a high level of min [TM] may signal the existence of a residual secretory tumor. Return to basal values usually means eradication or disappearance of the cancer, although sometimes only the secreting cell clone. Individual TM kinetics may be useful in the early detection of

recurrent disease or metastasis. The exponential increase in TM levels even within the reference interval usually indicates tumor regrowth. The growth rate can be assessed using the doubling time (dT), which is calculated from three measured values and reflects the rate of initial progression of recurrent disease (Figure 12.1).

12.3 Endocrine manifestations of tumors

Some tumors are able synthesize and secrete hormones or structurally and functionally similar substances. In tumors of endocrine organs such as insulinoma or adrenal adenoma, this **eutopic hormone production** is an expected finding. However, if the hormones are produced by tumor cells of non-endocrine origin, this is an **ectopic production of hormones** (Table 12.8). Modern molecular biological methods have revealed that many tissues can synthesize hormones that act in these tissues at the local level, in a paracrine manner similar to cytokines. It is not entirely clear what mechanisms support the production of those hormones and their precursors in tumor cells.

Most of those paraneoplastic endocrine syndromes are caused by the production of peptide hormones or hormonal precursors in tumor cells of neuroendocrine origin. Ectopic hormone production has several common properties which distinguish them from an overproduction of hormones by endocrine glands:

- Ectopic hormone production is rarely suppressible.
- Hormones are relatively ineffective or their molecules are incompletely post-translationally processed and have a lower biological effect, therefore they cause clinical syndromes only in advanced stages of malignancy.
- Tumors can mimic endocrine syndromes by secreting hormone-like molecules with biological activity (PTH and PTH-related peptide).

TABLE 12.8 EXAMPLES OF ECTOPIC HORMONE PRODUCTION BY TUMORS

ADH	ACTH	PTHrP
lung (SCLC)	lung (SCLC)	breast
duodenum, pancreas,	thymoma	epidermoid carcinoma
colon, bladder	pancreas	kidney, ovary, bladder
carcinoid	bronchial carcinoid	NSCLC
mesothelioma	pheochromocytoma,	endocrine: insulinoma
head and neck ca	medullary ca	pheochromocytoma,
lymphomas	neural tumors	lymphomas, multiple myeloma

Ectopic ACTH production

Cushing's syndrome (CS) is a set of clinical symptoms resulting from exposure of body tissues to supraphysiological concentrations of glucocorticoids (hypercortisolism). The syndrome may be caused by increased ACTH production in the pituitary gland (Cushing's disease), overproduction of cortisol in the adrenal cortex (unilateral tumor or bilateral hyperplasia) or ectopic ACTH production in other tumors (5 – 15% of cases). The most common ectopic sources of ACTH are neuroendocrine lung tumors (small cell lung cancer and bronchial carcinoid),

thymomas, pancreatic tumors and medullary thyroid cancer. In addition to ACTH, its precursors (POMC - proopiomelanocortin; pro-ACTH) or CRH (corticoliberin) are sometimes formed, which also stimulate the overproduction of cortisol in the adrenal cortex.

Clinical manifestations of paraneoplastic CS may include hypertension, hypokalemia, muscle weakness, or generalized oedema. Weight gain with typical centripetal fat distribution, moon face, acne, and hyperpigmented striae are less common. Unlike other paraneoplastic endocrine syndromes (SIADH and hypercalcemia), clinical signs occur before the diagnosis of cancer. The appearance of paraneoplastic CS may similarly herald tumor recurrence. Laboratory tests listed in Table 12.9. may help distinguishing ectopic CS from Cushing's disease caused by pituitary tumors. Imaging methods (CT, MR, scintigraphy with ^{111}In-octreotide taken up by somatostatin receptors) and endoscopic methods focused on the chest and abdomen are used for the localization of the primary tumor.

TABLE 12.9 LABORATORY FINDINGS IN CUSHING DISEASE AND ECTOPIC CS

Test	Cushing disease	Ectopic CS
Cortisol in serum and urine	↑	↑↑↑
P-ACTH	↑–↑↑	↑↑↑
S-cortisol after high-dose DXM suppression	↓	no response
Response of S-cortisol after CRH	augmented response	no response
Screening of other TM (βHCG, AFP, calcitonin, 5-HIAA, chromogranin A	negative	positive

Paraneoplastic hypercalcemia

Hypercalcemia occurs in up to 10% of patients with advanced malignancies and usually indicates a poor prognosis. The three most common mechanisms participate in its creation:

1. Secretion of parathyroid hormone-like peptide (PTHrP) by tumor cells is the cause of up to 80% of cases and often occurs in epidermoid tumors. Upon binding to PTH receptors in bone and kidney, PTHrP increases bone resorption and calcium reabsorption in the kidney. Ectopic production of intact PTH by tumors is very rare.
2. Direct, osteolytic activity at the site of bone metastases accounts for another 20% of causes. It is often present in breast, lung and ovarian cancers and primary bone tumors, such as multiple myeloma and lymphomas. Osteolytic lesions leading to hypercalcemia are caused by the activation an of osteoclastogenic cytokines (e.g. TNF-α, IL-1, IL-6).
3. Rarely, hypercalcemia can be caused by tumor secretion of vitamin D, especially in some types of lymphoma.

Typical laboratory findings in cancer patients with hypercalcemia:

- Increased total serum calcium; in hypoalbuminemic patients, it is recommended to evaluate calcium corrected for normal albumin;
- Low or normal PTH concentration;
- Increased activity of ALP or its bone isoenzyme - may support the diagnosis of hypercalcemia due to tumor bone resorption;
- Elevated PTHrP, if available.

Ectopic secretion of antidiuretic hormone

Syndrome of inappropriate antidiuretic hormone secretion (SIADH), manifested by hypoosmotic euvolemic hyponatremia and relatively increased natriuresis, affects about 1 – 2% of cancer patients. The most common causes of SIADH are small cell lung cancers (10 – 45% of these tumors), less frequently tumors of the head and neck, pancreas, prostate, brain, and leukemia. Tumor cells produce an antidiuretic hormone (ADH) regardless of the body's existing osmolality. This inappropriate secretion leads to increased reabsorption of solute-free water and the formation of concentrated, hyperosmolar urine. In addition, natriuretic peptides, which in some cases are formed directly in tumor cells together with ADH or are excreted physiologically in response to subclinical hypervolemia, also contribute to the development of massive hyponatremia. The diagnostic criteria of SIADH were presented in the chapter 2 (INFO 2.2).

Hypoglycemia

Hypoglycemia associated with the presence of a tumor is a rare but serious complication in cancer patients. Several mechanisms are involved in its formation, which can be combined:

1. Excess insulin production by tumors arising from the pancreatic islets of Langerhans (insulinomas) or less often by extra pancreatic tumors. Insulinoma is the most frequent tumor associated with clinical and laboratory hypoglycemia. Insulinomas can occur as a part of multiple endocrine neoplasia type 1 (in 5%); about 5 – 10% of these tumors are malignant.
2. Insufficient gluconeogenesis, which maintains normal glycemia by supplying glucose during starvation. As the majority of gluconeogenesis takes place in the liver, hypoglycemia occurs in patients with severe primary or metastatic liver cancer.
3. Tumor secretion of peptides, most commonly IGF-2 (insulin-like growth factor 2), somatostatin and glucagon-like peptide 1 (GLP-1), increases glucose utilization in cells by several different mechanisms. This type of non-islet cell tumor hypoglycemia (NICTH) is manifested by recurrent or persistent clinical signs of hypoglycemia with values <1 mmol/L. It mainly affects elderly patients with advanced tumors in the abdominal cavity, such as retroperitoneal sarcomas, mesotheliomas, hepatomas, malignant lymphomas or kidney tumors.

INFO 12.2 Laboratory findings in paraneoplastic hypoglycemia

IGFs are peptides structurally similar to insulin, which bind to insulin receptors. Both IGF-1 and IGF-2 physiologically bind to the binding protein (IGFBP3) and together form a complex that is too large to cross the capillary wall and reach insulin receptors.

However, some tumors produce the precursor protein IGF-2 (big IGF-2), which circulates in the blood partially unbound and its free molecules cross the endothelial barrier to insulin receptors in the tumor itself, but also in the liver and skeletal muscles. The laboratory distinction between the causes of tumor-induced hypoglycemia is based on the characteristic findings:

- Serum insulin and C-peptide concentrations are elevated or normal (disproportionately high for a given hypoglycemia) in insulinoma and undetectable in NICTH.
- Growth hormone, IGF-1 and IGFBP3 levels are normal in insulinoma and low in NICTH.
- The IGF-2 to IGF-1 ratio is generally in the reference range for insulinoma compared to elevated IGF-2 and the IGF-2 to IGF-1 ratio (>10) for NICTH.

