CLINICAL-BIOCHEMICAL TESTING

BASICS AND INTERPRETATION OF SELECTED FINDINGS

University undergraduate scripts

Daniel Čierny, 2025

Clinical-Biochemical Testing – Basics and Interpretation of Selected Findings

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Biochemistry is the science of life. All our life processes – walking, talking, moving, feeding – are essentially chemical reactions. So biochemistry is actually the chemistry of life, and it´s supremely interesting.

Aaron Ciechanover (1947 - ...)

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Preface

Currently available written resources summarise a mass of theoretical knowledge from clinical biochemistry and laboratory medicine. The textbook *Clinical Biochemistry (Murphy, Srivastava, Deans)* contains the information needed to understand and apply clinical biochemistry in a clinical context for undergraduates across a range of courses including medicine, nursing, biomedical science, pharmacy and life sciences. The textbook *Clinical Biochemistry* (Ďurovcová & Mareková) from Pavol Jozef Šafárik University in Košice provides detailed information on selected areas supplemented by interesting case reports, addressed not only to medical students, but also to to young doctors and laboratory diagnosticians in specialisation training.

The present university scripts entitled "CLINICAL-BIOCHEMICAL TESTING – BASICS AND INTERPRETATION OF SELECTED FINDINGS" are the result of the author's efforts to summarise basic knowledge about the characteristics of laboratory tests, the methods of investigation of routine biochemical parameters, and to supplement currently available, high-quality literature with a practical interpretation of selected clinical biochemistry findings. The first two chapters describe the basic characteristics of laboratory testing and the entire process of biological material processing from sampling to obtaining laboratory results in the clinical-biochemical laboratory. The third chapter contains concrete cases of patients and diseases in questions and exercises that are aimed at developing the reader's practical skills in relation to the indication and interpretation of biochemical tests. For a fully comprehensive and accurate interpretation, the explained biochemical findings are complemented by clinical examination data and imaging findings.

The study material is aimed mainly at students in the fourth year of general medicine at the Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, who enrolled in the subject *Clinical Biochemistry and Laboratory Medicine*, since they have repeatedly asked for a "simple" textbook where the data from lectures and practicals can be found. However, the herein provided summarised information concerning laboratory practice, the indication and interpretation of biochemical investigations should also be useful for young 'graduate' physicians at the beginning of their clinical career. The author's aim was to interpret and explain the "fate" of the specimen in the clinical-biochemical laboratory, the principles of the

methods used, and the significance of the various basic parameters in an interesting and practically oriented manner.

The practical part of the textbook contains one interesting case from the laboratory and 14 case reports. While giving the clinical information about the patient and detailed results of laboratory tests, the questions are given and followed by correct answers and explanations. Thus, the theoretical data about biochemical markers are not presented in the traditional form of an information summary, but they are contained in a total of more than 90 answers to questions purposely targeted to develop both the knowledge and practical skills of the reader. The work contains in total 39 illustrations, 4 tables and 29 detailed anonymous laboratory test result sheets. All illustrations, tables and diagrams present in the publication were created and formulated by the author.

Contents

List of abbreviations and symbols

ACR	albumin-to-creatinine ratio
AFP	alpha-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMS	alpha-amylase
ASLO	antistreptolysin O
AST	aspartate aminotransferase
BE	base excess
bp	base pair
CDT	carbohydrate-deficient transferrin
CK	creatine kinase
CKD-EPI	Chronic Kidney Disease – Epidemiology Collaboration
C_{kreat}	creatinine clearence
CK-MB	isoform of creatine kinase (muscle, brain)
CMIA	chemiluminescence immunoassay
CNS	central nervous system
CRP	C-reactive protein
DOF	β1-N-deoxyfuranosylhemoglobin
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ECLIA	electrochemiluminescence immunoassay
EDTA	ethylenediaminetetraacetate
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
FPIA	fluorescence polarization immunoassay
G6P-DH	glucose-6-phosphate dehydrogenase
GLP	good laboratory practice
GMT	gamma-glutamyltransferase
HbA1c	glycated hemoglobin

HDL	high-density lipoproteins
hr	hour
HRM	high resolution melting analysis
CHS	cholinesterase
ICD-10	International Classification of Diseases - Tenth Revision
IFCC	International Federation of Clinical Chemistry
Ig	immunoglobulin
IL6	interleukin 6
KDIGO	Kidney Disease Improving Global Outcomes
LBP	lipopolysaccharide binding protein
LDL	low-density lipoproteins
LPS	lipase
LPSC	lipopolysaccharide
MODS	multiorgan dysfunction syndrome
NIS	hospital information system (*nemocničný informačný systém*)
NSE	neuron-specific enolase
NT-proBNP	N-terminal pro-brain natriuretic peptide
OG	osmolal gap
PCR	polymerase chain reaction
POCT	point of care testing
PSA	prostate specific antigen
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
sdLDL	small dense LDL
SIADH	syndrome of inappropriate antidiuretic hormone secretion
SIRS	systemic inflammatory response syndrome
SVaLZ	common laboratory and treatment workplaces (*spoločné vyšetrovacie a liečebné zložky*)
ÚKB	Department of Clinical Biochemistry (*Ústav klinickej biochémie*)

List of figures and tables

1 The importance of clinical biochemistry in medicine

Let’s consider a few basic questions that highlight the often underestimated importance of laboratory testing in medical practice. Excluding urgent life-threatening situations, let me ask: a) How often does a physician hospitalise or treat a patient without assessing their basic blood and urine biochemical test results? b) Does a surgeon start an operation without having at least some basic information about the state of the patient's internal environment? There are many similar questions and I believe the answer is clear. I dare say that the results of laboratory tests are increasingly and undoubtedly becoming a very important tool - or even the only one - for clinical decision-making.

Clinical biochemistry applies knowledge of biochemical processes in the human body to clinical practice. The main focuse of clinical biochemistry is to determine the concentration and activity of "chemical substances and enzymes" in body fluids. This provides the physician with answers to questions in the differential-diagnostic process, for screening diseases, predicting the prognosis and selecting the right treatment or monitoring its effectiveness. Currently, thousands of compounds can be analysed in body fluids, including substrates, products of metabolism, enzymes, hormones, drugs, toxic substances, antigens, antibodies, and many others.

Clinical biochemistry is a branch of laboratory medicine, and together with clinical hematology, clinical immunology and allergology, clinical microbiology, clinical pharmacology and other disciplines, they are sometimes also referred to as "SVaLZ" (Common laboratory and treatment workplaces, *Spoločné vyšetrovacie a liečebné zložky*). An inevitable part of the work of the laboratory personnel is to collaborate with the medical staff from the hospital clinics and departments, to implement new laboratory tests, and to provide consultative services regarding the sampling and processing of biological material or the interpretation of the results. Laboratory staff are also considerably involved in various research projects and pharmaceutical clinical trials. Unfortunately, in recent decades there has been a gradual shift in laboratory medicine farther from the patient, and the work of laboratories is often considered to be just a kind of service producing numerical results. In spite of that, a good modern laboratory provides a complex service of the highest quality, with the aim of delivering results as quickly as possible and offering an opportunity for consultation.

2 Laboratory testing

The aim of laboratory testing, which is a fundamental component of the comprehensive services provided by the laboratory, is quickly to deliver accurate and precise results. In most cases, the ordering physician is the person who uses the laboratory result to complete the overall findings (including medical history, clinical examination and imaging findings) and subsequently selects the appropriate follow-up procedures in patient management. Laboratory testing consists of several phases, which are shown in Fig. 1 .

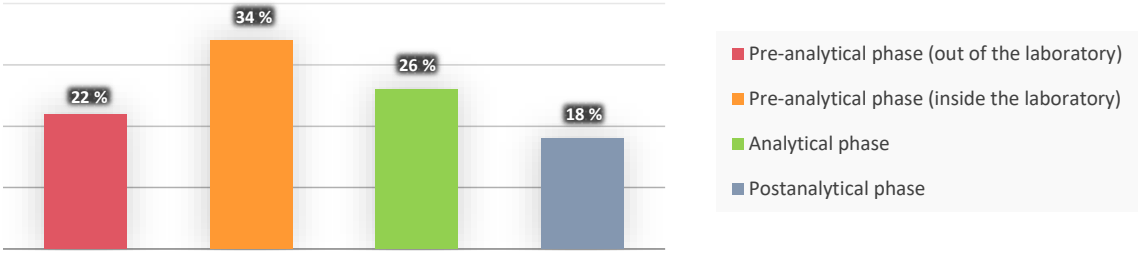

Figure 1 Phases of laboratory testing by time The largest portion of the laboratory testing is comprised of the pre-analytical phase, which accounts for more than 50% of the entire process and is made up of two parts: outside the laboratory (test indication, patient preparation, sample collection, transport and initial sample processing) and inside the laboratory (sample receiving, labeling and processing). This is followed by the analysis itself, i.e. the measurement of the concentration of the required analytes in the sample using different methods. The post-analytical phase involves collecting the results, evaluating and verifying their accuracy, transmitting the results to the indicating physician and interpreting them.

2.1 Pre-analytical phase of laboratory testing

The pre-analytical phase consists of the procedures and transactions from the indication of the testing to the initiation of the sample analysis. This phase significantly influences the quality of the results and accounts for more than 60% of errors. It involves the selection of appropriate biochemical markers, patient preparation, the sampling of biological material, the control of specimen identification, specimen transport to the laboratory and the storage and processing of the specimen in the laboratory before the start of the analysis.

2.1.1 Patient preparation and sampling of biological material

The most frequently delivered biological material to the clinical-biochemical laboratory is venous blood. The patient must be adequately informed about the purpose of sampling and must agree by signing an informed consent form. Blood collection is usually performed in the morning; the patient should be in a resting and lying position, fasting i.e. not having eaten for 10-12 hrs, having avoided excessive physical activity and stress, with the intake of only essential drugs, on awakening drinking a glass of water or unsweetened tea. Before sampling, the patient should not smoke, and the diet for at least the previous 24 hours should not contain excessive amounts of alcohol, carbohydrates, fats and other unusual foods. It is not suitable to exercise after applying a tourniquet to a limb before collection. Also, no infusion should be given near the area of sampling. Currently, a closed vacuum collection system is used for blood sampling, and the majority of biochemical markers are analysed from blood serum. After checking the suitability of the collected material and labelling the tube and the application form, it is necessary to let the blood clot for a sufficient time (ideally 15-30 minutes from sampling). Otherwise, erythrocyte hemolysis and additional fibrin precipitation may occur, and this might obstruct the analyser pipette or needles, resulting in a smaller sample volume being drawn and falsely decreased results. However, modern analysers already have a system to detect the presence of a clot in a sample, alerting the staff to remove the clot and to repeat the analysis. Biological material should be transported avoiding mechanical stress (e.g. excessive shaking, vibration) and without temperature fluctuations (thermo containers), as this can cause damage and hemolysis of the sample. The delivery of the specimen by the patient themselves is not appropriate, and the laboratory should not accept such material.

Question A Which blood tubes and procedures do we use to obtain serum and plasma, and what is the difference between these liquids? Which analytes in the clinical-biochmical lab do we measure only from plasma?

Answer A Serum and plasma are both obtained by centrifuging whole blood at approximately 4,000 revolutions per minute (rpm) for 5-10 minutes at 4°C, separating the blood elements from the liquid phase. Blood serum is obtained from clotted blood; blood plasma is obtained from uncoagulated blood and thus it contains coagulation factors, but differs also in the concentrations of other analytes. In serum, we observe higher levels of

potassium, lactate, ammonia, lipids, and higher activities of lactate dehydrogenase, alkaline phosphatase, and gammaglutamyltransferase.

Blood samples for **serum separation** are collected in tubes with a clot activator (SiO_2 coated on the inner walls). These tubes can also contain an inert separation gel, which prevents the blood coagulum from being re-suspended with the serum, allows longer storage of the sample and simplifies handling, as there is no need to transfer the serum to the next empty tube.

Blood samples for **plasma separation** are collected in tubes with anticoagulants (e.g. citrate, ethylenediaminetetraacetate salts - EDTA, lithium heparinate, oxalate). In practice, tubes with potassium salts of EDTA (K_2EDTA, K_3EDTA) are most commonly used, not only for blood plasma isolation, but also for blood count analysis in a hematological laboratory. Particular attention should be paid when analysing the potassium level, which may be falsely elevated when using these tubes. The different types of collection tubes used are shown in Fig. 2. The venous blood fractions obtained by centrifugation in the two most commonly used types of tubes are shown in Fig. 3.

Question B Which other types of blood collection tubes do you know and for what analyses are they used?

Answer B Tubes containing citrate with a precisely adjusted concentration (3.8%) are used for coagulation tests. Test tubes containing NaF + EDTA, inhibiting coagulation and glycolysis, are used for the accurate determination of plasma glucose and lactate concentrations. When using tubes containing lithium heparinate, plasma suitable for the determination of ions is obtained.

a b c d e f

Figure 2 Closed evacuated system tubes used for blood collection in practice

a - clot activator → coagulation → centrifugation → blood serum

b - clot activator + separation gel → coagulation → centrifugation → blood serum

c - K_3EDTA → non-clotting blood → centrifugation → blood plasma

→ blood count

d - citrate → uncoagulated blood → centrifugation → blood plasma

e - NaF + EDTA → uncoagulated blood with inhibition of glycolysis → centrifugation → blood plasma

f - lithium heparinate → uncoagulated blood → centrifugation → blood plasma

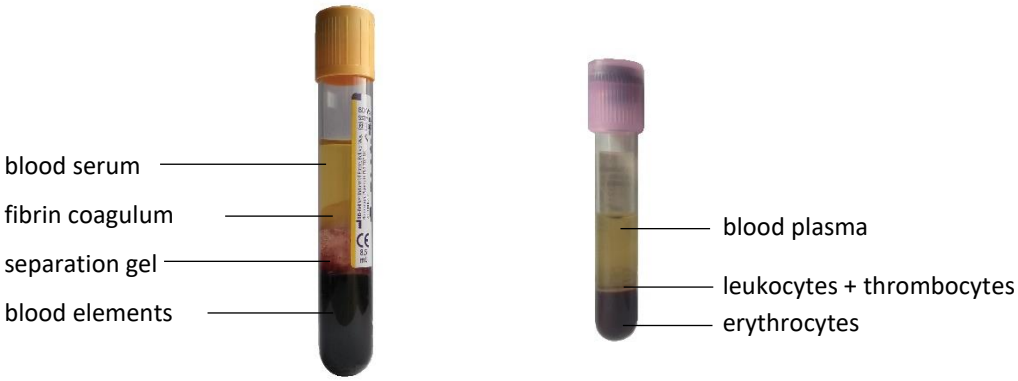

Figure 3 Centrifuged venous blood In the tubes, we can see the blood fractions after clotting and centrifugation. An unwanted fibrin coagulum in the serum is formed when the blood is not allowed to clot for a sufficient time. The plasma collection tube (EDTA) does not contain any separation gel, therefore care must be given not to mix or smear the plasma with sedimented blood elements during pipetting.

Question C Can all biochemical markers be precisely determined in both blood serum and plasma? Are there any analytes that require measurements solely from plasma?

Answer C In the clinical-biochemical laboratory, many analytes can be determined in both serum and plasma, when the appropriate reagent kit and analytical procedure provided by the manufacturer are used. The fact is that the majority of analytes are measured from serum,

as mentioned earlier. However, there are also biochemical parameters that are recommended to be determined only from plasma but not from serum, either due to the principle of the methodology or due to other issues. In our laboratory, the "**measurement only in plasma**" is currently applied to: lactate, ammonia, presepsin, troponin I, NT-proBNP (N-terminal pro-brain natriuretic peptide).

In addition to venous blood, biochemical markers can be analysed also from arterial or capillary blood, e.g. to determine the patient's acid-base balance status (so-called ASTRUP). Capillary blood is also used to measure the glycemic profile of diabetic patients. Capillary blood for acid-base balance measurement must be arterialised, which involves fingertip hyperemisation by massage or warm water before sampling. Non-coagulability is ensured by heparin coated on the inner walls of syringe or capillaries.

2.1.2 Processing of biological material

One of the crucial steps in the pre-analytical phase outside the laboratory is the **correct identification of the biological material**. The 'identification label' is attached to the collection tube and must contain the patient's name, birth number, diagnosis, health insurance company code, and the name of the ordering department and the physician. Incorrectly labelled biological material cannot be accepted by the laboratory staff. An exception is made for the admittance of specimens in cases of *periculum mortis*, or in *statim* patients with an unknown emergency condition. However, the ordering department is obliged to provide an additional identification of these specimens. When marking biological material, care should also be taken to ensure that samples from different patients are not interchanged.

Each correctly collected and labelled sample has to be accompanied by a printed request form, which is produced directly after selecting the parameters from the electronic request form in the hospital information system (NIS, *nemocničný informačný systém*) (Fig. 4).

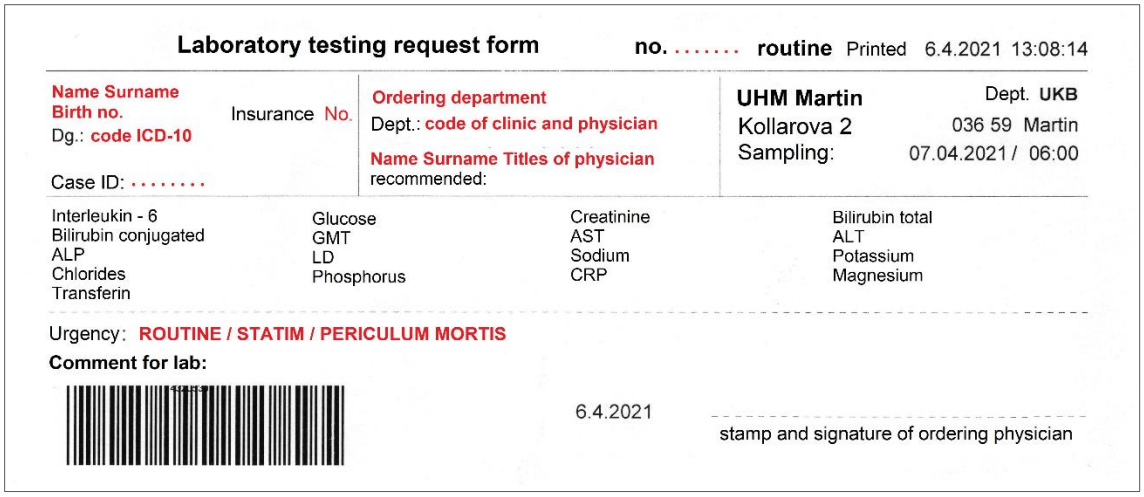

Figure 4 The request form accompanying each correctly labelled specimen upon delivery to the laboratory The request form must contain: the patient's name and surname, birth number, health insurance company code, ICD-10 (International Classification of Diseases - Tenth Revision) diagnosis code, information about the ordering department and physician, specimen number, time and date of biological material collection, list of parameters to be measured, urgency, barcode, stamp and signature of the indicating physician.

Scanning the request form's barcode at the laboratory's reception registers the sample and assigns it an identification number in the laboratory's information system (openLIMS). Simultaneously, the sample is tagged with a new barcode enabling it to be recognised in all instruments and analysers in the laboratory (Fig. 5).

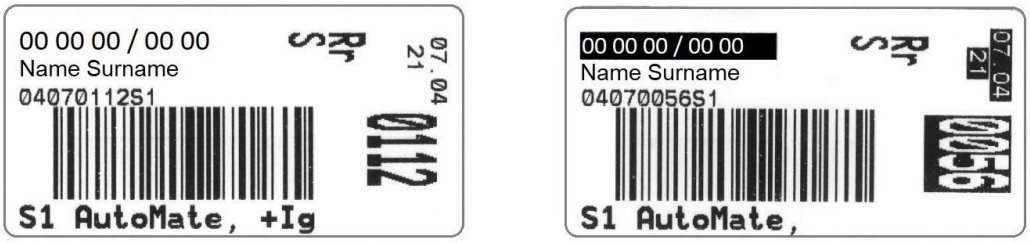

Figure 5 Barcode label created in the laboratory for all received tubes The label contains a barcode that is recognised by analysers, the patient's data (name and surname, birth number), the date of sample acceptance, the sample number (black background indicates a "*statim*" sample). The number code under the name allows the sample to be found by an automatic sorting line (AutoMate) in case of a "reexamination request": the first four digits - month, day, second four digits - sample number, S1 - serum. +Ig means that the sample will have an aliquot created for the measurement of immunoglobulins (Ig).

After centrifugation, the sample is transferred to the AutoMate automatic sorting line which recognises barcodes and distributes the samples into the different laboratory sections according to the markers to be measured (Fig. 6). Simultaneously, the "aliquots" are created when necessary, i.e. a portion of the sample is pipetted into another tube with an equivalent barcode label. This step enables the parallel analysis of the sample in several sections, thus

reducing the delivery time of the results. If additional markers need to be analysed and the sample is already in the laboratory, it is sufficient to send a new request form containing the required parameters and a "reexamination" note. Analyses of time-stable parameters can be performed during the entire storage period of the sample in the laboratory, reducing the burden of repeated blood draws on the patient. Samples are stored in a refrigerated room for 72 hours, after which they are discarded as biological waste.

Figure 6 AutoMate Automatic sorting line The individual racks in the sorting line (numbered 1 - 5) hold samples directed to the different analysers, or samples that have already been analysed and are destined for archiving in the refrigerated room (number 6). On the left and in the back centre we can see the moving arms that sort the samples.

In modern laboratories, the so-called serum indexes - hemolytic, lipemic and icteric - are measured for each sample prior to analysis and reported in the final results. These numerical indexes that indirectly indicate the concentration of hemoglobin, lipids and bilirubin, are obtained by measuring the absorbance at their absorption maximum wavelengths. Serum samples with elevated levels of these substances are shown in Fig. 7. In cases with very high concentrations, interferences or interactions with the measurement of other biochemical parameters may lead to incorrect results.

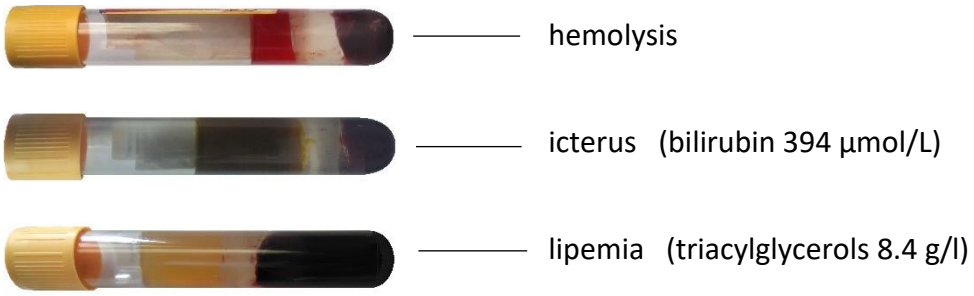

Figure 7 Possible interference in serum Elevated serum concentrations of hemoglobin (red colour), bilirubin (yellow to dark green colour) and lipids (milky opacity) may cause mismeasurement of other analytes concentrations by interaction with an ongoing chemical reaction or photometry.

Question D Which other biological specimens besides blood can be sent to the clinical biochemistry laboratory for testing, and what collection tubes do we use?

Answer D In addition to blood, in the clinical-biochemical laboratory we analyse urine, cerebrospinal fluid, gastric juice, punctate (pleural, peritoneal, synovial, ascites), aspirate from pancreatic cysts or from ductus pancreaticus, fluid - liquor vs. rhinorrhea, dialysate, stool. Further information regarding the examination of each type of biological material will be provided in the following chapters. The above-mentioned types of biological material are transported to the laboratory in clean collection tubes without any adjuvants; if the sample is to be centrifuged (liquor), the use of more durable polypropylene tubes is recommended.

2.1.3 Interesting case – "blue blood"

A venous blood sample from an 83-year-old female with breast cancer was delivered to the laboratory to perform pre-operative measurements prior to a sentinel lymph node biopsy. After centrifugation, the serum was atypically stained blue (see Fig. 8). The laboratory technicians had not seen such a peculiar feature in more than 30 years of their practice.

Figure 8 The atypically blue serum sample

Question E How would you explain this finding?

Answer E At first inspection of the specimen, we might consider that serum staining is caused by hyperbilirubinemia (so-called verdine posthepatic icterus). However, the serum conjugated bilirubin level was normal (4.7 µmol/L), and the total bilirubin level was only slightly elevated (22.2 µmol/L). Therefore, we considered the staining of the serum by medications or some other substance. It was later shown that the patient had been subcutaneously injected with the dye 'patent blue', which, after its transport through the lymphatic vessels, is routinely used to localise the sentinel lymph node.

2.1.4 Currently available laboratory tests

At the Department of Clinical Biochemistry of JFMED CU and UHM, it is currently possible to determine several panels of parameters, that can be ordered in the hospital information system by clicking on the "Patient request forms" icon (Fig. 9). For clarity, we present all these panels with short descriptions in Figs. 10 - 15. Moreover, in cooperation with other clinics of the JFMED CU and UHM, there is a long-term effort to continuously enlarge the number of available biochemical markers, following current trends in laboratory medicine.

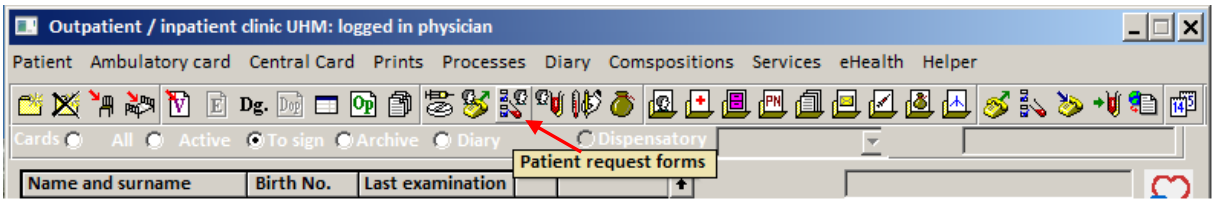

Figure 9 Patient request forms Clicking on the icon shown by the red arrow opens a window, after typing "add request", the individual panels of parameters that can be ordered are displayed.

Biochemical examinations - part 1

Metabolites and enzymes	Electrolytes and iron	Proteins	Cerebrospinal fluid	Urine	Endocrinology
Albumin	Potassium (K)	Alpha-1 antitrypsin	Albumin	Osmolality	C-peptide
Bilirubin total	Ferritin	Alpha-1-acid glycoprotein	Total protein		Estradiol
Bilirubin conjugated	Phosphorus (P)	ASLO	Elpho of proteins		FSH
Total protein	Magnesium (Mg)	Beta-2 microglobulin	Erythrocytes		Insulin
Glucose	Chlorides (Cl)	C1-inhibitor	Glucose		Cortisol
Creatinine		Ceruloplasmin	Chlorides (Cl)	Bence-Jones qualit.	LH
Uric acid		Haptoglobin	IgA	Hamburger urine sediment	Parathormone
Urea	Osmolality	Rheumatoid factor	IgG	Urine sediment	Prolactin - PRL
	Sodium (Na)		IgM	Proteinuria selectivity	Progesterone
ALP	Transferrin		Lactate	Typing of hematuria	HGH
	Calcium (Ca)		Spectrophotometry		T3 free
	Calcium ioniz. (Ca) ***		AST in cerebrospinal fluid	Albumin to creatinine ratio	T4 free
ALT	Iron (Fe)		LD in cerebrospinal fluid	Creatinine single sample	Testosterone
Amylase	**Iron absorption test**		Mononuclear leukocytes	Albumin single sample	TgAb
Amylase pancreatic	Iron 0.	CRP	Polymorphonuclear leukocytes		TPO Ab
AST	Iron I.	Interleukin - 6		Total protein to creatinine ratio	hTSH
CK	Iron II.	Procalcitonin	Kappa free light chains CSF	Creatinine single sample	Thyreoglobulin
GMT	Iron III.	Presepsin	Kappa index	Total protein single sample	
Cholinesterase	**Imunoglobulins**				**Cardiac markers**
LD	C3-complement	**Only for gynecology**	**Urine**	**Stool**	CK-MB
Lipase	C4-complement	Interleukin-6 in amniotic fluid	AMS	Occult blood test *****	Homocysteine
	IgA		Beta-2 microbulin	Muscle fibers	Myoglobin
Electrophoresis	IgG		Total protein	Starch grains	hs Troponin I
Elpho - serum proteins	IgM	**Oligoclonal bands**	Potassium (K)	Fat drops	NT-proBNP
Elpho - urinary proteins	IgE	**in cerebrospinal fluid**	Phosphorus (P)	Calprotectin	
Elpho - hemoglobin	Kappa free light chains	Oligoclonal IgG	Glucose		
	Lambda free light chains		Magnesium (Mg)		
			Chlorides (Cl)		
Imunofixation				*** calculation	
IFX in serum (IgA,IgG,IgM)	**Lipids**		Creatinine clearence	**** diuresis volume necesary	
IFX in urine (IgA,IgG,IgM)	Cholesterol		Creatinine	***** three samples necessary	
IFX in serum (IgD)	Cholesterol HDL		Uric acid		
IFX in urine (IgD)	Cholesterol LDL				
	Triacylglycerols		Microalbuminuria		
			Urea	Low-cost examinations	
			Sodium (Na)	Medium-cost examinations	
			Calcium (Ca)	Expensive examinations	

Figure 10 Biochemical examinations - part 1 An electronic request form in the hospital information system is created by marking the indicated parameters. When ordering the laboratory tests, it is also advisable to consider their costs, which are depicted in colours. Biochemical tests (Part 1) include serum markers - metabolites and enzymes, lipids, electrolytes, iron, immunoglobulins, serum proteins, electrophoretic testing, immunofixation, inflammatory markers, cardiac markers, and endocrinological tests (hormones). In addition, in this window we can order chemical and microscopic examinations of urine and cerebrospinal fluid (including a specialised test for oligoclonal bands of immunoglobulins). Selected stool examinations are also available.