All three types of hypoglycemia endanger patients, especially during periods without food, typically during night's sleep. In the differential diagnosis of paraneoplastic hypoglycemia, it is recommended to examine serum concentrations of insulin, C-peptide, proinsulin, IGF-1 and IGF-2 (INFO 12.2).

12. 4 Other manifestations of tumors detectable by laboratory findings

During the rapid progression of the tumor, the nutritional status of the host organism changes to meet the demands of the growing tumor. Numerous metabolic changes occur in cells (INFO 12.3). However, we do not detect these changes by routine laboratory tests, we only assume them.

INFO 12.3 Metabolic changes in tumor cells

- Increased transport and uptake of glucose by cells - increased expression of glucose transporters - GLUT1 and GLUT3, caused mainly by hypoxia.
- Increased rate of glycolysis - activation of key regulatory enzymes and shift of glucose metabolism from oxidative phosphorylation towards anaerobic glycolysis (Warburg effect), leads to the accumulation of lactate.
- Increased de novo synthesis of purine and pyrimidine nucleotides.
- Activation of DNA and RNA synthesis.
- Secretion of proteases (tissue metalloproteinases - MMPs, cathepsins, collagenases, elastase, etc.).
- Reduced production of protease inhibitors.
- Autocrine secretion of growth factors.
- Changes in the composition of cell membranes - change in the size and shape of cells.
- Changes in gene expression – re-expression of proteins and enzymes characteristic of embryonic cells that can be used as TM.

In addition to general and local manifestations, a growing tumor can lead to a variety of abnormalities in **routine biochemical examinations**. The most common causes of pathological laboratory results in cancer patients are:

1. **Obstruction of blood vessels or ducts:** For example, occlusion of the bile duct by a pancreatic tumor causes an increase in the activity of the cholestatic enzymes ALP and GMT in serum as a result of cholestasis, as well as hyperbilirubinemia.
2. **Tissue destruction**: The liver is a common site of tumor metastasis, manifested only by an isolated increase in ALP or GMT. If the rate of tumor destruction is higher, AST and ALT activity also increases. Bone metastatic tumors similarly lead to an increase in biochemical bone markers, e.g. bone isoenzyme ALP or CTX.
3. **Rapid growth and cell turnover:** It often occurs in leukemias and lymphomas and is associated with an increase in uric acid and LD. Uric acid results from the degradation of purine nucleotides in nucleic acids, while the increased activity of LD, a ubiquitously occurring glycolysis enzyme, reflects rapid cell turnover. Some large solid tumors with

insufficient blood supply provide sufficient energy through anaerobic glycolysis, leading to increased lactate production and lactic acidosis.

4. **Renal failure:** It is a relatively common finding in cancer patients and has several causes. These include tumor obstruction of the urinary tract, hypercalcemia, hyperuricemia, monoclonal free light chains (Bence-Jones proteinuria) or nephrotoxicity of cytotoxic drugs.

Tumor lysis syndrome

Tumor lysis syndrome (TLS), one of the most serious acute conditions in oncology, is caused by the sudden breakdown of a large number of tumor cells, most often after specific chemotherapy. It can also complicate other types of anticancer treatment (radiotherapy, hormonal or biological treatment) and can occasionally occur spontaneously. There is a higher risk of developing TLS in haematological malignancies or other fast-growing tumors. From the disintegrated tumor cells, their intracellular content is released into the bloodstream, which results in characteristic laboratory findings (Table 12.10). Risk factors for the development of TLS are pre-existing chronic kidney disease, oliguria, dehydration, pre-existing hyperuricemia and acidosis.

TABLE 12.10 LABORATORY FINDINGS IN TUMOR NECROSIS SYNDROME

Finding	Dg criteria	Consequence
Hyperuricemia	>480 μmol/L In children >ULRI	Precipitation in renal tubules– AKI vasoconstriction, oxidation, inflammation
Hyperkalemia	>6.0 mmol/L	bradycardia, fibrilation, hypotension, asystolia
Hyperfosfatemia	>1.5 mmol/L in adults >2.1 mmol/L in children	intra- and extra-renal precipitation of calcium-phosphates: AKI, gangrenous changes of skin, iriditis, arthritis, secondary hypocalcemia
Hypocalcemia (Ca corrected for S-ALB)	<1.75 mmol/L	Neuro-muscular irritability: carpopedal spasms, muscle cramps, bradycardia -asystolia
Cytokines		SIRS, multi-organ dysfunction/failure

The consequences of cell breakdown - hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcemia - can potentially result in serious clinical consequences. In hyperkalemia, the patient is at risk of arrhythmias and sudden cardiac death. Phosphates released from cells precipitate with calcium in soft tissues. Accumulation of calcium-phosphates and especially uric acid leads to kidney damage and can cause AKI. Increased neuromuscular excitability, arrhythmias and seizures are consequences of secondary hypocalcemia.

Uric acid, the end product of purine nucleotide degradation, not only precipitates in renal tubules at acidic urine pH, but also damages the kidney by a mechanism independent of crystallization, such as vasoconstriction with reduced renal perfusion, oxidation, and inflammation. Besides, the breakdown of tumor cells leads to a massive release of cytokines, which cause the systemic inflammatory response syndrome (SIRS) and often multi-organ dysfunction to failure.

The basic principles of TLS treatment are rehydration with regular monitoring of renal and cardiac function, administration of urate-lowering medications (allopurinol - xanthine oxidase inhibitor, rasburicase - recombinant uric acid oxidase), calcium supplementation and elimination of potassium and phosphorus (ion exchangers). Alkalization of urine is not recommended as phosphates and uric acid precursors (xanthine and hypoxanthine) precipitate at pH >7.5, negating the potential advantage of better solubility of uric acid at alkaline urine.

Tumor cachexia

Tumor cachexia is a multifactorial condition involving the loss of skeletal muscle and bone tissue that does not respond to conventional nutritional support. The main clinical signs of cachexia are unintentional weight loss (corrected for water retention) of more than 10% in adults and growth retardation in children not due to an endocrine disorder. Weight loss is assessed at the time of diagnosis in 80% of patients with upper GIT tumors and in approximately 60% of lung cancers.

The aetiology of tumor cachexia is complex, caused by anorexia, dysregulated metabolic status, and increased basal energy expenditure. Plenty of factors derived from the metabolism of the tumor and the host organism are involved in the development of cachexia, which eventually results in a chronic inflammatory state and catabolism with subsequent clinical signs of malnutrition (Figure 12.2).

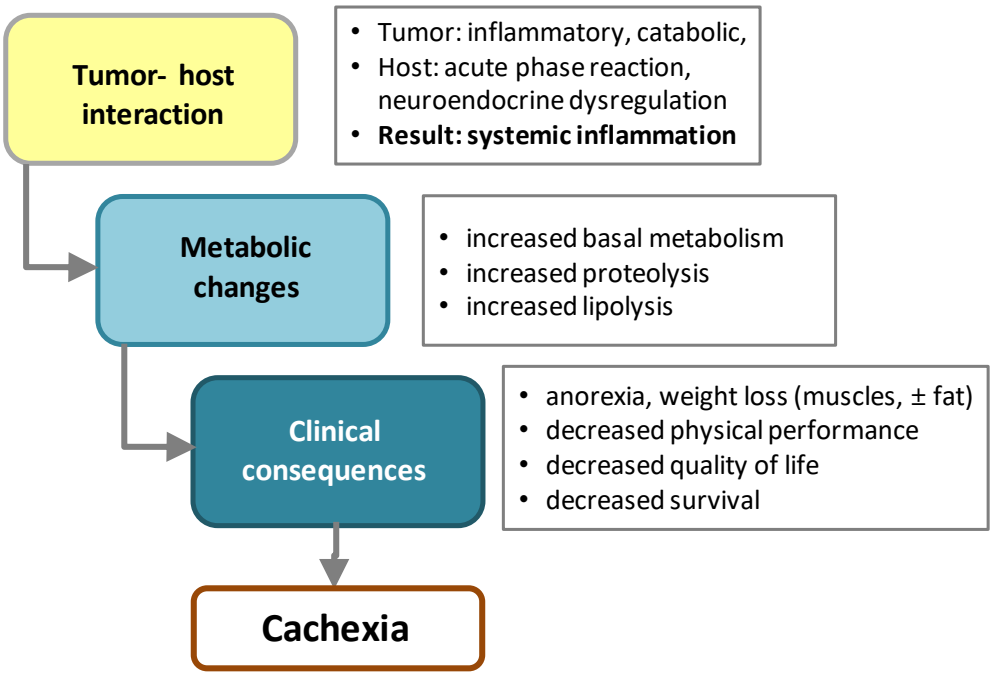


FIGURE 12.2 Scheme of origin and clinical consequences of tumor cachexia

Typically, cachexia affects the **heart muscle**, leading to heart failure and arrhythmias. Complex metabolic changes in the **liver** are characterized by insulin resistance, enhanced gluconeogenesis, which consumes lactate derived from glycolysis in tumor cells, altered proteosynthesis in favour of positive acute phase reactants, and decreased albumin synthesis. The function of the brain, digestive and immune systems is regularly affected.