Biochemical examinations - part 2			
Tumor markers	**Toxicology**	**Medicaments**	**Other examinations**
AFP		Cyclosporin 00 hr	5-HIAA **
Beta HCG		Cyclosporin 02 hrs	
CA 125	Amphetamin	Digoxin	Ammonia
CA 15-3	Barbiturates		HbA1c - glycated hemoglobin
CA 19-9	Benzodiazepines		Chlorides in sweat
CA 72-4	Ethanol in serum		Lactate
CEA	Ethanol in urine	Valproic acid	Osteocalcin
CYFRA 21-1	Extasy	Sirolimus	BETA-CTx
	Cocaine	Tacrolimus	total P1NP
	Metamphetamine		Bile acids
NSE		Vancomycin	
PSA		Gentamicin	Quantiferon
	Morphine	Methotrexate	ALP - liver isoensyme
SCCA	Tetrahydrocannabinol - THC		ALP - bone isoensyme
	Tricycl. antidepressants		
		Vitamins	**Gastric juice**
		Vitamin B_{12} - active	BAO
		Vitamin D - total	MAO1
		Folate	MAO2
			** 5-hydroxyindolylacetic acid
			Low-cost examinations
			Medium-cost examinations
			Expensive examinations

Figure 11 Biochemical examinations - part 2 Displayed Biochemical tests (Part 2) include tumour markers, toxicological screening of urine, medicaments and vitamins in serum, gastric juice and other examinations.

Punctate			
Transudate	**Exudate**	**Synovial fluid**	**Dialysate**
AMS	AMS exudate	C3 complement	Total proteins
Total proteins	Total proteins exudate	C4 complement	Potassium
Erythrocytes	Erythrocytes exudate	Total proteins	Erythrocytes
Glucose	Glucose exudate	Erythrocytes	Phosphorus
Cholesterol	Cholesterol exudate	Glucose	Glucose
LD punctate	LD exudate	IgA	Chlorides
Leukocytes	Leukocytes exudate	IgG	Creatinine
pH punctate	pH exudate	IgM	Uric acid
Rheumatoid factor	Rheumatoid factor exudate	Uric acid	Leukocytes
		LD	Urea
		Leukocytes	Natrium
Punctate		pH	Calcium
Creatinine		Rheumatoid factor	Cmid urea3
			fD urea2
			iD urea1
			Qd dialysate flow rate
			td time of dialysis
			Tid dialysis interval
			Ultrafiltration dial.

Figure 12 Punctate The biochemical examination of punctate enables the differentiation of inflammatory exudate from transudate; we also examine the synovial punctate and dialysate.

Serology	
HAV Ab	Chlamydia pn.IgA
HAV IgM	Chlamydia pn. IgG
HBcAb	Chlamydia pn. IgM
HBc IgM	Chlamydia tr. IgG
HBsAb	Chlamydia tr IgA
HBsAg	Herpes-Simplex virus IgM
	Herpes-Simplex virus IgG
HCV Ab	Mycoplasma pn. IgG
HIV combo	Mycoplasma pn. IgM
Syphilis	Mycoplasma pn. IgA
anti EBNA-IgG	VZV IgG chickenpox
TOXO IgG	VZV IgM chickenpox
TOXO IgM	Tick-borne encephalitis IgM
CMV IgG	Tick-borne encephalitis IgG
CMV IgM	Borrelia IgM
Rubella IgG	Borrelia IgG
Rubella IgM	

Figure 13 Serology At the Department of Clinical Biochemistry, serological tests to detect antibodies and antigens of various infectious agents are also performed.

Specific tests			
oGTT	Glucagon test	Thirst test	Insulin test
Glucose I.	Glucose 1	Osmolality 1	Glucose 1
Glucose II.	Glucose 2	Osmolality 2	Glucose 2
Glucose III.	Glucose 3	Osmolality 3	Glucose 3
Glucose IV.	Glucose 4	Osmolality 4	Glucose 4
	Glucose 5	Osmolality 5	Glucose 5
Glucose urine I.	Glucose 6	Osmolality 6	Glucose 6
Glucose urine II.	Glucose 7		Glucose 7
Glucose urine III.	Glucose 8		Glucose 8
Glucose urine IV.	Glucose 9		Glucose 9
			Glucose 10
Acetone urine I.	Cortisol 1		
Acetone urine II.	Cortisol 2		
Acetone urine III.	Cortisol 3		
Acetone urine IV.	Cortisol 4		
	Cortisol 5		
Protein urine I.	Cortisol 6		
Protein urine II.			
Protein urine III.	Growth hormone - STH 1		
	Growth hormone - STH 2		
	Growth hormone - STH 3		
	Growth hormone - STH 4		
	Growth hormone - STH 5		
	Growth hormone - STH 6		

Figure 14 Specific tests It is possible to perform laboratory measurements for the oral glucose tolerance test, glucagon test, insulin test and thirst test.

DNA analysis	
DNA	
ACE c.2306-109_2306-108insBAT(rs4646994)/angiotensin Iconverting enzyme	IL1RN c.152-516_152(STR) (rs2234663) - interleukin 1 receptor antagonist
AGT p.M268T (rs699) /angiotensinogen	IL23R p.R381Q (rs11209026) - interleukin 23 receptor
APOB p.E4181K (rs1042031) / apolipoprotein B (EcoR I)	ITGA2 c.780-847G>A (rs2910964) - glycoprotein Ia
APOB p.R3527Q (rs5742904) - apolipoprotein B	ITGA2 p.E534K (rs1801106) - glycoprotein Ia
APOB p.T2515= (rs693) - apolipoptrotein B (Xba I)	ITGB3 p.L59P (rs5918) - glycoprotein IIIa
APOE p.C130R (rs429358) - apolipoprotein E	LCT c.117+326C>T (rs4988235) - lactase
	LPL c.1019-1582C>T (rs285) - lipoprotein lipase
ATP7B p.H1069Q (rs76151636) - copper-transporting P-type ATPase	LPL c.1322+483T>G (rs320) - lipoprotein lipase
CETP c.118+279G>A (rs708272) - cholesteryl ester transfer protein	LPL p.N318S (rs268) - lipoprotein lipase
COL1A1 c.104-441T>G (rs1800012) - collagen, type I, alpha 1	LPL p.S474X (rs328) - lipoprotein lipase
CTLA4 c.-157-162C>T (rs5742909) - cytotoxic T-lymphocyte-associated protein 4	MTHFR p.A222V (rs1801133) - methylenetetrahyfdrofolate reductase
CTLA4 p.T17A (rs231775) - cytotoxic T-lymphocyte-associated protein 4	MTHFR p.E429A (rs1801131) - methylenetetrahyfdrofolate reductase
CYP2C8 p.K399R (rs10509681) - cytochrome P450, family 2, subfamily C, polypeptide 8	NOD2 c.3019_3020insC (rs5743293) - nucleotide-binding oligomerization domain containing 2
CYP2C9 p.I359L (rs1057910) - cytochrome P450, family 2, subfamily C, polypeptide 9	NOD2 p.R908G (rs2066845) - nucleotide-binding oligomerization domain containing 2
CYP2C9 p.R144C (rs1799853) - cytochrome P450, family 2, subfamily C, polypeptide 9	NOD2 p.W702R (rs2066844) - nucleotide-binding oligomerization domain containing 2
CYP4V2 p.Q259K (rs13146272) - cytochrome P450, family 4, subfamily V, polypeptide 2	PAI-1 g.4332_4333insG (rs1799889) - plasminogen activator inhibitor, type I
ESR1 c.453-351A>G (rs9340799) - estrogen receptor 1 (Xba I)	PON1 p.Q192R (rs662) - paraoxonase 1
ESR1 c.453-397T>C (rs2234693) - estrogen receptor 1 (Pvu II)	THBD p.A43T (rs1800576) - thrombomodulin
F11 c.1481-188C>T (rs2289252) - coagulation factor XI	TNF c.-233+8274C>T (rs1800629) - thumor necrosis factor
F11 c.56-282T>C (rs2036914) - coagulation factor XI	TPMT p.A154T (rs1800460) - thiopurine S-methyltransferase
F12 c.-4T>C (rs1801020) - coagulation factor XII	TPMT p.A80P (rs1800462) - thiopurine S-methyltransferase
F13A1 p.V35L (rs5985) - coagulation factor XIII A1	TPMT p.Y240C (rs1142345) - thiopurine S-methyltransferase
F2 c.*97G>A (rs1799963) - protrombin	TYMS c.*447delT (rs16430) - thymidylate synthase
F5 Leiden p.R506Q (rs6025) - activated protein C cofactor (Leiden mutation)	VDR c.1024+238G>A (rs1544410) - vitamin D (1,25- dihydroxyvitamin D3) receptor
FGB c.-39-424G>A (rs1800790) - fibrinogen beta chain	VKORC1 c.-226-1413C>T (rs9923231) - vitamin K epoxide reductase complex, subunit 1
HABP2 p.G534E (rs7080536) - hyaluronan binding protein 2 (FSAP)	(rs3135388) - HLA-DRB1*15
HFE p.C282Y (rs1800562) - hemochromatosis protein	DNA isolation
HFE p.H63D (rs1799945) - hemochromatosis protein	
HFE p.S65C (rs1800730) - hemochromatosis protein	

Figure 15 Examinations of DNA In addition to the above-mentioned biochemical examinations, the Department of Clinical Biochemistry also performs molecular biological testing: the detection of gene polymorphisms associated with various diseases. The currently provided genetic tests can be divided into several panels: thrombophilic disorders, pharmacogenetics, hereditary hemochromatosis, disorders of lipid metabolism, osteoporosis, *sclerosis multiplex*.

2.2 Urgency of the examinations

According to requirements for urgency, we distinguish between "routine" examinations, urgent examinations ("*statim*", Lat. immediately) and examinations from vital indications ("*periculum mortis*", Lat. life-threatening). Routine examinations are performed in hospitalised patients in whom biochemical parameters are checked daily or less frequently without urgency. In many cases, the dynamics of a marker's level changes over time is rather more important than their absolute concentration.

"*Statim*" examinations (Fig. 16) are performed in patients with suspected more serious acute conditions, in which a rapid diagnosis is crucial for further correct and time-efficient management (susp. myocardial infarction, acute pancreatitis, acute abdomen, etc.). Test results should be delivered no later than one hour after the specimen's admission to the laboratory, ideally as quickly as possible if allowed by the analytical properties of the measured parameters.

If a specimen from a vital indication (labelled "*periculum mortis*") is received by the laboratory, it is immediately prioritised over all other specimens, and the results are delivered no later than within half an hour, usually in dozens of minutes. However, when requesting the results of urgent examinations by telephone, it is necessary to take into account the time necessary to process the sample - i.e. transport to the laboratory, 5 minutes centrifugation, the time needed for the markers' analysis in the machine (we are unable to accelerate the ongoing chemical reaction at a given temperature and catalysis). For the above reasons, I would like to ask my medical colleagues to be polite and correct without unnecessary shouting at laboratory staff when asking for results over the telephone. When the results of a "*periculum*" examination become available, they are immediately reported to the ordering department.

In addition to laboratory examinations, there are also markers beeing measured directly at the place of hospitalisation or at the patient's bedside, called POCT (*point of care testing*). These tests are usually carried out by a nurse in the clinic where the patient is being cared for. The advantage of these tests is the immediate availability of the result; there is no need to transport the sample to the laboratory and no other related procedures. Typical examples of POCT examinations include glucose measurement by glucometer, acid-base balance examination, urine examination using diagnostic strips, rapid CRP tests, etc. However, it is important to note that the quality of POCT tests also needs to be under the supervision of an accredited clinical-biochemical laboratory.

Urgent examinations (night shifts, weekends) - STATIM, PERICULUM MORTIS				
Metabolites and enzymes	**Cerebrospinal fluid**	**Toxicology**	**Glycemic profile**	**Intrinsic environment**
Albumin	Total protein	Amphetamin	Glycemic profile 1)	ABB artery
Bilirubin total	Erythrocytes	Barbiturates		ABB vein
Bilirubin conjugated	Glucose	Benzodiazepines		ABB capillary
Total protein	Chlorides (Cl)	Ethanol in serum		ABB umbilical artery
Glucose	Mononuclear leukocytes	Ethanol in urine		ABB umbilical vein
Creatinine	Polymorphonuclear leukocytes	Extasy		
Uric acid	Spectrophotometry			
Urea	Lactate	Cocaine		
Lipase		Metamphetamine		Methemoglobin **
ALP				Carboxyhemoglobin **
ALT	**Urine**	Morphine		Oxyhemoglobin **
AMS	Sediment	Tetrahydrocannabinol - THC		Deoxyhemoglobin **
AST	Sodium urine	Tricycl. antidepressants		
CK	**Cardiac markers**		**Punctate transudate**	
GMT	CK-MB		AMS transudate	
	Myoglobin			
Electrolytes	hs Troponin		**Punctate exudate**	
Potassium (K)	NT pro BNP		AMS exudate	
Magnesium (Mg)	**Medicaments**			
Chlorides (Cl)	Digoxin			
Osmolality	Valproic acid			
Sodium (Na)				
Calcium (Ca)				
Calcium ion. (Ca2+) ***	Vancomycin			
Inflammatory markers	Gentamicin			
CRP		**Other examinations**		**Low-cost examinations**
Interleukin - 6		Ammonia		**Medium-cost examinations**
Prokalcitonin		Lactate		**Expensive examinations**
Presepsin				
			1) please note the time of sampling	** take syringe or 2 capillaries
*** - calculation				

Figure 16 Urgent examinations These tests are also available during night shifts, i.e. 24 hours a day, 7 days a week. They include blood tests: basic metabolites and enzymes, electrolytes, inflammatory markers, cardiac markers, intrinsic environment (acid-base balance), glycemic profile, medicaments, other investigations. In addition, urgent examinations also include the analysis of urine (with basic toxicological screening) and cerebrospinal fluid.

2.3 Analytical phase of the laboratory testing

The insertion of a properly collected, labelled, delivered and processed sample into the analyser begins the analytical phase of the laboratory testing. In the laboratory, it is carried out in accordance with GLP (*good laboratory practice*) procedures aimed to eliminate errors in the analytical process.

Depending on the necessity, availability and promptness of execution, we distinguish between basic, special and highly-specialised laboratory tests.

Basic examinations are quickly and continuously available to in-patient and out-patient clinics and departments. Special examinations include the determination of hormones, oncomarkers, medicaments, vitamins, electrophoretic methods, immunofixation, typisation of proteinuria, examination of catecholamines, trace elements, etc. These measurements are performed in the hospital laboratory, but most of them only during normal 8-hour working days, not during night shifts. In some cases, it is necessary to collect samples for a certain

period of time, and the examination is performed only when enough samples have been accumulated to utilise all the spots in the manufacturer's reagent kit. For example, the *QuantiFERON* diagnostic test, used to confirm a tuberculosis infection in a patient, is performed only when 22 samples have been collected in the laboratory (this can sometimes take up to two weeks for economic, time and practical reasons). A similar situation occurs when collecting samples for the examination of oligoclonal IgG bands, electrophoresis of plasma or urinary proteins, etc.

Highly-specialised laboratory tests are performed in laboratories centralised within the regions or country. This fact results from the small number of patients requiring these tests, the economic complexity of the measurement methods and the difficult interpretation of the results. Also, the indication of additional markers measurements requires experience that can only be gained by consolidating these tests and patients in specialised centres.

2.3.1 Principles of analytical methods in the clinical-biochemical laboratory

After the pre-analytical processing of the sample in the laboratory, all samples are sorted and, if necessary, also aliquoted in the AutoMate automatic sorting line, as mentioned above. This is followed by the transfer of the samples to the analysers that are in the appropriate sections: biochemical laboratory, immunochemical laboratory, molecular biological laboratory, electrophoretic laboratory, urinalysis laboratory.

Samples delivered to the "*statim*" laboratory bypass this process because they are immediately processed and prioritised over others. The urgent care laboratory also contains acid-base analysers, analysers assessing glycemia, a microscope for cerebrospinal fluid cytologic examination, and special analysers for measuring cardiac and inflammatory markers.

2.3.1.1 Biochemical laboratory

When the tube is inserted and the sample identification code is read in the automated biochemical analyser, hundreds of marker measurements are performed per hour using photometric methods. Commercially available reagents and wash buffers from certified manufacturers are regularly used to refill the analysers. The analyser is equipped with its own

automatic sample pipettor, stirrers, reagent dispenser, reaction cuvettes, incubation bath, washing system, transport system (sample input - work area - output) and measuring equipment (light beam source, detector). The principle of the analyses can be compared to the measurement of absorbance, which was carried out during exercises from JFMED Medical Chemistry and Biochemistry on the "SPEKOL" instrument. A common component of modern biochemical analysers is a module for direct and very fast (in minutes) measurement of the serum minerals levels (Na, K, Cl), which is using ion-selective electrodes (Fig. 17).

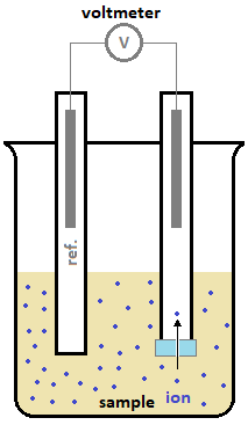

Figure 17 Principle of ion concentration measurement using ion-selective electrode The ISE module contains a reference and a measuring electrode that is selectively permeable for the ion under measurement (see blue part of the electrode). During the transport of the ion and its binding to specific sites of the membrane, an electrical potential difference is generated and compared to the reference electrode (ref.), being proportional to the ion concentration.

Photometric methods are very often used for determining numerous analytes (e.g. glucose, enzymes - AST, ALT, ALP, GMT, AMS, LPS, CK, CK-MB, urea, uric acid, bilirubin, total protein and albumin, creatinine, CRP, calcium, iron, magnesium, phosphate ...). The advantage is the very small sample volume required for the measurement of one analyte (2 - 50 µl) and the quickness of the result delivery. As an example, the measurement of serum glucose is performed using a commercially available kit compatible with a biochemical analyser. The kit contains the reagents hexokinase and G6P-DH (glucose-6-phosphate dehydrogenase), and the reaction proceeds in several steps as shown in Fig. 18.

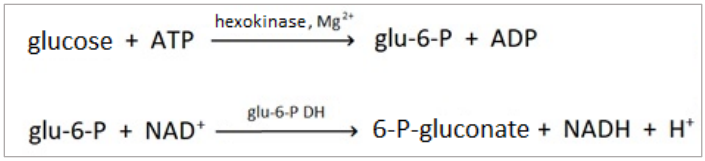

Figure 18 Principle of serum glucose measurement by an automatic biochemical analyser 1, Glucose is phosphorylated by hexokinase to glucose-6-phosphate (glu-6-P). 2, Glucose-6-phosphate dehydrogenase (glu-6-P DH) specifically oxidises glu-6-P to 6-phosphogluconate with simultaneous reduction of NAD^+ to NADH. The light beam intensity is decreased after passing through the cuvette, where the NADH is formed from the sample and reagents. This is reflected by a change in absorbance (340/380 nm), which is proportional to the concentration of glucose in the sample.

In addition to the spectrophotometry described above, many other optical methods are used in automated biochemical analysers, of which turbidimetry, nephelometry and chemiluminescence will be described very briefly. Turbidimetry is an optical method that is based on the measurement of the intensity of transmitted light that is attenuated by scattering on particles; it can be simply compared to the measurement of solution opacity intensity. Nephelometry measures the intensity of diffusely scattered light at an angle different from light beam direction (Fig. 19). In chemiluminescence, a luminometer measures the intensity of photons emitted from an excited substrate that is induced by a chemical reaction. If the reaction is coupled with anodic oxidation, the method is called electrochemiluminescence.

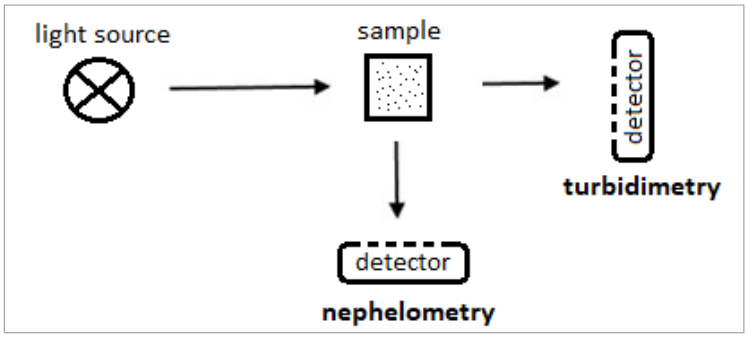

Figure 19 Nephelometry and turbidimetry These are optical methods, the principle of which is to measure the impairment of the light beam intensity by the particles in the sample.

Serum osmolality is directly dependent on the concentration of minerals and low molecular weight substances in the serum. The measurement of osmolality is performed by osmometer using the cryoscopic principle. As the concentration of dissolved substances (osmolality) increases, the very accurately measured freezing point of the solution decreases (approximately 0.001858 °C by dissolution of 1 mmol of substance per 1 kg of serum).

2.3.1.2 Immunochemical laboratory

Immunochemical methods are based on the principle of antigen-antibody reactions. They are mostly used to determine the concentration of protein compounds (tumor markers, hormones, immunoglobulins), as well as drugs, vitamins, etc. As an analogy to biochemical methods, we often use immunoturbidimetry, immunonephelometry, electrochemiluminescence immunoassay (ECLIA), as well as enzyme-linked immunosorbent assay (ELISA), immunofixation and many newer patented methods such as chemiluminescence immunoassay (CMIA), fluorescence polarization immunoassay (FPIA), etc. In comparison to biochemical analyses, the sample volume required for immunochemical analyses is larger (approximately 50-300 µl). This should be kept under consideration when ordering the testing of several immunochemically determined markers (e.g., hormone profile, tumor marker panel), when it may be necessary to send more than one tube of blood to avoid sample shortage and the need for a repeated collection and re-sending of biological material. In addition, the examination time (i.e. also turn-around-time) required for antigen-antibody binding and other reaction steps is significantly longer. Assays of this type, with the exception of cardiac markers, are usually only performed during routine daytime hours, and only in seriously indicated cases during nighttime hours (severe patient conditions, life-threatening situations, initiation of acute therapy). As an example of immunochemical methods, we describe a simplified principle of serum vitamin D level determination by the CMIA method in an automated immunochemical analyser (Fig. 20).

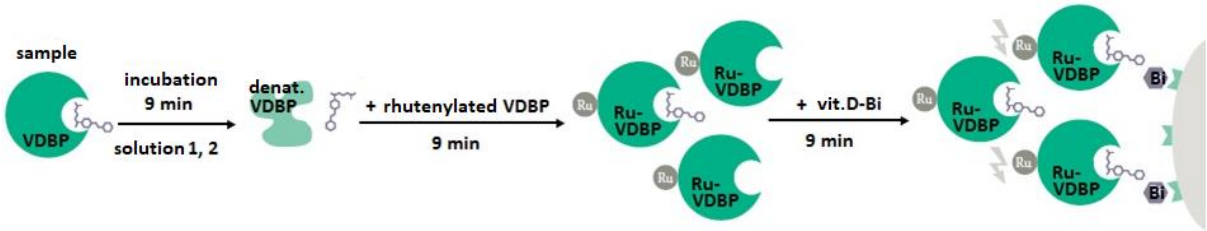

Figure 20 Determination of vitamin D (calcidiol) serum level by electrochemiluminescence assay The vitamin D (vit. D) in serum, reflecting its metabolism in human body, is transported by the vitamin D binding protein (VDBP). After incubation of the sample with reaction solutions 1 and 2, the VDBP in the sample is denatured and vitamin D is released. Subsequently, ruthenylated VDBP (Ru-VDBP) is added in excess to form immunocomplexes with the released vitamin D in the sample. The unreacted ruthenylated VDBP is further incubated with biotinylated vit. D (vit. D-Bi), which forms complexes with streptavidin present on the microparticles of the measuring electrode. In the mentioned reactions, electrochemiluminescence, i.e. the emission of photons during the chemical reaction at the electrode, is induced, the intensity of the emitted light being inversely proportional to the concentration of vitamin D in the sample.

Immunochemical methods are also used in toxicological screening for the presence of drugs in urine, which is carried out 24 hours a day in the statim laboratory. These are immunochromatographic tests capable of detecting various types of drugs and medicaments (amphetamines, barbiturates, benzodiazepines, ecstasy, cocaine, methamphetamine, opioids, tetrahydrocannabinol (THC), tricyclic antidepressants, etc). The single-use cartridge used for testing is shown on the Figure 21.

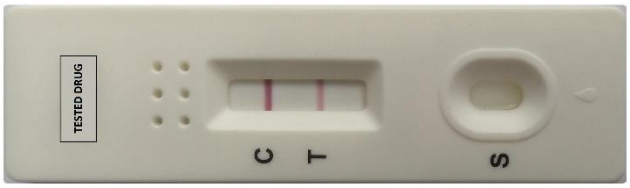

Figure 21 Immunochromatographic tests used for the toxicological screening of drugs in urine After applying 200 µl of the sample to the well in the cartridge (S), capillary flow of the sample and competition with the drug conjugate for the binding site on the specific antibody occurs. A control line (C) is always present if the test has been performed correctly. If the drug is present in the sample at a sufficient concentration (sensitivity of tens to hundreds ng/ml, e.g. THC 15 ng/ml), it prevents the binding of the conjugate and the line in the test area (T) does not appear - the test is positive. If the drug is not present, the conjugate binds, a coloured line appears in the T area - the test is negative.

2.3.1.3 Electrophoretic laboratory

The principle of electrophoresis is the separation of particles according to their size and charge in an electric field. In the electrophoretic laboratory, we perform the electrophoresis of proteins (serum, urine, cerebrospinal fluid), examination of hemoglobins and alkaline phosphatase isoforms. The separation is performed in a specific medium, usually an agarose gel, then the separated protein fractions are stained, and the gel is dried and scanned. The densitometric evaluation of the obtained image enables us to assess the percentage of each protein fraction from the serum (Fig. 22). The electrophoretic examination of the urine allows us to determine the type of proteinuria. In the cerebrospinal fluid, we electrophoretically examine the so-called oligoclonal bands of immunoglobulins which are synthesised "intrathecally" by one clone of plasma cells in the central nervous system as a result of a pathological immune response. This test is positive if we find 2 or more bands of antibodies in the CSF that are not present in the serum.

Immunofixation electrophoresis is used to confirm the presence and determine the type of paraprotein in serum or urine in patients with monoclonal gammopathies, or to identify von Willebrand factor multimers. After the electrophoretic separation of proteins, the separated proteins are immunoprecipitated with monoclonal antibodies (antisera) against the target proteins, followed by thermal fixation, drying, visualization and evaluation.

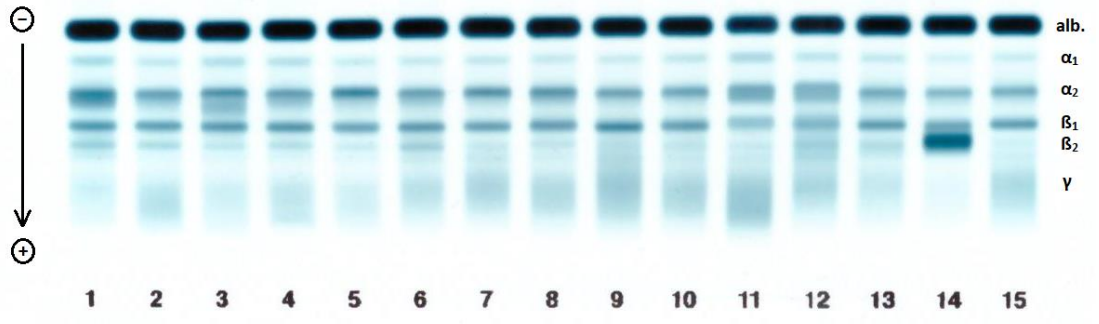

Figure 22 Electrophoresis of human serum proteins The figure shows an electropherogram of human serum, each number represents a sample from one patient. After sample deposition, the proteins migrate due to their negative charge from the cathode to the anode, then they are stained, the gel is dried, scanned and evaluated densitometrically. Protein fractions - albumin (alb.), globulins (α1, α2, ß1, ß2, γ) are visible as so-called "bands".