Diagnostic criteria for cachexia are not uniform, but always include reduced BMI (<20 in subjects younger than 70 years) and other clinical and laboratory parameters informing about the following attributes of this condition, which are:

- chronic inflammation: CRP >10 mg/L, elevated IL-6, leukocytosis;
- anemia: Hb <120 g/L;
- protein depletion (reduced intake and proteosynthesis in the liver), S-Alb and prealbumin below the lower limit of RI;
- reduced food intake: anorexia, fatigue;
- loss of muscle mass: decreased muscle strength, decreased S-creatinine, increased U-creatinine.

Case studies and self-assessment questions

Case report 12.1

An 8-year-old boy was examined in an ORL clinic for several months worsening snoring, fatigue, sore throat, enlarged tonsils, and painless enlargement of the cervical lymph nodes. He was given dexamethasone (4 mg IM) for considerable congestion of the nasal mucosa and prescribed loratadine. Over the next 48 hours, the boy's weakness got worse and he vomited repeatedly, so his parents brought him to the emergency department with signs of dehydration. Laboratory tests are listed in the table.
CT of the lungs revealed a small mediastinal mass. Intensive rehydration treatment with isotonic NaCl and rasburicase and $Al(OH)_3$ were given at the beginning of hospitalization. The patient was transferred with a diagnosis of acute lymphoblastic leukemia to the intensive care unit, where oliguria developed and biochemical findings reached a maximum on day 3. Renal function returned to normal without the need for hemodialysis.

Serum test	Day 1	Day 3	RI
Na	133		135 – 145 mmol/L
K	5.9		3.6 – 5.3 mmol/L
Urea	10.8		1.8 – 6.7 mmol/L
Creatinine	88.4	349	27 – 88 μmol/L
Uric acid	732	1055	140 – 340 μmol/L
P	2.7	3.6	1.1 – 1.9 mmol/L
Ca	1.7	1.45	2.1 – 2.7 mmol/L
WBC	81 200		4.5 – 13.5 × 10^9/L
Circulating lymphoblasts in peripheral blood smear			

Questions:

a. What is the laboratory diagnostic criteria for tumor lysis syndrome?
b. What is the significance of rasburicase administration in the patient?
c. Does the patient meet the criteria for acute renal failure?

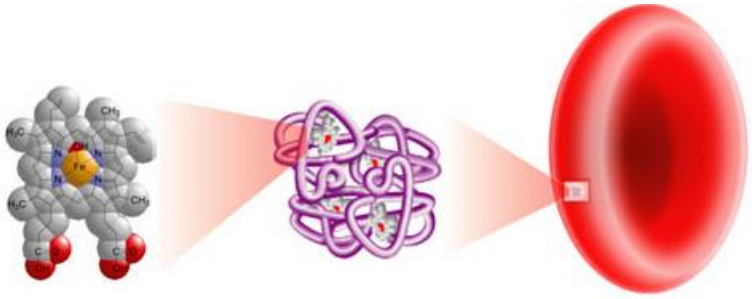
Self-assessing questions

1. What is the main clinical use of biochemical tumor markers?
2. List examples of the use of TM in cancer screening.
3. What are the characteristic features of ectopic hormone production?
4. What mechanisms are involved in the development of malignant hypercalcemia?
5. Name the characteristic biochemical findings in tumor lysis syndrome.

KEY INFORMATION

- ☑ Tumor markers are biochemical substances produced mainly by the tumor itself or even by normal cells of the organism in response to the presence of the tumor.
- ☑ Normal values of tumor markers do not rule out the presence of a tumor. Their increased concentration not always caused by a tumor.
- ☑ The main clinical significance of TM is in predicting and monitoring the response to anticancer therapy, early detection of relapse, and assessment prognosis of disease.
- ☑ The use of traditional TMs in the screening and diagnosis of cancer is limited.
- ☑ It is important to measure TMs by the same method in one laboratory when monitoring cancer patients.
- ☑ Cushing's syndrome (hypercorticism) and SIADH (euvolemic hypotonic hyponatremia) are common among paraneoplastic endocrine syndromes.
- ☑ Advanced tumors are also manifested with other pathological biochemical findings, such as hypercalcemia, hypoglycemia, obstructive hyperbilirubinemia.
- ☑ Tumor lysis syndrome involves an acute oncological condition that threatens the patient with acute renal failure and cardiac arrhythmias.
- ☑ Typical biochemical findings of TLS are hyperuricemia, hyperkalemia, hyperphosphatemia and hypocalcemia.

13



Heme metabolism disorders: iron and porphyrine

CONTENT

Iron (Fe) is an essential element necessary not only for erythropoiesis, but also for cell respiration, cell proliferation and differentiation, regulation of gene expression, cell signalling and also for some immune functions. Iron deficiency, especially in the first two years of life, affects the mental and physical development of children and can endanger cognitive and motor functions. In the elderly, iron deficiency can result in poor physical performance and undesirable complications in pregnancy. Excess iron can damage parenchymal organs, especially the liver, heart and pancreas. Understanding Fe metabolism has changed dramatically over the past two decades due to knowledge of its cellular and systemic homeostasis and the identification of many key proteins that regulate Fe metabolism.

This chapter focuses on:

- disorders of iron metabolism and heme with emphasis on laboratory tests used for their diagnosis;
- some biochemical aspects of anemia.

13.1 Basic physiology

Basic iron metabolism

The body of an adult contains approximately 3 – 4 g (50 – 70 mmol) of iron in three compartments, between which it is intensively exchanged. Free iron (Fe^{2+}) is a transient and highly reactive element that is involved in radical reactions, which contributes to the formation of reactive forms, especially oxygen (hydroxyl radicals), which can damage the body. Therefore, free iron is inactivated by binding in complex molecules, proteins and several antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase or catalase. The intake and distribution of iron are strictly regulated, as there is no mechanism in addition to bleeding to eliminate excess Fe from the body. In transport and storage proteins, iron occurs in the form of Fe^{3+}. It passes through cell membranes only by active transport in the form of Fe^{2+} and this form, it also occurs in hemoglobin (oxidized and reduced).

Iron occurs in the body in three different forms:

1. The **functional form** is mainly iron-binding **heme** in hemoglobin (about 75% of total Fe). A minor portion of heme iron is found in muscle myoglobin, Fe-containing enzymes (e.g. catalase) and cytochromes.
2. The **storage form** (about 25% of total iron) consists of the protein's ferritin and hemosiderin, found in each cell, but especially in the macrophages of the liver, spleen and bone marrow, as well as in enterocytes. **Ferritin** is a safe form of intracellular stores iron in the soluble and readily available form. One molecule of ferritin holds up to 4 000 Fe^{3+} atoms. **Hemosiderin** is a product of incomplete lysosomal degradation of ferritin. Ferritin is stored in the cytoplasm of cells, so release of iron is easier than from hemosiderin found in lysosomes.
3. The **transport form** is plasma iron bound to **transferrin** - a single-chain β-globulin that contains two binding sites for iron, which are usually saturated in 20 – 30%. Iron bound with transferrin makes up only <0.1% of the total amount in the body, e.g. 50 – 70 μmol, and this form is determined as serum iron (S-Fe).