2.3.1.4 Molecular genetics laboratory

In the molecular genetics laboratory, we analyse mutations in DNA (deoxyribonucleic acid) that are associated with specific diseases. We are currently investigating several panels of gene polymorphisms in relation to thrombophilic states, pharmacogenetics, hereditary hemochromatosis, disorders of lipid metabolism, osteoporosis and *sclerosis multiplex*. DNA isolation and purification is required before analysis. We are currently using an automatic isolation machine that utilises the principle of DNA binding to paramagnetic particles. At a single run, this machine can process 32 samples, which are successively collected according to the clinicians' requirements. In general, DNA mutation analysis is not an emergency test; the results are usually delivered within several weeks. However, delivering the result as quickly as possible can be important in pharmacogenetics, where the choice of the appropriate drug dosage related to its potential toxicity depends on the genotype of the patient. In addition, DNA can also be isolated using affinity columns or other special isolation kits. The general principle of DNA isolation is illustrated in Fig. 23.

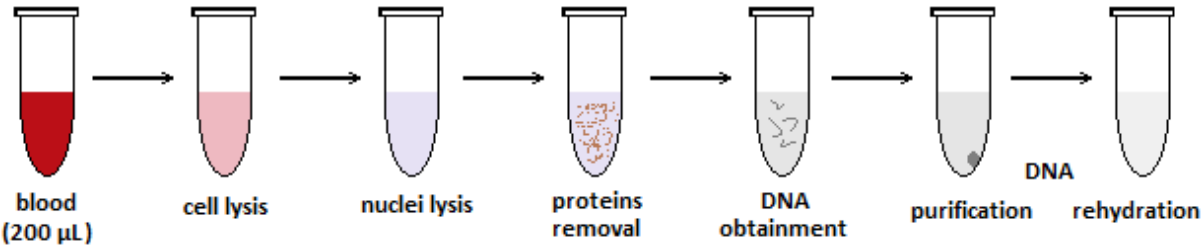

Figure 23 DNA isolation - general principle and steps A sample of whole blood containing DNA in the leucocyte nuclei is mixed with a solution that causes lysis of the cell membranes. The intracellular contents are released, the nuclei of the cells are disrupted by releasing the DNA and proteins are removed from the solution. The DNA is obtained by precipitation with a solution of concentrated alcohol, or by trapping the DNA in a molecular resin using affinity columns, or by binding the DNA to paramagnetic particles in an automated isolation apparatus. Subsequently, the DNA is purified several several times, hydrated with a special buffer solution and stored for further use in a refrigerator; for long-term storage (decades) it is kept frozen.

The mutation analysis itself is performed using the polymerase chain reaction (PCR), which serves primarily to obtain a sufficient number of DNA copies for further analysis, or to directly detect the mutations (special types of PCR).

Question F What are the components of the reaction mixture and main steps of the PCR reaction? Which special enzyme do we use?

Answer F The reaction uses the enzyme Taq polymerase, which is isolated from the bacterium *Thermus aquaticus* and is thermostable. The reaction mixture requires the presence of the Taq polymerase, a DNA sample, nucleotides, buffer, and magnesium ions. The main PCR steps are: DNA denaturation (formation of single-stranded DNA), primer binding (annealing) and polymerisation (elongation). In one cycle, the amount of DNA is doubled; after 30-40 cycles we have millions of copies of DNA.

Currently the most-used methodology to detect DNA mutations in our laboratory is melting point analysis (HRM analysis, High Resolution Melting analysis); we also use the allele specific PCR or restriction fragment length polymorphism (RFLP) analysis. The principles of these methods are explained in Fig. 24.

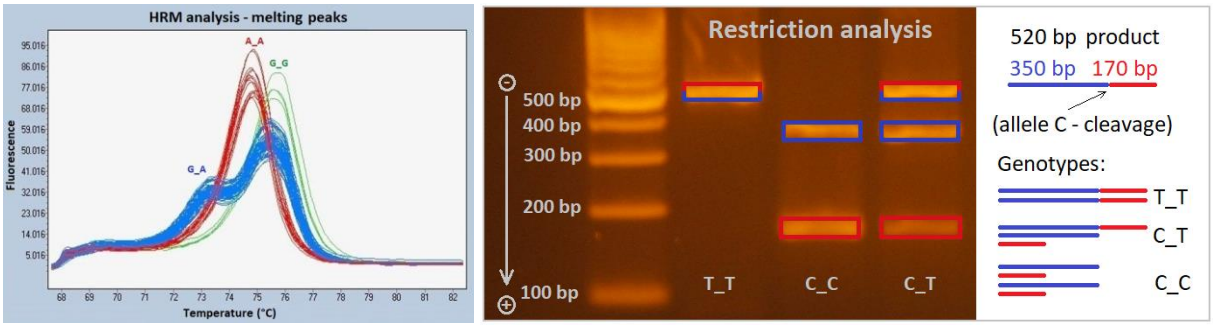

Figure 24 Principles of DNA mutation analysis The left part of the figure represents the result of the so-called **DNA melting point curve analysis (HRM)**. The specific DNA fragment of about 50 bp (base pairs) in which the target G → A mutation (substitution of guanine for adenine) is located is amplified by real-time PCR. For each cycle that runs, the fluorescence that is measured is proportional to the amount of produced DNA. After amplification, DNA is heated to yield single-stranded DNA, which results in a significant change of the sample fluorescence. According to the DNA melting temperature, we can determine the genotype: genotype A_A - lower melting temperature (adenine - thymine are bound by two hydrogen bonds), genotype G_G - higher melting temperature (guanine - cytosine are bound by three hydrogen bonds), heterozygous genotype G_A is formed by a combination of both curves. The right part of the figure shows the result of the so-called **restriction fragment length polymorphism (RFLP) analysis**. The target DNA sequence is amplified and subsequently cleaved using restriction endonuclease. Then the resulting fragments are separated by agarose gel electrophoresis. In this case, a restriction enzyme cleaved the DNA when the normal C allele was present; in the presence of a mutation (T allele), cleavage did not occur. This means that the T_T genotype is characterised by the presence of uncleaved DNA fragments with a full fragment length of 520 bp (two T alleles), the C_T genotype by cleavage of one strand into fragments of 350 bp + 170 bp (one C allele) in addition to 520 bp (one T allele), and the homozygous C_C genotype only by cleaved fragments of 350 bp + 170 bp (two C alleles).

Allele-specific PCR uses several primers, one of them specific for the mutant allele, the other for the normal allele. In the presence of a mutation, the amplification yields a product of a different size than the product of the normal allele amplification. In addition, another pair of primers is used to synthesise a DNA fragment, the presence of which ensures the correctly performed PCR reaction. The fragments are then visualised and the result is similar to the restriction analysis, i.e. a control fragment and DNA fragments of various lengths correlating with the presence or absence of the mutation.

2.3.1.5 Examination of the acid-base balance

The acid-base balance can be tested from whole blood, which must be transported to the laboratory packed with ice immediately after collection, but cannot be frozen. Most often, so-called arterialised capillary blood, obtained from the fingertip after its hyperemisation, is delivered to the laboratory. The blood is drawn into a heparin-coated capillary containing a thin steel nail inside, allowing the sample to be mixed with a magnet after collection and just

before analysis. The capillary is closed on both sides with plastic caps and must be free of air bubbles, which might significantly affect the accuracy of the result due to gases diffusion.

As well as capillary blood, arterial blood is also relatively often drawn into special 1 ml capped syringes for acid-base balance testing; in the past, conventional 2 ml syringes flushed with heparin prior to collection were used (Fig. 25). The collection must be anaerobic, i.e. the syringes must be capped and free of air bubbles. Venous blood can be collected in a similar manner.

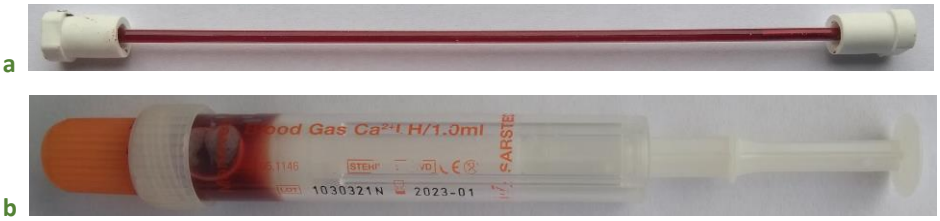

Figure 25 Blood samples sent to the laboratory for analysis of the acid-base balance a - arterialised blood in the capillary must be anaerobically closed and free of bubbles, a thin stainless steel nail stirrer can be seen at the right margin of the capillary. b - arterial blood delivered in a special capped collection tube containing Ca^{2+} heparin; there should be no bubble in the tube, since it could negatively affect the result accuracy.

The tested sample is slowly aspirated into a thin plastic tube in the acid-base analyser and successively comes into contact with the measuring electrodes, where electrochemical processes occur. The basic principle of the measurement is shown in Fig. 26.

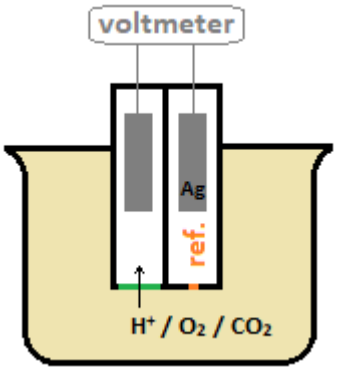

Figure 26 Principle of measurement of pH and partial pressures of blood gases in the sample The **pH** measurement is performed with a machine called a pH meter, which determines the activity of hydrogen ions (H^+) in the solution. The body of the instrument, which is submerged in the sample, contains a glass electrode (green) and a reference electrode (ref., orange). The glass electrode is made of special, thin glass that is selectively permeable to H^+; the reference electrode contains a silver probe in a solution with a precisely defined concentration of Cl^-. In an acidic medium, excess H^+ diffuses through the glass electrode and the occuring

chemical reaction is accompanied by the generation of an electric potential proportional to pH. The measurement of partial pressures of **blood gases** (O_2, CO_2) is also based on the potentiometric principle using a silver reference electrode. Oxygen diffuses through a selectively permeable teflon membrane and is reduced at the platinum electrode. Carbon dioxide diffuses through the silicone membrane and reacts with the bicarbonate solution changing the H^+ concentration.

2.4 Diagnostic properties of laboratory markers

The diagnostic properties of laboratory methods characterise their properties from a clinical point of view, i.e. their relationship to the diagnosis. These include diagnostic sensitivity, diagnostic specificity and diagnostic efficiency, which can range from 0-1 (0-100%). They are directly related to the discrimination value (*'cut-off'*).

Diagnostic sensitivity indicates the proportion of the number of sick individuals with a positive test to the total number of sick individuals tested. It can be defined as the probability that a test result will be positive if the person being tested is diseased.

Question G Serum alpha-fetoprotein (AFP) levels were examined in 20 patients with histologically verified hepatocellular carcinoma, 12 of whom had elevated levels above the "cut-off". What is the diagnostic sensitivity of AFP in the diagnostics of hepatocellular carcinoma?

Answer G The diagnostic sensitivity of AFP in the diagnostics of hepatocellular carcinoma is 60%.

Diagnostic sensitivity $= \frac{\text{true positive sick individuals}}{\text{all sick individuals}} = \frac{12}{20}$ = 0.6 (60%)

Diagnostic specificity is related to healthy individuals and is defined as the ratio of the number of healthy individuals with a negative test to the total number of healthy individuals tested. Diagnostic specificity is a measure of the ability of a test to exclude the presence of disease and expresses the probability of a negative result in a healthy person.

Question H Serum AFP levels were examined in 50 medical students without any health problems, 2 of whom had elevated levels above the reference limit. Subsequently, further investigations were performed in these two subjects excluding the presence of liver carcinoma. What is the diagnostic specificity of AFP?

Answer H The diagnostic specificity of AFP in the diagnostics of liver carcinoma is 96%.

$$\text{Diagnostic specificity} = \frac{\text{true negative individuals}}{\text{all examined healthy individuals}} = \frac{48}{50} = 0.96\ (96\%)$$

Both the diagnostic sensitivity and specificity of a marker used in practice should ideally be greater than 90%. The ability of the method to classify correctly both healthy and diseased individuals is expressed by the **diagnostic effectiveness**. The higher the diagnostic efficiency of the method, the more likely it is that the result will be negative in healthy individuals and that it will be positive in diseased individuals.

Question I Determine the diagnostic effectiveness of serum AFP level testing in the diagnostics of liver carcinoma. Its level was elevated in 30 out of 50 diseased patients and in 2 out of 50 healthy subjects.

Answer I The diagnostic effectiveness of AFP in the diagnostics of liver carcinoma is 78%.

$$\text{Diagnostic effectiveness} = \frac{\text{true positive} + \text{true negative}}{\text{all examined individuals}} = \frac{30 + 48}{50 + 50} = 0.78\ (78\%)$$

The diagnostic properties of the laboratory method are directly related to the so-called discrimination value (cut-off), which distinguishes healthy and sick individuals. The cut-off can be determined using different methods by testing a large cohort of healthy and diseased individuals. If the value of the marker tested is above the cut-off, the individual is classified as positive (diseased), and if the value is below the cut-off, the individual is classified as negative (healthy).

As a simplified illustrative example, let us consider the examination of neuron-specific enolase (NSE) in a set of 10 neuroblastoma patients and 10 healthy subjects. Table 1 demonstrates, using the NSE level values, how successive changes in the cut-off value settings affect the diagnostic sensitivity and specificity of the marker. Using a cut-off of 16 ng/ml (from clinical practice), the diagnostic sensitivity of the marker is 60% and the diagnostic specificity is 90%. When the cut-off value is reduced to 10 ng/ml, the sensitivity increases to 100% (detecting all patients) and the specificity decreases to 60% (at the cost of increased false positivities in healthy subjects). Conversely, when the cut-off is increased to 21 ng/ml, the sensitivity in the sick patients significantly reduces to 40% (i.e. there is a high false negativity),

while the specificity in the healthy individuals is 100% (i.e. zero false positivity). An optimal compromise would be to set the cut-off value at 14 ng/ml, at which the NSE level test has both diagnostic sensitivity and specificity of 90% (illustrative example).

However, if we would like to use NSE as a **screening marker** in neuroblastoma diagnostics, it would be better to set the cut-off value slightly lower than the optimum, in order to exclude false negatives in diseased individuals. For example, if we used a cut-off value of 10 ng/ml, all sick individuals (10 out of 10) would be identified, and at the same time 4 healthy individuals (4 out of 10) would be false positive. These would then undergo further imaging tests to rule out the disease. An example of an excellent screening marker is the use of prostate specific antigen (PSA) in prostate cancer diagnostics. A higher false positivity rate is required in order to identify all individuals with any pathological processes in the prostate; other methods are subsequently used to exclude the diagnosis in healthy individuals.