Absorption, recirculation and storage of iron

The absorption of iron from food takes place mainly in the duodenum, in the brush border of enterocytes, which regulate the intensity of absorption and storage of iron in the form according to the needs of the organism (Figure 13.1). From the iron content in a mixed diet (10 – 20 mg/day), only about 5 – 10% is absorbed, with heme Fe^{2+} being absorbed better than non-heme Fe^{3+} of plant origin, which must be reduced (in the acidic environment of gastric juice) to Fe^{2+}. In iron deficiency and conditions with increased erythropoiesis, absorption by active transporters can increase up to 20 – 30% in iron deficiency and with increased erythropoiesis. On the contrary, the absorption of iron is reduced if a diet is rich in calcium, phytates, oxalates, polyphenols (tannins in tea, coffee, cocoa), as well as if iron body stores are increased.

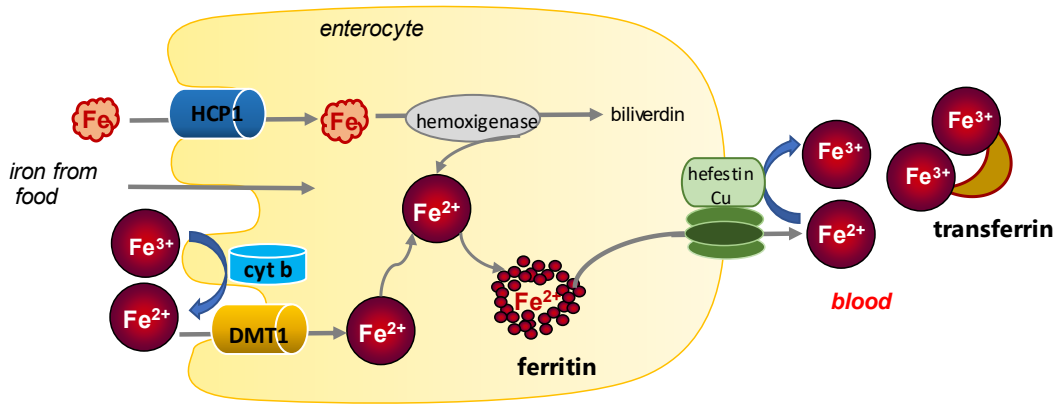


FIGURE 13.1 Absorption of iron in enterocytes. DMT1 – divalent metal transporter 1, HCP1 – heme carrier protein1, FPN – ferroportin, cytB – cytochrome M

The absorbed iron is again oxidized to Fe^{3+} by the activity of ceruloplasmin. The part of iron is stored in the form of ferritin in enterocytes, the rest is incorporated into transferrin and transported to cells that use it in different ways: erythroid precursor cells for hemoglobin synthesis, muscle for myoglobin synthesis and other cells incorporate iron into storage proteins.

Iron from disintegrated erythrocytes, stored as ferritin in the macrophages of the bone marrow, spleen, and liver, can also be reused for erythropoiesis. Physiological excretion of iron from the body is possible only in feces (unabsorbed Fe from food) as well as exfoliated skin and intestinal mucosa cells, which change relatively quickly (up to 1 mg/20 µmol per day). Women also lose Fe in blood (menstruation, pregnancy).

Hepcidin, a peptide hormone (25 AK) produced by the liver, is a central regulator of iron balance. Its physiological role is to inhibit the supply of iron from enterocytes and macrophages to the plasma, which is ensured by reducing the number of transport channels called **ferroportin** on the surface of these cells. Increasing iron stores and inflammation of any origin stimulate synthesis of hepcidin. Hepcidin production during the inflammatory response is induced by cytokines, especially IL-6. On contrary, in iron deficiency (e.g. in anemia, hypoxia and increased erythropoiesis in the bone marrow) low concentration of hepcidin allows a smooth iron supply to the blood (Figure 13.2).

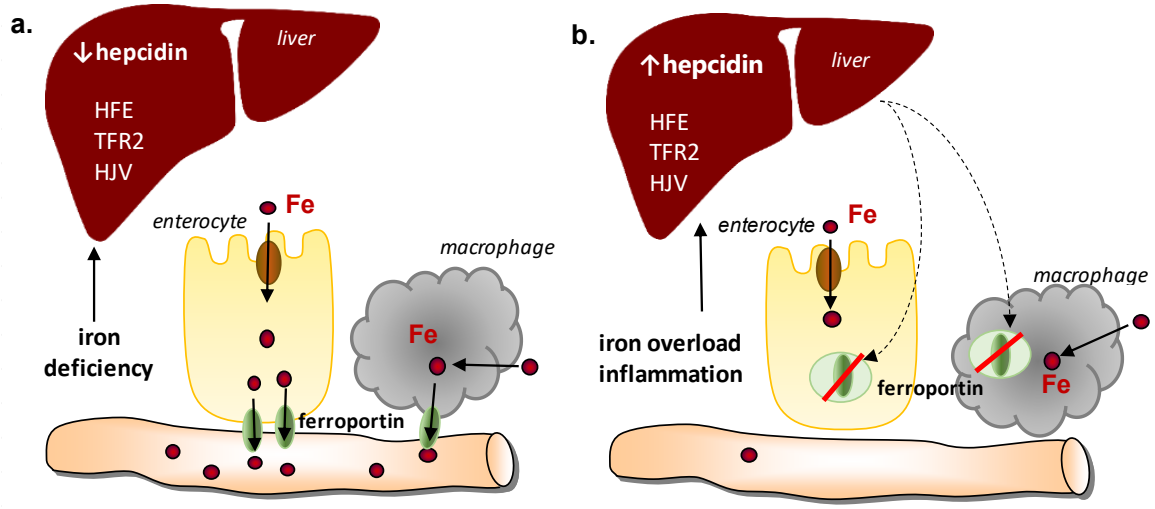


FIGURE 13.2 The role of hepcidin in iron absorption

At the systemic level, **transferrin saturation** with iron is a major factor regulating hepcidin production. At the cellular level, several mechanisms control the production of several regulatory proteins in iron homeostasis (INFO 13.1).

INFO 13.1 Proteins involved into iron homeostasis

IRP1 and IRP2 (iron regulatory protein 1, 2) are proteins that bind to non-coding regions of mRNA intended for the production of proteins involved in iron homeostasis, e.g. transferrin receptors, DMT1 (divalent metal transporter 1), ferroportin. These sections are called iron response elements (IRE). In condition with iron deficiency, IRP1/2 binds to the IRE, stabilizing the mRNA molecule, preventing its degradation, and thereby increasing the production of the encoded protein. There are different forms of mRNA (with or without IRE) in different tissues, which determines the different behaviour of cells concerning iron uptake and storage.

DMT1 is a transport channel for Fe^{2+} in enterocytes, but also erythroid precursors, hepatocytes and macrophages. It transports Fe from transferrin to the cytosol of cells and its transcription and synthesis increases with Fe deficiency in cells.

Ferritin protects cells from the toxicity of free iron. Ferritin mRNA also contains IRE and its translation increases with increasing intracellular iron content.

Ferroportin is the only known export channel for iron, found mainly on macrophages (spleen, liver), enterocytes, hepatocytes, erythrocytes and their precursors. It transports Fe^{2+} extracellularly and after oxidation with ceruloplasmin, Fe^{3+} binds to transferrin. Ferroportin is a target molecule for hepcidin.

13.2 Laboratory assessment of iron metabolism

The following laboratory tests are used to confirm suspected iron deficiency or excess in the body.

Serum iron (S-Fe)

The S-Fe test has a limited diagnostic value, because its concentration fluctuates significantly even in healthy individuals. It decreases in sideropenia and increases with an iron excess in the body, but these changes are evident only in advanced disorders of iron balance. Examination of S-Fe concentration is of diagnostic importance especially in case of suspicious iron intoxication or screening for haemochromatosis.

Among the many factors affecting S-Fe, the following are significant:

- **diurnal rhythm**: higher S-Fe level in the morning than in the afternoon and evening;
- **menstrual cycle**: higher values in the luteal phase and lower values at the end of the cycle;
- **p. o. contraceptives**: increase the concentration of S-Fe;
- **pregnancy**: reduces S-Fe by hemodilution as well as increased demands on Fe;
- **acute infections and trauma**: decreased S-Fe is due to increased hepcidin production and reduced transferrin synthesis (negative acute phase reactant);
- **chronic** inflammatory diseases and malignancies;
- **chronic liver disease**: elevated S-Fe levels are due to point necrosis of the liver parenchyma.

Transferrin and total binding capacity

Transferrin transfers Fe^{3+} to the cells containing transferrin receptors; 2/3 of the receptors are found on erythroid precursor cells, and 1/3 in other tissues. Physiologically, about 30% of the binding sites on transferrin are saturated with iron. **Transferrin** can be measured directly as a protein concentration, or indirectly through its ability to bind externally supplied Fe - as **total iron binding capacity** (TIBC). The physiological TIBC for iron varies in range 45 – 72 μmol/L. Because the half-life of transferrin is relatively long, changes in its concentration do not reflect short-term fluctuations in S-Fe. The ratio between S-Fe and TIBC indicates the percentage of **transferrin saturation**. There is an inverse relationship between TIBC and transferrin saturation.

Typical physiological and pathological changes in serum transferrin are listed in Table 13.1. Examination of transferrin saturation has a diagnostic value especially in the detection of latent hemosiderosis, which is indicated by values >60%. Examination of TIBC in patients with chronic kidney disease treated with erythropoietin provides better information on the availability of Fe than ferritin, which is elevated due to a decrease in GFR.

TABLE 13.1 CHANGES IN SERUM TRANSFERRIN AND TIBC

Physiological increase	Pathological changes
3rd trimester of pregnancy p.o. birth control pills estrogenes	Low: iron deficiency Chronic diseases with Fe deficiency Nephrotic syndrome High: iron overload

Ferritin

The concentration of ferritin in the serum is low; its source is apoptotic cells. The amount of iron bound in serum ferritin is minimal, so it does not influence the iron balance. Serum ferritin levels reflect the intracellular iron stores available for erythropoiesis.

Decreased serum ferritin is an early marker of iron deficiency. In interpretation, it is necessary to take into account the fact that it is a positive acute-phase reactant. Therefore, ferritin may not be reduced in sideropenic patients with concomitant inflammatory or malignant disease. Ferritin concentrations >100 μg/L reliably exclude iron deficiency. Elevated serum ferritin is a sign of iron excess in the body, regardless of its origin. Other causes of the increased serum ferritin are listed in the Table 13.2.

TABLE 13.2 CHANGES IN SERUM TRANSFERRIN AND TIBC

Increased synthesis due to Fe accumulation	Increased synthesis without Fe accumulation	Cell necrosis
Hemochromatosis Aceruloplasminemia Hemosiderosis (transfusions, iron infusions) Infective erythropoiesis Thalassemias	Malignancies Chronic inflammation Acute and chronic infections Hereditary hyperferritinemia Gaucher disease	Liver: necrosis, chronic viral hepatitis, alcoholic liver disease, NAFLD Hemolysis

Patients with CKD treated by hemodialysis have elevated serum ferritin levels, but this does not reflect iron available for hematopoiesis. Even with elevated ferritin levels, they suffer from multifactorial anemia and other hematological parameters (e.g. low hemoglobin content in erythrocytes and reticulocytes) inform better about iron deficiency.

Soluble transferrin receptors

Transferrin receptors are surface transmembrane proteins that take up transferrin-Fe complexes from circulation. The number of transferrin receptors expressed on cell membranes directly and proportionally reflects the need for iron in these cells (Figure 13.3). Circulating extra-membrane parts of these receptors can be determined in serum as so-called soluble transferrin receptors (**sTrfR**). Their serum concentration rises as **the earliest indicator of iron deficiency** and, besides, they are not affected by inflammation or chronic diseases such as transferrin and ferritin.

Elevated values are also found in patients with more intense erythropoiesis, e.g. in hemolytic anemias or thalassemia's. Decreased sTrfR values reflect decreased erythropoiesis and also occur in iron excess or protein malnutrition. The disadvantage of the parameter is the low availability and standardization of methods.

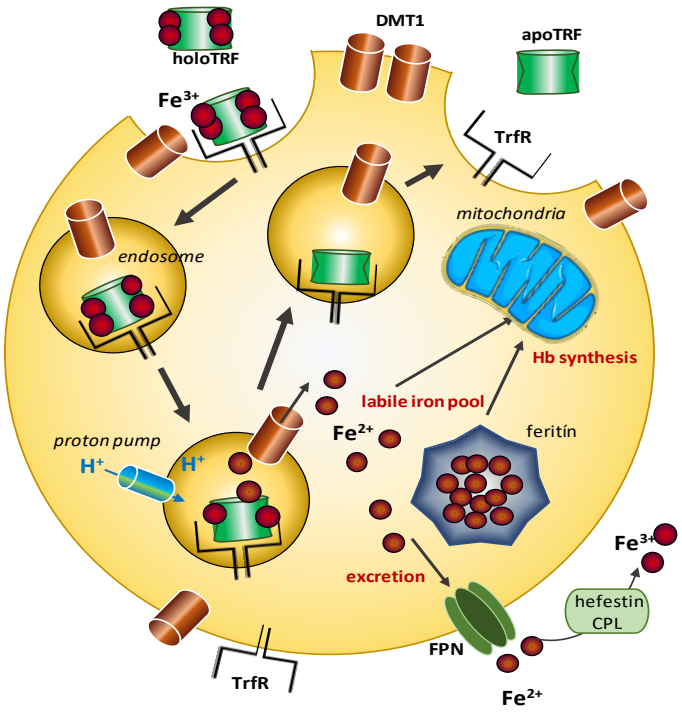


FIGURE 13.3 Transferrin receptors and their role in the cellular iron uptake

Iron deficiency

Iron deficiency (sideropenia) is the most widespread form of nutritional deficiency worldwide and causes 50% of all anemias. Anemia is microcytic, hypochromic with MCV <80 fL. Sideropenia occurs as a result of:

- insufficient dietary intake of iron, including its low bioavailability with high fiber, and phytate content in food;
- absorption disorders (malabsorption syndromes and conditions after bowel surgery);
- increased iron losses.

Several laboratory parameters inform about the iron deficit (Table 13.3). The best diagnostic test to assess iron deficiency is low ferritin. Other parameters are recommended in patients with CKD (e.g. % hypochromic erythrocytes and reticulocytes).

TABLE 13.3 LABORATORY FINDINGS IN DIFFERENT STAGES OF SIDEROPENIA

Sideropenia	Pre-latent	Latent	Manifest
Iron stores	↓	↓↓	↓↓ – 0
S-Fe	N	↓	↓↓
S-ferritin	↓	↓↓	↓↓ – 0
S-transferrin	N	N	N–↑
% transferrin saturation	N	↓	↓↓
sTrfR	↑	↑	↑↑
TIBC	N	↑	↑↑
Sideroblasts in bone marrow	N	N – ↓	↓↓ – 0
Anemia	absent	absent	present

Iron excess

Excess iron is not as common as iron deficiency. The most common causes are chronically increased oral or parenteral intake and increased absorption in enterocytes in genetically determined hemochromatosis (Table 13.4).

Laboratory signs of excess iron in the body are:

- increased S-Fe concentration;
- increased transferrin saturation;
- increased serum ferritin.

If transferrin saturation rises above 35 – 40%, a part of the circulating iron exists in potentially toxic form - in complexes with citrate or albumin. Iron easily leaves that bond and enters the cells, where due to its high reactivity, it can cause damage or even organ failure.

TABLE 13.4 CAUSES OF IRON EXCESS

Mechanism	Examples
High intake	Excessive content in diet, repeated transfusions
High intestinal absorption	Hereditary hemochromatosis
Increased but ineffective erythropoiesis	thalassemia, congenital sideroblastic anemia, myelodysplastic syndrome
Increased RBC destruction	congenital hemolytic anemias, sickle-cell anemia

Hereditary hemochromatosis is the most frequent autosomal recessive disease in whites, with a prevalence of 1 in 300 to 500 individuals. It is caused by several gene mutations (at least 5). The most common type 1 hemochromatosis is mostly seen in people of northern European descent. The type 1 form results from mutation in the HFE gene encoding synthesis of protein that binds to transferrin receptors and regulates their activity. Mutations in any of these genes impair the control of the intestine's absorption of iron and alter the distribution of iron to other parts of the body. As a result, iron accumulates in tissues and organs, which can

disrupt their normal functions. Organs affected by hemochromatosis include liver (cirrhosis), pancreas ("bronze" DM), heart (cardiomyopathy), joints (chondrocalcinosis), skin, endocrine organs (testicular atrophy, thyroid and pituitary). Gene penetration is low, so despite the spread of this mutation, clinical signs are not present in all affected individuals.