Table 1 The impact of cut-off marker value (NSE) on diagnostic sensitivity and specificity

		Healthy individuals										**Patients with neuroblastoma**									
NSE (ng/mL)		3	4	6	6	7	8	10	11	12	13	14	15	15	18	19	20	25	84	90	98
***Cut-off* (ng/mL)**	**16**	TN	TN	TN	TN	TN	TN	TN	TN	TN	FN	FN	FN	FN	FP	TP	TP	TP	TP	TP	TP
		Specificity = TN / all healthy = 9/10 = 0.9 (90%)										Sensitivity = TP / all diseased = 6 / 10 = 0.6 (60%)									
	10	TN	TN	TN	TN	TN	TN	FP	FP	FP	TP	TP	TP	TP	FP	TP	TP	TP	TP	TP	TP
		Specificity = TN / all healthy = 6/10 = 0.6 (60%)										Sensitivity = TP / all diseased = 10 / 10 = 1.0 (100%)									
	21	TN	TN	TN	TN	TN	TN	TN	TN	TN	FN	FN	FN	FN	TN	FN	FN	TP	TP	TP	TP
		Specificity = TN / all healthy = 10/10 = 1.0 (100%)										Sensitivity = TP / all diseased = 4 / 10 = 0.4 (40%)									
	14	TN	TN	TN	TN	TN	TN	TN	TN	TN	FN	TP	TP	TP	FP	TP	TP	TP	TP	TP	TP
		Specificity = TN / all healthy = 9/10 = 0.9 (90%)										Sensitivity = TP / all diseased = 9 / 10 = 0.9 (90%)									

Abbreviations: FN - false negative in diseased patients, FP - false positive in healthy individuals, TN - true negative in healthy individuals, TP - true positive in diseased patients

2.5 Reference values of the laboratory method

The reference ranges of the laboratory method allow us to categorise the patient's result as normal, increased or decreased. The reference interval (physiological range) is determined by the upper and lower reference limits and comprises 95% of the values obtained

by the marker level measurement in the reference population. Critical values of the result (decision limit) may be directly related to the patient's life-threatening condition and the laboratory technician is responsible for reporting them to the ordering department (nurse or physician) (Fig. 27).

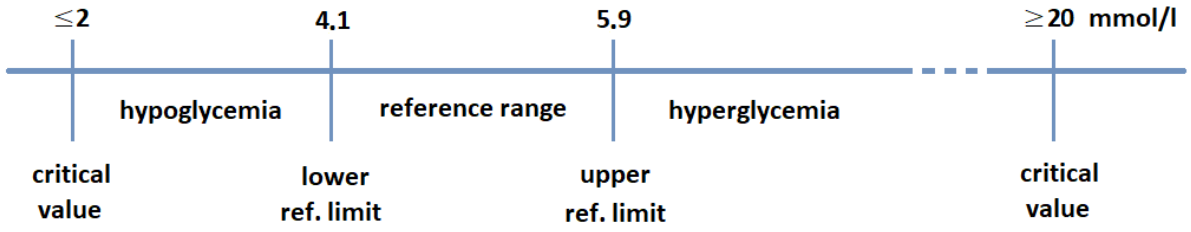

Figure 27 Glycemia reference range The lower and upper reference limits indicate the lowest and highest physiological value of the result and define the reference interval. Glycemia levels higher and lower than the critical values may be associated with a threat to the patient's life and increased mortality.

If we want to classify a test result as physiological or pathological using the reference limits, we need to carefully understand how the reference limits are created. A reference group is defined as a set of individuals presumed to be free of disease, and is obtained by random selection, according to age, sex, race and other characteristics. After the appropriate collection of biological material and analysis of the sample, the obtained results are then processed according to the distribution pattern by various statistical methods (Fig. 28). The fact that the reference limits contain 95% of the values obtained from the marker analysis in the reference population logically indicates that the result in 5% of healthy individuals lies physiologically outside the reference interval. Therefore, when interpreting the result of a biochemical test, we take into account not only the absolute value of the result and its relationship to the reference range - i.e. a normal physiological result, a pathologically increased or decreased result (mild, moderate, significant, extreme), but also a number of additional factors, which are described in Chapter 1.9. In Table 2, we present the actual reference values for selected basic biochemical parameters in healthy adults used in our laboratory.

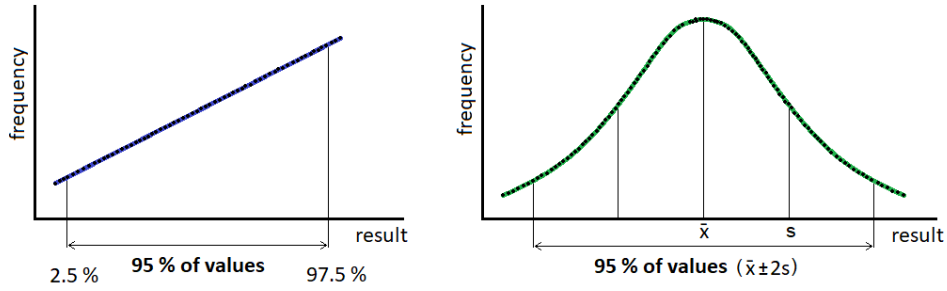

Figure 28 Determination of the reference range by the direct inductive method The reference interval includes 95% of the values obtained in a healthy reference population. For the direct non-parametric method (blue), the results are arranged in ascending order and the reference interval is made up of values between the 2.5 and 97.5 percentile. The direct parametric method (green) is used when the values are normally distributed (Gaussian curve) and the reference interval is defined as the mean of the result ± standard deviation ($\bar{x} \pm 2s$).

Question J The results of marker levels analysis in serum are expressed in different units. Define the amount of substance (1 mol) and the enzyme activity (1 katal).

Answer J The amount of substance **1 mol** is defined by Avogadro's constant and contains 6.022×10^{23} particles. An enzyme has the activity **1 katal** if it catalyses the conversion of 1 mole of substrate per 1 s, this has to be under optimal conditions (complete saturation with substrate, temperature, pH) and in the presence of catalyst activators. Since both units are relatively large, in biochemical practice we use the smaller units - µkat, nkat, mmol and µmol, respectively per litre of serum.

Table 2 Reference intervals of selected biochemical laboratory tests for healthy adults

	Name	*Sample*	*Abbreviation*	*Reference interval*	*Unit*	*Sex*
Glucose	**Glycemia**	serum	S_GLU	**4.1 – 5.9**	mmol/L	
	Glycosuria	urine	U_GLU	**0**	mmol/L	
Minerals and trace elements	**Natrium**	serum	S_Na	**136 – 146**	mmol/L	
	Potassium	serum	S_K	**3.5 – 5.1**	mmol/L	
	Calcium – total	serum	S_Ca	**2.20 – 2.65**	mmol/L	
	Calcium – ionized	serum	S_CaI	**1.03 – 1.3**	mmol/L	
	Chlorides	serum	S_Cl	**101 – 109**	mmol/L	
	Magnesium	serum	S_Mg	**0.73 – 1.06** **0.77 – 1.03**	mmol/L	male female
	Phosphorus inorganic	serum	S_P	**0.81 – 1.45**	mmol/L	
	Iron – total	serum	S_Fe	**12.5 – 32.2** **10.7 – 32.2**	µmol/L	male female
Nitrogen waste products	**Ammonia**	plasma	S_AMON	**16 – 53**	µmol/L	
	Creatinine	serum	S_KRE	**59 – 104** **45 – 84**	µmol/L	male female
	Urea	serum	S_UREA	**2.8 – 7.2**	mmol/L	
	Uric acid	serum	S_KMOC	**208 – 428** **155 – 357**	µmol/L	male female
Plasma proteins	**Total protein**	serum	S_TP	**66 – 83**	g/L	
	Albumin	serum	S_ALB	**35 – 52**	g/L	
Bile pigments	**Bilirubin total**	serum	S_TBIL	**5 – 21**	µmol/L	
	Bilirubin - conjugated	serum	S_BILk	**0.1 – 3.4**	µmol/L	
Lipid panel	**Triacylglycerols**	serum	S_TRG	**0.4 – 1.7**	mmol/L	
	Cholesterol	serum	S_CHOL	**< 5.17**	mmol/L	
	Cholesterol HDL	serum	S_HDLC	**1.03 – 2.00** **1.20 – 2.20**	mmol/L	male female
	Cholesterol LDL	serum	S_LDLC	**1.00 – 3.30**	mmol/L	
Acid-base balance	**Lactate**	plasma	pl_LAK	**0.5 – 2.2**	mmol/L	
	Base excess	blood	krv_BE(B)	**-2.5 – 2.5**	mmol/L	
	Actual bicarbonate	blood	krv_HCO_3	**22.0 – 26.0**	mmol/L	
	Partial pressure CO_2 (non-bound)	blood	krv_PCO_2	**4.64 – 6.00**	kPa	
	Partial pressure O_2	blood	krv_pO_2	**9.8 – 13.3**	kPa	
	Hemoglobin oxygen saturation	blood	krv_O_2 sat	**95 – 99**	**%**	
	pH	blood	krv_pH	**7.36 – 7.44**	-	
	pH	urine	U_pH	**4.8 – 7.4**	-	
Plasma enzymes	**Aspartate Aminotransferase**	serum	S_AST	**< 0.85**	µkat/L	male
	Alanine Aminotransferase		S_ALT	**< 0.60**		female
	Gamma-glutamyl transferase	serum	S_GMT	**< 0.92** **< 0.63**	µkat/L	male female
	Amylase	serum	S_AMS	**0.46 – 1.66**	µkat/L	
	Alkaline phosphatase	serum	S_ALP	**0.50 – 2.15**	µkat/L	
	Lactate dehydrogenase	serum	S_LD	**1.83 – 4.12**	µkat/L	
	Lipase	serum	S_LPS	**0.22 – 1.00**	µkat/L	
Cardiomarkers	**Creatine kinase**	serum	S_CK	**< 2.85** **< 2.42**	µkat/L	male female
	Creatine kinase – isoform MB	serum	S_CKMB	**< 0.4**	µkat/L	
	High-sensitivity Troponin I	plasma	S_hsTnI	**< 19.8** **< 11.6**	ng/L	male female
	Myoglobin	serum	S_MYOG	**< 70**	µg/L	
Inflammatory markers	**C-reactive protein**	serum	S_CRP	**< 5**	mg/L	
	Procalcitonin	serum	S_PROC	**< 0.5**	µg/L	
	Presepsin	plasma	S_PRES	**< 337**	ng/L	
	Interleukin 6	serum	S_IL6	**< 6.4**	ng/L	
Other markers	**Prostate-specific antigen**	serum	S_PSA	**< 4**	µg/L	
	Vitamin D	serum	S_VIDc	**30 – 100**	µg/L	
	Osmolality	serum	S_OSM	**275 – 295** **280 – 300**	mmol/kg	0 – 60 y > 60 y

2.6 Analytical characteristics of laboratory methods

The analytical properties of laboratory methods will be described by basic informations, directly related to the result quality and their presentation to the ordering physician. An important analytical characteristic of the laboratory method is its **accuracy**, which is affected by the deviation of the result from the true value. The main components of accuracy are **precision** and **trueness** (Fig. 29).

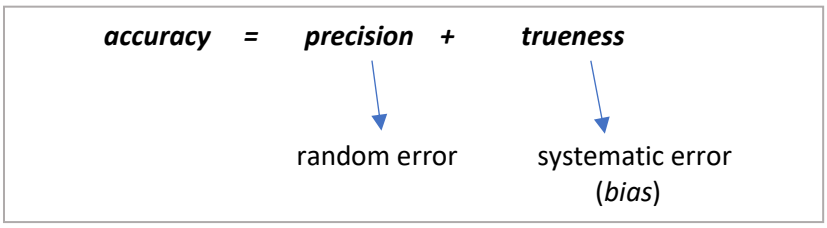

Figure 29 Basic analytical properties of the laboratory method and errors The accuracy of the result is a combination of precision and trueness. Accuracy is affected by random error and trueness by systematic error.

The **precision** of a laboratory method is defined as the degree of concordance between results obtained by the repeat analysis of a single sample. We distinguish between **precision in a series** - repeatability (multiple analyses performed at the same time) and **precision over time** - reproducibility (analyses performed once a day over a longer period of time), which determines the stability of the method. The precision of the method is affected by **random error**, which cannot be completely eliminated and is caused by the influence of temperature, variations in the machine performance and other factors. The maximum acceptable method uncertainty is defined by a coefficient of variation of 10%. The **trueness** of a laboratory method expresses the tightness of the result mean value to the true value and is affected by **systematic error (bias)**. The relationship between precision and trueness is shown in Fig. 30.

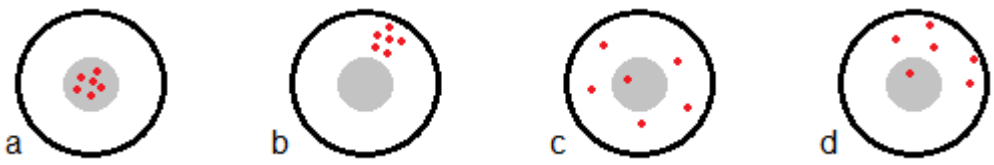

Figure 30 Relationship between accuracy, precision and trueness of the laboratory method There are four possibilities for the results' correctness: a - precise true (represented by the shots in the middle circle of the target), b - precise false, c - imprecise individually false, arithmetic mean true, d - imprecise false

Since every measurement is burdened by a measurement error that cannot be completely eliminated, there is a parameter called the **measurement uncertainty**. This is defined as the interval that surrounds the true value and may contain the measured variable result. It is used very little in practice because, for example, at 5% measurement uncertainty of serum cholesterol, the common result of 5 mmol/L would have to be reported by the laboratory as 5 mmol/L ± 5% (i.e. 4.75-5.25 mmol/L).

The **analytical sensitivity** of method determines the smallest difference in concentrations that can be measured. The greater the response (e.g. change in absorbance) to a small change in concentration of the measured substance, the higher the analytical sensitivity. Practically, analytical sensitivity is related to terms such as limit of detection and limit of quantification, which directly affect the character of the laboratory result and are shown in Figure 31.

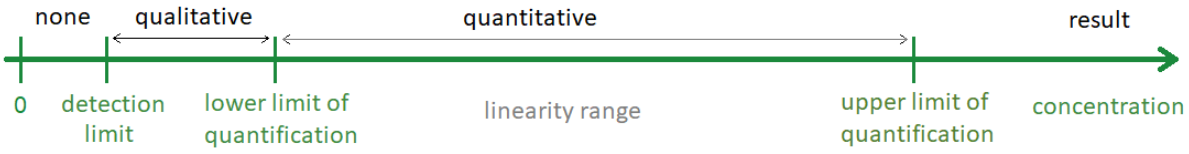

Figure 31 Analytical sensitivity of the method and the character of provided results When the analyte concentration is below the detection limit, no result can be given. At a concentration between the limit of detection and the lower limit of quantification, the laboratory shall give a qualitative result (positive/negative). The lower and upper limit of quantification define the range of concentrations where a quantitative result can be given at the specified units with the required precision. This range is typically characterised by a linear dependence of the absorbance change (or other measured variable) on the concentration of the substance. If the analyte concentration is below the lower limit of quantification and a quantitative result is required, the laboratory will provide the result as '< lower limit of quantification'. Above the upper limit of quantification, dilution of the sample is necessary to reduce the concentration into the linearity range; the dilution factor (usually 10-fold, sometimes up to 100-fold, exceptionally more) has to be taken into account when calculating the result. If the concentration of the analyte cannot be measured even at the highest permissible dilution, the laboratory will give the result as '> upper limit of quantification'.