Hemosiderosis is a histological term for increased iron excess, which is stored as hemosiderin in the monocyte-macrophage system. For simplicity, the term also covers non-genetic forms of iron excess, e.g. after repeated transfusions or excessive intake of iron-containing preparations. **Iron poisoning** occurs as an acute intoxication with iron-containing products mostly in children (abdominal pain, nausea, vomiting, later encephalopathy, and renal and hepatic failure). An increased concentration of S-Fe is detected in the laboratory; values >90 µmol/L require treatment with chelating agents (desferrioxamine) that bind iron in circulation and the complexes are excreted in the urine.

Laboratory diagnosis of hemochromatosis is mainly supported by the following findings, which indicate increased iron stores:

- increased transferrin saturation (>50% in men and >40% in women);
- increase in serum ferritin except in early stages of the disease (>300 µg/L or >670 pmol/L in men, and >200 µg/L or >450 pmol/L in women).

Pathological laboratory findings occur frequently in the preclinical stage of the disease. Homozygous men are affected more (up to 80%) than women (50%). Individuals with ferritin >1 000 µg/L (>2 250 pmol/L) have a high risk of clinical manifestation of the disease, especially cirrhosis and hepatocellular carcinoma.

A recent large study (65 000 volunteers in Norway) confirmed that individuals with increased transferrin saturation and increased ferritin are unlikely (19% in males and 26% in females) to be homozygous for the most common C282Y mutation. In most cases, ferritin is elevated for other reasons (Table 13.5).

TABLE 13.5 BIOCHEMICAL FINDING IN DISORDERS OF IRON BALANCE

Cause	Fe	Transferrin	Ferritin	sTRFR	Fe in BM
Iron deficit	↓	↑	usually ↓	↑	↓
Acute illness	↓	N	N–↑	N	N
Chronic disease	↓	↓	N–↑	N	usually ↑
Iron overload	↑	↓	↑	↓	↑
Liver disease	↑	↓	↑	N–↑	↑-N
Hemolysis	↑	N–↓	↑	↑	↑

Hemochromatosis is confirmed by **genetic testing** for a mutation in the HFE gene. A liver biopsy is not necessary to confirm the diagnosis, but can be used to evaluate fibrosis and rule out other liver diseases. The content of iron in the bone marrow is usually normal in hereditary hemochromatosis, rather the accumulation of iron in parenchymal organs predominates. Increased iron content in the monocyte-macrophage system occurs in ineffective or defective erythropoiesis (haematological disorders, malignancies and chronic inflammatory diseases).

13.3 Other biochemical aspects of anemia

Sideropenic anemia is only one of the possible forms of anemia, ie a decrease in the concentration of hemoglobin in the blood below the reference interval. Regardless of the cause, all types of anemia negatively affect the supply of oxygen to the tissues. Anemias are classified according to **erythrocyte size** into microcytic, normocytic, and macrocytic, or based on **etiopathogenetic criteria** (Table 13.6). Possible causes of anemia are:

1. **Insufficient RBC production**:
   a. for nutritional deficiency of substances necessary for hematopoiesis (Fe, folic acid, vitamin B12, protein malnutrition),
   b. from congenital or acquired causes with sufficient essential factors (chronic disease anemia, aplastic and dysplastic anemia malignant bone marrow infiltration);

2. **Increased RBC loss**: due to bleeding or hemolysis.

In addition to the complete blood count and the morphological examination of the peripheral blood or bone marrow smear, other, especially immunological and biochemical examinations are used in the laboratory diagnosis of anemia. Biochemical tests are particularly important in **macrocytic anemias** due to folic acid deficiency and folic acid deficiency derivatives (folates) or vitamin B12 (Figure 13.4) as well as **in hemolytic anemias.**

TABLE 13.6 BIOCHEMICAL FINDING IN DISORDERS OF IRON BALANCE

Parameter	Microcytic	Normocytic	Macrocytoic
Hb	<120 g/L in women, <135 g/L in men		
MCV (fL)	<80	80 – 100	>100
Examples of anemia	sideropenic, hemoglobinopathies (thalassemia's), chronic hemorrhagic a., ACD (20%), sideroblastic anemia	acute hemorrhagic, hemolytic anemia, ACD (80%), aplastic anemia, hypothyroidism	megaloblastic (folate and VIT B12 deficiency, drugs with anti-folate effect), alcoholism, liver disease, myelodysplastic syndrome

Folic acid and vitamin B12 deficiency

Folic acid and vitamin B12 are components of the coenzymes needed for DNA and RNA synthesis. The deficiency of both substances causes macrocytic to megaloblastic anemia due to impaired maturation of the nucleus of erythrocyte precursor cells. Slow maturation of the nuclei leads to an increase in the cytoplasm volume. The deficiency affects hematopoiesis as a whole, it is also manifested by hypersegmentation of neutrophil nuclei, mild leukopenia, and thrombocytopenia.

Folates are a mixture of compounds found in the diet mainly in leafy vegetables, nuts, and animal liver. Only less than 1% is in the form of folic acid, the remainder being tetrahydrofolates (THF, 5-methyl THF, and 10-formyl THF). The body of an adult human has a supply of folate sufficient for about 4 months with a daily metabolic requirement of about 100 µg. Folate deficiency occurs when:

- insufficient intake (lack of leafy vegetables or their heat treatment);

- malabsorption (celiac disease, etc.);
- increased need (pregnancy, lactation, puberty, malignancy) or loss;
- when taking medicines with an anti-folate effect (anticonvulsants, methotrexate).

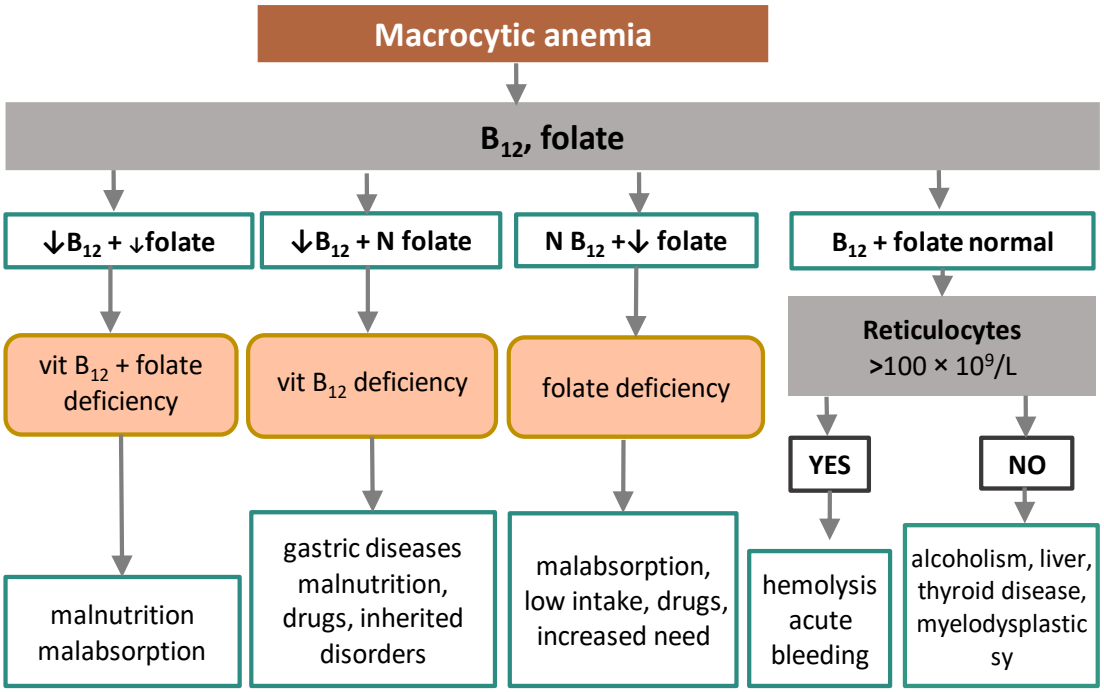


FIGURE 13.4 Differential diagnosis of macrocytic anemia

Vitamin B12 (cobalamin) is a compound similar to porphyrin with centrally bound cobalt instead of iron. Its only source for humans is food of animal origin. The vitamin stores in an adult's body last for 3 – 4 years until a deficit develops. The resorption of cobalamin in the terminal ileum requires an intrinsic factor (IF) formed in the gastric mucosa to protect it from degradation during transport through the ileum. After reabsorption, it binds to a transport protein (transcobalamin) in the bloodstream. This form is called **holotranscobalamin,** or an active vitamin, because it releases cobalamin into the cells, where hematopoiesis takes place or is stored (liver and other internal organs). The end products of intracellular metabolism of cobalamin - methylcobalamin and adenosylcobalamin - are involved in the metabolism of homocysteine and methyl malonyl-coenzyme A. As methylcobalamin and adenosylcobalamin are essential for the proper metabolism and function of neurons, cobalamin deficiency is manifested by a variety of neurological symptoms.

The causes of vitamin B12 deficiency are in particular:

- Insufficient intake in food (vegetarians, vegans);
- Impaired absorption - gastric factors (atrophic gastritis, antibodies against IF or parietal cells of the gastric mucosa, gastrectomy, proton pump inhibitors);
- Impaired absorption - intestinal causes: resection of the ileum, celiac disease, chronic inflammatory diseases (Crohn), rare genetic defects of the cubilin receptor in enterocytes.

Laboratory diagnostics of deficiency includes measurement of vitamin B12 in serum, in certain cases also an examination of holotranscobalamin, homocysteine, intrinsic factor, and its antibodies.

Biochemical manifestations in hemolytic anemias

Hemolysis is a premature breakdown of RBCs that shortens their normal lifespan (approximately 120 days). It can take place intravascularly or extravascularly - in the macrophages of the spleen, liver, and bone marrow, both mechanisms often combined. Hemolytic anemia occurs when the rate of destruction exceeds compensatory erythropoiesis in the bone marrow. It is characterized by the following triad - **anemia, reticulocytosis, and jaundice.** Depending on the cause, hemolytic anemias have specific morphological findings in the blood smear (spherocytes, RBC fragmentation, Heinz bodies, etc.).

Biochemical signs of **extravascular hemolysis** and hemoglobin breakdown are:

- increase in serum unconjugated bilirubin with jaundice if its concentration exceeds 50 μmol/L;
- only urobilinogen is positive in urine, unconjugated bilirubin is not filtered in urine;
- increase in S-Fe and transferrin saturation.

In **intravascular hemolysis**, hemoglobin is released from RBC into the plasma, resulting in:

- **hemoglobinuria** - a positive reaction to the blood during a chemical examination of the urine;
- iron deposition in tubular cells in the form of **hemosiderin** - it is possible to detect iron in epithelial cells of urine sediment even 6 weeks after the episode of hemolysis;
- decrease in serum **haptoglobin** - glycoprotein binding the α-chain of disintegrating hemoglobin, subsequently the complex is taken up in the reticuloendothelial system;
- decrease in serum **hemopexin:** free heme forms complexes with protein haptoglobin and, to a lesser extent, albumin. These complexes are taken up and metabolized by the liver, which reduces hemopexin levels to unmeasurable levels. Other causes of reduction may be advanced liver disease and renal protein loss.
- **free hemoglobin** present in plasma or serum means that available haptoglobin has been consumed and represent a sign of significant hemolysis.

Other tests are used to investigate the cause of hemolytic anemia, e.g. Coombs (direct antiglobulin) test, an examination of pathological hemoglobin, flow cytometry (hereditary spherocytosis, paroxysmal nocturnal hemoglobinuria) and examination of enzyme deficits in venereal anemia.

13.4 Heme metabolism disorders

Heme is an iron-containing pigment essential for life, synthesized in the liver and maturing erythroid cells in the bone marrow. Disorders of heme synthesis due to enzyme defects lead to diseases such as **porphyria** and some types of **anemia** (e.g., X chromosome-linked sideroblastic anemia). Degradation of heme is a strictly controlled process that takes place in the spleen and liver. By opening the cyclic heme molecule, linear tetrapyrrole biliverdin is formed, subsequently reduced to bilirubin (see Chapter 5). Porphyrias (*gr. porphyros* = purple) are rare metabolic diseases caused by a congenital or acquired defect of enzymes necessary for heme biosynthesis. Each enzymopathy leads to the accumulation of typical precursor tetrapyrroles, porphyrins, which are intermediates in heme biosynthesis (Figure 13.4).

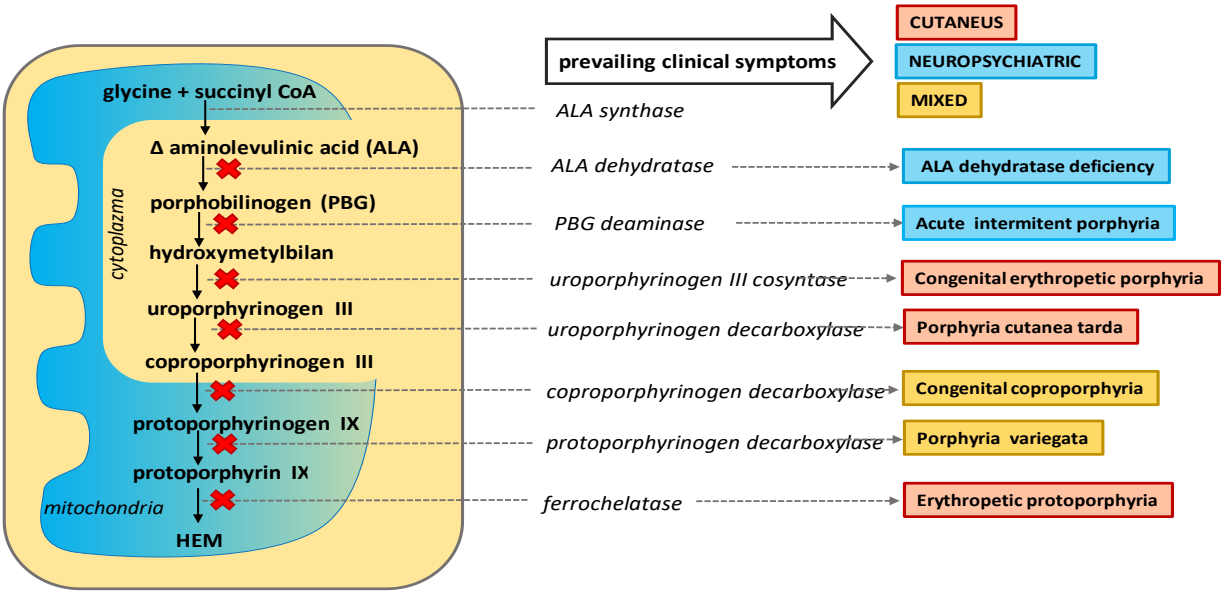


FIGURE 13.4 Examples of enzyme defects in heme biosynthesis leading to porphyrias

Porphyrias can be classified according to several criteria:

- the main site of overproduction of heme precursors (**liver, bone marrow**),
- clinical signs (**cutaneous** or **neuro-visceral**)
- the course (**acute** or **chronic**).

The penetration of the disease is not high, only about 10 - 20% of individuals with a congenital enzyme defect have clinical manifestations during their lifetime. **Acute porphyrias** are manifested by dramatic neuro-visceral attacks that affect almost exclusively adults, predominantly women (escalating abdominal pain, nausea, vomiting, hypertension, tachycardia, peripheral motor polyneuropathy, encephalopathy, psychiatric disorders). The attacks are provoked mainly by hormonal changes, commonly prescribed drugs, starvation, alcohol and stress. The causes of acute porphyrias are enzyme defects in the early phase of heme biosynthesis, which lead to the accumulation of mainly aminolevulinic acid and porphobilinogen in body fluids. The most common form is acute intermittent porphyria (AIP).

Cutaneous porphyrias occur as acute or chronic diseases. The enzyme defects in the later stages of heme biosynsynthesis lead to the accumulation of intermediate metabolites, porphyrinogens, whose oxidation products - porphyrins - cause skin **photosensitivity**. When exposed to light at a wavelength of about 400 nm, excitation of porphyrins occurs, which react with molecular oxygen to form reactive forms, causing damage to cellular organelles and skin manifestations (INFO 13.2).

The cutaneous manifestation of *erythropoietic protoporphyria* (EPP) begins in childhood and is manifested by burning, itching, erythema of the skin exposed to sunlight. Other types of porphyria are characterized by skin irritation, blisters and bulges, later scars, hyperpigmentation and hypertrichosis. The most common of all porphyrias is *porphyria cutanea tarda* (PCT), which occurs sporadically, only in 10 - 20% of cases familiarly. In addition to genetic causes, porphyric syndromes can also occur as **secondary manifestations** of other diseases, such as an acute liver failure or poisoning (e.g. lead) or chronic liver disease, hemoblastosis, or the syndrome of polyvalent chemical sensitivity (allergic, toxic and immunological manifestations of various chemicals).