Analytical specificity (selectivity) expresses the extent to which the determination of a substance in biological material is affected by other substances reacting with the reagent (called interferences). It can also be defined as the ability to discriminate between the analyte being measured and other compounds in the sample. An example of interference may be immunochemical methods for the determination of similar substances (opiates - morphine, codeine, heroin i.e. diacetylmorphine) where a sort of group cross-reactivity can be present.

2.7 Assessment of the quality in laboratory practice

The aim of quality control is to ensure the analytical accuracy of the laboratory results obtained. Quality control is performed at two levels - internal and external.

Internal quality assessment is assured by the laboratory and consists of daily or periodic monitoring of the measurements' stability. Precision (reproducibility) is monitored by analysing a control sample with a known concentration of the analyte, while the results should be matched within a precisely defined range. The trueness control is also performed regularly using commercially available sera with a precisely defined range of expected values (both physiological and pathological). If the quality control is not successful, recalibration of the method and re-quality control is mandatory until the method achieves the required precision and trueness.

External quality assessment ensures the so-called interlaboratory equivalency of results, i.e. it compares the measurement results of the provided sera between laboratories among each other. It ensures that if we measure e.g. glycemia in three different laboratories in Slovakia, or in another laboratory in the Czech Republic, the values will correlate with each other with a certain maximum acceptable variation. External quality control is carried out by national or supranational companies that supply the laboratory with a sample containing a certain analyte of unknown concentration. Once measured, the result is uploaded into an online system where, after comparison and evaluation, the laboratory receives a certificate issued by that company. External quality control is performed at various time intervals, usually approximately every 6 months, or at a higher frequency for more sensitive analytes (e.g. once a month for acid-base balance).

2.8 Post-analytical phase of laboratory testing and interpretation of results

The so-called post-analytical phase of the laboratory testing starts when the result comes out of the analyser. The test result is automatically electronically transferred to the laboratory information system (LIS). The first person in contact with the result is the laboratory technician operating the analyser, who pre-evaluates the result and checks its **analytical accuracy**. The laboratory technician shall re-perform the analysis if the result does not correlate logically with the previous record, or has an extremely low or high value (here a

sample dilution is necessary). If the result is suspected to be affected by interference, the laboratory technician assesses the severity of the interference, may delete the result and require the ordering department to retake the sample. As an example, hyperkalemia in a hemolysed sample may artificially reach values as high as 10 mmol/L or more.

After the above-described analytical accuracy control, the result is submitted to a so-called **medical control**. This is carried out by doctors and other authorised laboratory staff who are competent to transmit the results to the ordering clinicians through the hospital information system. This control evaluates the correlation of the results with previous results, the patient's diagnosis and other findings from clinical examination and medical imaging methods. This data can be obtained from the patient's documentation in the hospital information system or directly by phone consulting the responsible physician. After the validation, the laboratory result is then transmitted to the hospital information system; if necessary, a clinical biochemist's commentary for the ordering physician can be added. In addition to the electronic delivery, for decades the results were also delivered to the ordering clinicians in a paper printed format, but this has recently been discontinued and currently the results are delivered only electronically. **Critical values**, i.e. extremely elevated or extremely decreased marker concentrations, have to be reported quickly after detection by telephone to the ordering clinic by the laboratory staff, with a record of the report being made in the laboratory information system.

After the analysis in the laboratory, the sample is loaded back into the automatic sorting line, which then places the sample into a storage rack and gives it a particular number. These samples are stored in the laboratory for 72 hours in a refrigerated room, where they are available for further analysis in the case of the clinician's re-ordering requirements.

The interpretation of laboratory results is usually carried out by the patient's treating physician, but should be interdisciplinary. The interpretation of the results is made in relation to physiological reference ranges and, due to the above mentioned methods of their acquisition, the fact that 5% of healthy individuals may have a result outside the reference range should be taken into serious consideration. It is obvious that not every result within the reference range correlates with the health of the patient and, conversely, not every result outside the reference range is a guarantee that the investigated individual is sick. Laboratory results are not just numbers on paper and they have to be interpreted carefully. As well as the

absolute value of the result, comparing the dynamics in the changes of results over time is of greater importance. When interpreting the result of a laboratory testing, data from the patient's medical history, findings of clinical examination and other imaging and investigation methods must be seriously taken into account. If there is any doubt as to the accuracy of the result, it is possible and sometimes even necessary for the ordering physician to collaborate with the medical laboratory staff, as there are many factors that can influence the precision and trueness of the obtained result.

The “life-story” of a biological sample in the clinical-biochemical laboratory is described in Fig. 32.

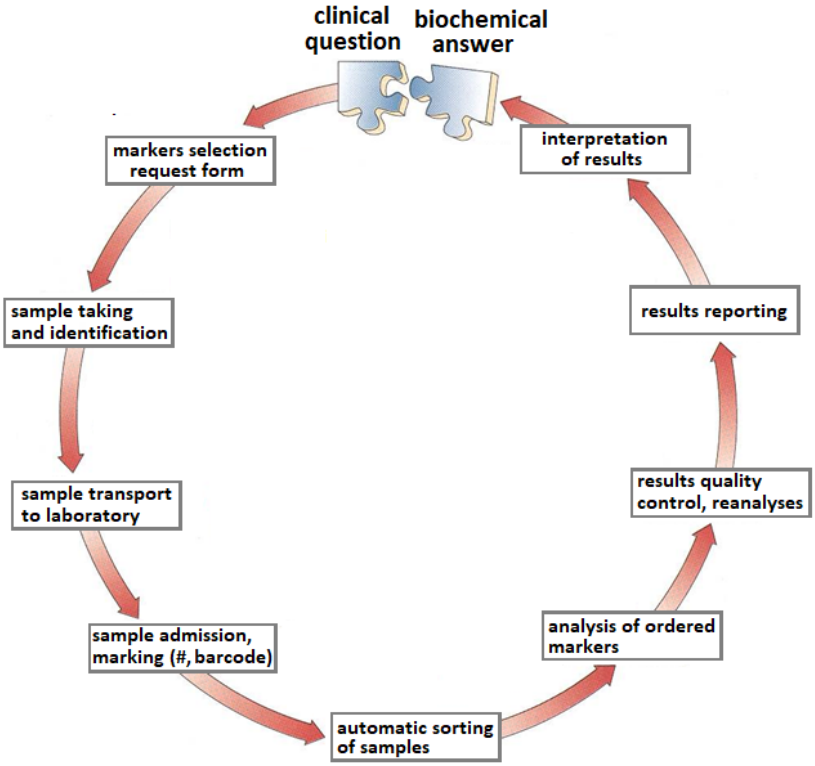

Figure 32 “Life-story” of a biological sample in the clinical-biochemical laboratory – summary After the occurrence of a clinical question that needs to be answered by the examination of biochemical markers, a request is entered in the hospital information system. The sample is correctly taken, labelled and transported to the laboratory. At the laboratory, on arrival, the sample is assigned its unique barcode, that includes a specific sample number in the laboratory information system, and that is further recognised by the laboratory machines. In the automatic sorting line, the samples are categorised for the different sections of the clinical biochemical laboratory; the aliquots are also created if necessary. After the sample itself is analysed in the analysers, the results are controlled multiple times by the laboratory staff, and transmitted via the laboratory and hospital information system to the ordering physician. The sample is then stored for potential further analyses. If critical test results are detected, they have to be reported also by telephone. At the end of the 'biochemical response' formation, the results are interpreted by the ordering physician in the light of the clinical data and the findings of other examinations.

2.9 Sources of errors in laboratory testing

As described in the previous chapters, the overall process of generating laboratory results is complex and involves a large number of actions, implying the possibility of creating errors. The production of a correct result is greatly influenced by all the persons, materials and processes with which the sample has come into contact. The factors related to the patient from whom the sample comes are also not negligible. In Table 3 below, the possible errors are described according to the phase of the laboratory testing at which they may occur, with a brief description of their effect on the result obtained. As can be seen, most errors occur in the pre-analytical phase, primarily outside the laboratory. Since the laboratory can only influence this phase through proper briefing and communication with the ordering and collecting healthcare professionals, all actions during this phase of the examination need to be performed as carefully and responsibly as possible. Precisely defined rules regarding the collection, processing, and transport of biological material by healthcare staff are available in the laboratory manual (UHM intranet).

Table 3 Sources of laboratory errors and their consequences

	Source of the error	Consequence
Pre-analytical phase (outside the laboratory)	patient - non-affectable factors: race age, sex pregnancy diurnal rhythm	 ↑ CK in blacks, ↑ salivary AMS in Asians different physiological values (see Table 2) ↓ creatinine, TP, albumin, ↑lipids, proteins, ALP early morning peak cortisol levels
	patient - affectable factors: obesity diet smoking alcoholism physical activity, i.m. injection, trauma medication, drugs diseases	 ↑ lipids, uric acid, hyperinsulinism postprandial - ↑ insulin, glycemia, TAG, ↓ K^+, P ↑ lipids, cortisol, CEA, fibrinogen, carbonyl Hb ↑ AST, ALT, GMT, uric acid, cortisol, lipids ↑ lactate, CK, AST, myoglobin ... ...
	the use of oxidants for skin disinfection before the blood sampling	interference with methods which use peroxidase (glycemia, cholesterol...)
	blood sampling - rapid aspiration by needle, excessive shaking of sample	hemolysis - ↑ K^+, P, CK, AST, LD, interference with measurements
	blood collection - long strangle by tourniquet	↑ CK, AST, lactate, albumin, K^+, Ca^{2+}
	blood sampling - patient in standing position	↑ aldosterone, adrenaline, albumin, proteins, lipids
	sampling from the limb / i.v. catheter with infusion	↑ glucose, Na^+, Cl^-, K^+ or other administered substances

	use of anticoagulants EDTA / citrate / oxalate for Ca^{2+} measurement	↓ Ca^{2+} by its binding
	use of K_3EDTA for K^+ determination	false hyperkalemia
	strong shaking of blood in the tube, long transport of blood	hemolysis
	storage of blood in the fridge overnight, freezing of blood before centrifugation	hemolysis
	high/low temperature of blood transport	inactivation of enzymes, hemolysis
	intense light exposure	degradation and falsely ↓ bilirubin, folate
	mislabeling of sample	results uncorrelated to the patient's condition and previous findings
	incorrect collection tube	inability to determine analyte concentration, result: 'repeat sampling'
	loss of sample	impossibility of sample processing
Pre-analytical phase (in the laboratory)	insufficient blood clotting time (< 15 - 30 min) before serum separation	fibrin fibres formation → needle occlusion, aspiration of smaller sample volume → falsely ↓ result
	non-use of refrigerated centrifuge for thermally unstable analytes	falsely ↓ PTH, osteocalcin ...
	sample interchange	results with uncorrelated to the patient's condition and previous results
	repeated freezing and thawing of the sample	false change in analyte concentration
	breakage of the tube / spilling of the sample	impossibility to obtain the test results
Analytical phase	incorrect calibration or quality control	incorrect and inaccurate results
	use of expired/low quality kit	incorrect and inaccurate results
	not considering sample dilution when calculating the final analyte concentration	falsely ↓ result
	rapid sample aspiration before the entire volume is achieved (ASTRUP)	falsely ↓ or no result, insufficient sample volume for further analysis
	insufficient sample volume loaded into the analyser	incomplete analysis - result "short sample", request "to resample"
	marker measurement from instead of plasma (or vice versa)	falsely ↑ or ↓ result
	aspiration and analysis of a smaller sample volume (clot needle obstruction)	falsely ↓ result - repeating the analysis
	incorrect subjective assessment of the "optical" test	incorrect result
Post-analytical phase	incorrect data transfer / copying	submitting an incorrect result
	not identifying an incorrect result during analytical/medical control	submitting an incorrect result
	not considering pre-analytical factors	incorrect interpretation of results
	not considering patient´s history, clinical and imaging methods findings	incorrect interpretation of results
	late result delivery, failure to report critical results	life threatening to the patient

Abbreviations: ↑ - increase, ↓ - decrease, a. – acid

3 Interpretation of selected clinical-biochemical findings

In the previous chapters, we described the basic characteristics of the samples examination in the clinical-biochemical laboratory, the principles of the used methods and the production of the results, including the bacis rules for their interpretation. Every medical graduate should have an adequate knowledge of these "laboratory practice essentials", since he or she will be in daily, routine interaction with laboratory results.

In the following chapters, we will present and interpret in depth the results of biochemical examinations in selected cases. For an easier orientation in relation to the reference ranges, we highlight increased result values in red, decreased values in blue; normal values are shown in black. If the reference range is different from those for healthy adults in Table 2, we also indicate the referring patient’s age in parentheses. Since there are many literature sources providing a plethora of detailed theoretical information which is also taught as part of the Clinical Biochemistry and Laboratory Medicine course at the JFMED CU, the data necessary for the correct interpretation of particular markers is purposive and condensed. The author's effort was always to provide the basic characteristics of an as yet undefined marker, which are also necessary for the appropriate interpretation of the obtained results. The following case reports are presented mainly for medical students and young graduates in general medicine.

3.1 Case 1 Acid-base balance

A 59-year-old female patient with long-term treated chronic obstructive pulmonary disease was hospitalised for an exacerbation of the disease. The patient subjectively reported a worsening of long-term difficulties: accentuation of dyspnoea and cough, feeling of chest tightness, expectoration of purulent sputum. Objectively, there was a productive cough, a fever of 38 °C, and auscultatory signs of bronchopneumonia and weakened basal respiration. The results of the acid-base balance examination from capillary blood are:

Laboratory marker	Result	Reference range	Units
Acid-base balance			
pO_2	11.43	9.8 – 13.3	kPa
pCO_2	9.07	4.64 – 6.00	kPa
pH	7.299	7.36 – 7.44	-
BE	3.8	-2.5 – 2.5	
HCO_3^-	32.6	22 – 26	mmol/L
O_2 sat	95.3	95 – 99	%

Question 3.1.1 Name the four basic acid-base balance disorders you know and characterise which parameters other than pH are primarily changed?

Answer 3.1.1 There are four basic acid-base balance disorders and their combinations - metabolic acidosis and metabolic alkalosis (primary change in HCO_3^- concentration), respiratory acidosis and respiratory alkalosis (primary change in pCO_2).