INFO 13.2 Porphyrias and legends

Many believe that cases of acute cutaneous porphyria may have contributed to the rise of vampire legends in the past. Some manifestations of the disease (e.g. photosensitivity of the eyes and skin) led to avoidance of daylight in affected individuals. Heavy blisters caused scarring of the face, red discoloration of the tooth enamel, receding gums and protruding teeth. Hypertrichosis or increased lanugo-like facial hair made them mysterious creatures. Drinking blood may have been recommended in the past to alleviate anemia, and garlic is known to worsen the cutaneous manifestations of porphyria. It is believed that one of the builders of the Paris Opera, who lived in the underground to protect himself from daylight, may have suffered from some form of porphyria and was the inspiration for the French writer, Gaston Leroux, to write the novel The Phantom of the Opera.

13.5 Laboratory diagnostics of porpyhrias

In clinical practice, porphyrin testing is indicated in three situations:

1. suspected chronic porphyria (PCT only);
2. acute attack of suspected acute porphyria;
3. suspicion of porphyria in a patient outside of an acute attack, when the probability of porphyrins detection in biological material is low.

The diagnosis of porphyrias is based on evidence of porphyrin metabolism intermediates in erythrocytes, urine, and feces (Table 13.6). Porphyrins are highly non-stabile on light; all biological material must be therefore protected from light. The typical finding is a dark red-brown color of urine (the color of port wine) after oxidation in air. In material collected during an acute attack, the probability of a positive test result is much higher.

TABLE 13.7 BIOCHEMICAL FINDING IN FREQUENT FORMS OF PORPHYRIA

Type of porphyria	RBCs	Urine	Feces
Congenital erythropoietic porphyria	Uro-, copro-	Uro-, Copro-	Copro-
Erythropoietic porphyria	Proto-	-	Proto-
Acute intermittent porphyria	-	ALA, PBG	-
Porphyria variegate	-	ALA, PBG, copro-	Copro-, Proto-
Congenital coproporphyria	-	ALA, PBG, copro-	Copro-
Porphyria cutanea tarda	-	Uro-	Isocopro-
Hepatoerythropoetic porphyrias	Proto-	Uro-	Isocopro-
Toxic (secondary) porphyrias	-	Uro-	–

ALA – Δ aminolevulinic acid, PGB – porphobilinogen, Uro – uroporphyrin, Copro – coproporphyrin, Proto – protoporphyrin

Screening methods include the determination of **major metabolites of porphyrins in urine**. A positive screening test requires a more detailed analysis and **quantification of porphyrins**

in all biological materials using fluorescence and chromatographic methods. Due to the rare occurrence and complexity of laboratory analysis and interpretation of results, the centralization of diagnostics in selected laboratories is reasonable (INFO 13.3).

INFO 13.3 Laboratory methods in the diagnosis of porphyria

Screening tests:

1. Quantification of PBG and ALA in random fresh urine collected during or immediately after an attack. An adequate urine concentration must be observed; if U-Cr is too low (<2 mmol/L), sampling should be repeated. Examination is a marker of acute porphyria.
2. Total excretion of urinary porphyrins in 24-hour urine. Elevated values (>200 μg/24 h) occur in acute, chronic, and secondary diseases (coproporphyrinuria).

Special methods:

1. Spectrophotometric fractionation of plasma porphyrins - the ability of fluorescence after their excitation by light (400 nm) is used;
2. HPLC fractionation of porphyrins (uro-, copro-, protoporphyrin) in urine and feces. Examination of both urine and feces is important if any of the cutaneous forms of the disease are suspected;
3. Examination of enzyme activities, e.g. ALA dehydratase;
4. Examination the molecular level, in unclear and complicated cases.
5. Examination of latent porphyria in family members of the affected individual allows to inform the carrier of the disease about possible factors causing the clinical manifestation of the disease.

Case studies and self-assessment questions

Case report 13.1

A 46-year-old man is examined by a hepatologist for chronically elevated liver function tests. He has also been diagnosed with T2DM. Serological tests for viral hepatitis B and C are negative. His biochemical findings before liver biopsy are shown in the table.

Serum	Result	RI
Bilirubin	13	<20.0 μmol/L
ALT	3.05	<0.66 μkat/L
ALP	3.38	<1.75 μkat/L
GGT	4.25	<0.65 μkat/L
ALB	44	36 – 50 g/L
Fe	40	11 – 30 μmol/L
TIBC	42	54 – 80 μmol/L
Transferrin saturation	85	20 – 40%
Ferritin	2250	22 – 640 pmol/L

Questions:

a. What is the expected diagnosis based on biochemical findings?
b. What examination is needed to confirm the diagnosis?
c. What may be the cause of elevated serum ferritin?

Case report 13.2

A 34-year-old woman is examined for fatigue, weakness, nausea, shortness of breath after exertion, and frequent headaches. There is no history of previous chronic disease, surgery, allergy, she does not take any medications regularly. She has two children, last time she was examined by her gynecologist 3 years ago, when she had got an intra-uterine device. On physical examination, she is afebrile, without cyanosis and jaundice, adequately hydrated, has pale skin, and mucous membranes. The findings of the laboratory tests are given in the table.

FBC	Result	RI	Serum	Result	RI
RBC	3.27	3.8×10^{12}/L	Bilirubin	17	<20 μmol/L
Hb	79	120 – 160 g/L	ALT	0.23	<0.66 μkat/L
Htc	0.27	0.36 – 0.34	ALP	0.73	<1.75 μkat/L
MCV	80.2	82 – 100 fL	ALB	45.8	34 – 48 g/L
WBC	5.5	$3.8 – 10 \times 10^{9}$/L	Fe	8.8	7 – 34 μmol/L
PLT	274	$150 – 400 \times 10^{9}$/L	TIBC	89	41 – 77 μmol/L
			Transferrin sat.	12.6	20 – 40%
			Ferritin	21.1	22 – 640 pmol/L

Questions:

a. What is the expected diagnosis based on biochemical findings?
b. What examination is needed to confirm the diagnosis?
c. What is the most frequent cause of iron deficiency?

Self-assessing questions

1. Identify the main regulator of the iron balance and explain the mechanism of its effect?
2. List the tests used to diagnose iron deficiency.
3. What factors may affect serum iron level?
4. What biochemical tests would you indicate in a patient with macrocytic anemia?
5. Name the biochemical signs of hemolysis.
6. Explain the causes of porphyria?
7. When is the highest probability of positive laboratory results in an examination of porphyria?

KEY INFORMATION

- ☑ Absorption, transport, and iron stocks are strictly controlled. Most of the Fe in the body is in the form of heme.
- ☑ Daily absorption of iron rom food is about 1 mg, which is transported in the blood bound to transferrin, stored intracellularly in the form of ferritin and hemosiderin.
- ☑ Serum iron as an isolated test is of little diagnostic value, with the exception of iron intoxication.
- ☑ The best indicator of iron stores in the body is serum ferritin.
- ☑ Anemia from iron deficiency can occur due to insufficient food intake, malabsorption, or excessive blood loss.
- ☑ Iron excess in the body is less common iron deficiency. It arises on a genetic basis with increased intestinal absorption of iron (hemochromatosis) or for non-genetic reasons with excessive intake of iron (transfusion, chronic alcoholism).
- ☑ Porphyria's are disorders of heme biosynthesis that are clinically manifested by neuro-visceral attacks or cutaneous manifestations due to photosensitization.
- ☑ Acute porphyrias are life-threatening situations, there is evidence of increased urinary excretion of heme biosynthesis intermediates, especially porphobilinogen.
- ☑ Beside clinical presentation, the differential diagnosis of porphyrias is based on the detection of a different spectrum of produced heme precursors in body fluids.

Clinical Biochemistry

Academic textbook

Authors:	MUDr. Eva Ďurovcová, PhD. prof. Ing. Mária Mareková, CSc.

Publisher:	Pavol Jozef Šafárik University in Košice Publishing ŠafárikPress
Year:	2020
Pages:	275
Author's sheets:	19,03
Edition:	second

ISBN 978-80-8152-937-5 (e-publication)