Question 3.1.2 How do we proceed with the evaluation of the acid-base balance test results?

Answer 3.1.2 When evaluating the result of the acid-base balance examination, we can follow the procedure shown in Fig. 33.

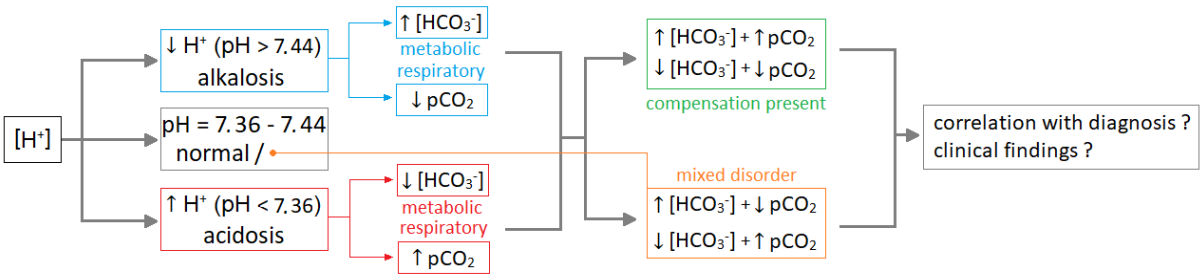

Figure 33 Simplified manual for the interpretation of acid-base balance results When indicating an acid-base balance examination (ASTRUP), we consider the assumed disorder with respect to the patient's clinical condition, diagnosis and symptoms. When the result is obtained, we firstly assess whether it is acidosis or alkalosis according to the pH. We then decide whether it is a metabolic disorder (change in bicarbonate concentration,

which correlates with pH) or a respiratory disorder (change in pCO_2, which correlates with pH). In the next step, we consider whether there is compensation (both HCO_3^- and pCO_2 concentrations altered in the same direction) or whether it is a mixed disorder (both HCO_3^- and pCO_2 concentrations altered in the opposite direction, pH may be normal). Finally, as in any interpretation of the results, we evaluate whether our assessment of the patient's acid-base balance status correlates with the suspected diagnosis, clinical picture and the results of imaging methods and other investigations. Abbreviations: pCO_2 - partial pressure of carbon dioxide, HCO_3^- - bicarbonate.

Question 3.1.3 What is the patient's acid-base balance disorder and what is the mechanism of its development? State the reasons.

Answer 3.1.3 Due to the decreased pH and hypercapnia, it is respiratory acidosis. The increase in the partial pressure of carbon dioxide results from hypoventilation and retention of CO_2, which reacts with water to form carboxylic acid.

Question 3.1.4 Why is the bicarbonate concentration elevated? How do you define the "BE" parameter?

Answer 3.1.4 The bicarbonate concentration is elevated because they are regenerated and retrieved by the kidneys as a compensatory mechanism of respiratory acidosis. Base excess (BE) is defined as the amount of bases in mmol/L that must be added or removed from the blood to normalise pH, assuming a respiratory disorder is not present. The BE value correlates significantly with the "metabolic component" of acid-base balance disorder. In most cases, when bicarbonates are in excess (the patient has metabolic alkalosis), the BE is also elevated and vice versa.

Question 3.1.5 What could happen to the patient's acid-base balance status when we put her on artificial ventilation and normalise the partial pressure of carbon dioxide? Is it possible to threaten her life by this procedure?

Answer 3.1.5 Once hypercapnia is eliminated, due to bicarbonate retention, there is an "overshooting " towards secondary metabolic alkalosis. In patients with chronic respiratory acidosis, artificial ventilation can cause life-threatening complications, as hypoxia is the only stimulus to the respiratory centre due to chronic hypercapnia. If hypoxia is suddenly removed, apnoea may be induced.

3.2 Case 2 Acid-base balance, kidney function

A 40-year-old female patient with no previously known significant medical history arrived at the hospital with shaking, fever, dull pain in her hip, and nausea; beforehand, she had vomited several times. Prior to the onset of symptoms, the patient was dysuric for several days and urinated more frequently; she was oliguric for the last two days (diuresis about 250 mL/24 h) at normal fluid intake. Objectively, the patient had a tight abdominal wall, positive left tapotement, tachypnoea (33 breaths/min), and a body temperature of 39.1 °C. The results of the acid-base balance examination from capillary blood are:

Laboratory marker	Result	Reference range	Units
Acid-base balance capillary			
pO_2	11.47	9.8 – 13.3	kPa
pCO_2	3.89	4.64 – 6.00	kPa
pH	7.184	7.36 – 7.43	-
BE	-16.1	-2.5 – 2.5	
HCO_3^-	10.7	22 – 26	mmol/L
O_2 sat	95.9	95 – 99	%

Question 3.2.1 Does the patient have an acid-base balance disorder? If yes, has a compensation already developed?

Answer 3.2.1 Considering the decreased pH and the decrease in bicarbonate concentration (including BE), this is a metabolic acidosis. Hypocapnia is the result of compensatory hyperventilation, by which the body excretes carbon dioxide through the lungs and thus rids itself of acids.

Question 3.2.2 Which cause of acid-base balance disorder would you suspect in the patient and which other biochemical markers would you investigate in order to confirm/exclude the diagnosis?

Answer 3.2.2 The medical history, clinical picture and physical findings are suggestive of a possible uroinfection with renal dysfunction and acids retention. The results of other biochemical examinations were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	133	136 – 146	mmol/L
Potassium	6.9	3.5 – 5.1	mmol/L
Calcium	2.26	2.20 – 2.65	mmol/L
Chlorides	106	101 – 109	mmol/L
Phosphates	2.09	0.81 – 1.45	mmol/L
Calcium ionizovaný	1.255	1.130 – 1.320	mmol/L
Serum			
Glucose	13.3	4.1 – 5.9	mmol/L
Creatinine	461	58 – 96	µmol/L
Urea	24.3	2.8 – 7.2	mmol/L
Uric acid	340	155 – 357	µmol/L
Total protein	60.0	66 – 83	g/L
Albumin	37	35 – 52	g/L
Bilirubin - total	7.5	5.0 – 21.0	µmol/L
Bilirubin - conjugated	0.8	0.1 – 3.4	µmol/L
AST	0.30	< 0.6	µkat/L
ALT	0.25	< 0.6	µkat/L
CRP	35.7	< 5	mmol/L

Question 3.2.3 Are the serum biochemical findings in concordance with the clinical picture and the suspected diagnosis? How do you explain the presence of hyponatremia?

Answer 3.2.3 Biochemical findings - elevated serum levels of potassium, phosphate, urea and creatinine combined with oliguria are indicative of acute renal failure, in which acid metabolites, nitrogenous and other substances accumulate in the organism. In the oliguric phase, as a result of reduced fluids excretion, dilutional hyponatremia is present.

Question 3.2.4 Which parameters are examined during chemical and microscopic examination of urine ("urine chemistry + sediment")? What kidney disease has caused the acute renal failure?

Urine chemistry			
pH of urine	5.0	4.8 – 7.4	
Specific gravity	1.013	1.001 – 1.035	kg/m^3
Protein	posit		
Glucose	negat		
Acetone	negat		
Bilirubine	negat		
Urobilinogen	negat		
Leucocytes chemically	posit		
Erythrocytes chemically	posit		
Nitrites	posit		

Urine microscopy			
Leukocytes	3590	0 – 15	/ µL
Erythrocytes	181	0 – 10	/ µL
Squamous epithelial cells	24	0 – 15	/ µL
Tubular epithelial cells	rare		
Bacteria	numerous		
Amorphous crystals	present		
Mucus threads	rare		

Answer 3.2.4 The cause of the renal failure in this patient was a uroinfection - complicated acute tubulointerstitial nephritis. This diagnosis was indicated by the clinical picture (tapotement, weakness, febrility, dysuria...), increased inflammatory activity in the serum and in particular by the urine findings (proteinuria, increased number of leukocytes, erythrocytes, bacteria and renal epithelial cells).

Question 3.2.5 Escherichia coli was confirmed by the cultivation of urine. What does the presence of nitrites in urine suggest? How would you explain finding bacteria in the urine without a pathological increase in the urinary leukocytes count?

Answer 3.2.5 Nitrites (NO_2^-) are used for the chemical detection of bacteria in urine since they are formed by the catalysed reduction of nitrates (NO_3^-) using enterobacterial enzymes. A false negativity of nitrites can be caused by an inadequate supply of dietary nitrates, or by a short storage of urine in the bladder, insufficient for bacteria to reduce dietary nitrates. An elevated number of bacteria in the urine without a finding of leukocytes is indicative of urine contamination during or after collection.

Question 3.2.6 How do serum urea and creatinine levels change in acute renal failure? Does the measurement of serum creatinine level have any limitations when using it as a marker of renal glomerular filtration?

Answer 3.2.6 An increased serum level of nitrogen waste products (azotemia) in acute renal failure is characterised by a greater elevation of urea than creatinine, while in chronic renal failure a greater elevation of creatinine is typical. The relationship between creatininemia and estimated glomerular filtration rate (eGFR) is demonstrated in Fig. 34.

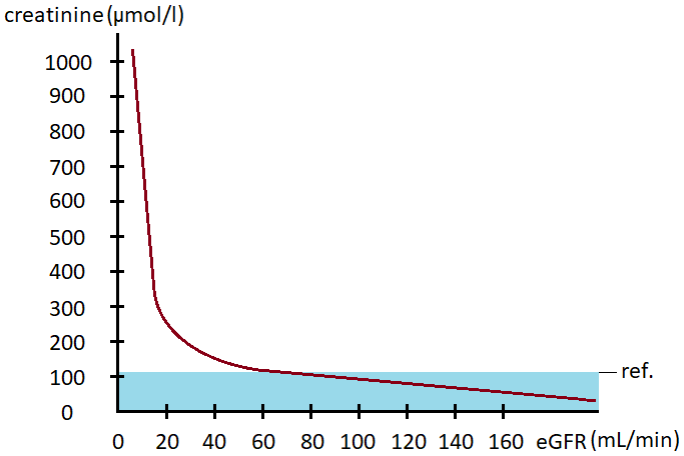

Figure 34 Relationship between glomerular filtration rate and serum creatinine level The increase in creatininemia above the reference range (ref.) occurs only when the estimated glomerular filtration rate (eGFR) decreases to approximately half of the normal value (physiologically, eGFR is approximately 120 mL/min/1.73 m^2). A normal serum creatinine level may be present even when there is a considerable decrease in the glomerular filtration rate.

Because of the described imprecise dependence of creatininemia on eGFR, a more convenient marker is the calculation of estimated glomerular filtration rate in mL/min using different formulae. In clinical practice, the Cockroft-Gault equation (age, weight, serum creatinine) has been used for a long time. In the above-mentioned patient, the calculated eGFR was 11 mL/min (less than 10% of normal GF).

3.3 Case 3 Acid-base balance, markers of inflammation and sepsis

An 85-year-old male patient underwent abdominal surgery (distal pancreatectomy) because of an insulinoma with already detected metastases. One week after surgery, he developed sepsis and multiorgan dysfunction syndrome (MODS) with liver damage, myelinolysis in the central nervous system (CNS) and cardiac arrest with subsequent successful resuscitation. The patient is currently comatose in the intensive care unit. The results of biochemical examinations are:

Laboratory marker	Result	Reference range	Units
Acid-base balance capillary			
pO_2	10.26	9.8 – 13.3	kPa
pCO_2	6.94	4.64 – 6.00	kPa
pH	7.112	7.36 – 7.43	-
BE	-13.4	-2.5 – 2.5	
HCO_3^-	16.2	22 – 26	mmol/L
O_2 sat	90.3	95 – 99	%
Minerals in serum			
Sodium	143	136 - 146	mmol/L
Potassium	4.7	3.5 – 5.1	mmol/L
Chlorides	107	101 - 109	mmol/L
Serum			
Procalcitonin	26.49	< 0.5	mmol/L
Lactate plasma	2.88	0.5 – 2.2	mmol/L

Question 3.3.1 Can you explain which acid-base balance disorder is present?

Answer 3.3.1 The pH value is decreased, the patient has acidosis. The partial pressure of carbon dioxide is elevated, and the bicarbonate level is decreased. Since these are both altered in the opposite direction, this is a mixed acid-base balance disorder, a combination of respiratory and metabolic acidosis that correlates well with the clinical data. Cardiac arrest caused tissue hypoxia with a consequent increase in lactate production; hypoventilation due to apnoea resulted in CO_2 retention.

Question 3.3.2 Calculate the anion gap and then define its significance.

Answer 3.3.2 The anion gap (AG) determines the type of metabolic acidosis; the reference range is approximately 14–18 mmol/L and it is calculated as follows:

$$AG = ([Na^+] + [K^+]) – ([Cl^-] + [HCO_3^-]) = (143 + 4.7) – (107 + 16.2) = 24.5 \text{ mmol/L}$$

Thus, the patient has metabolic acidosis with an elevated AG value, which is typically present when there is an excess of acids of exogenous or endogenous origin, in this case lactate. Another type is metabolic acidosis with a normal AG value, which is present when bicarbonate is lost by the gastrointestinal system or kidneys (Fig. 35).

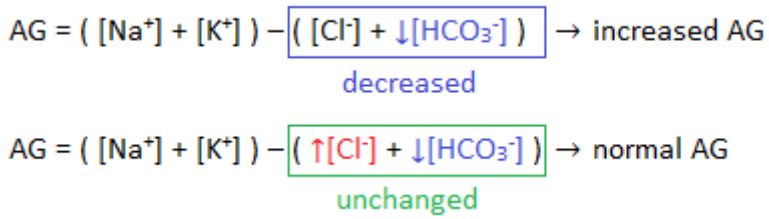

Figure 35 Explanation for the causes of AG alterations in metabolic acidosis In metabolic acidosis with elevated AG, bicarbonate is neutralised during the buffering of organic acids; therefore after subtracting the decreased bicarbonate value, the AG increases. In metabolic acidosis with normal AG, the lost bicarbonate in the anion column is counterbalanced by chlorides, and the total value of the right subtracted bracket as well as AG does not change.

Question 3.3.3 Interpret the results of serum procalcitonin level measurement.

Answer 3.3.3 Procalcitonin (PCT) is a glycoprotein, identical to the normal thyroid calcitonin prohormone, being produced in the liver and other tissues in septic states. In serum, its level increases significantly during severe bacterial infections (not in viral infections). Normal serum PCT concentration is < 0.5 µg/L, values 0.5–2 µg/L being the so-called grey zone are present in chronic inflammatory diseases, the level 2–10 µg/L suggest an increased likelihood of sepsis and septic shock, values > 10 µg/L are mostly present in severe sepsis and MODS (our patient 26.49 µg/L). During severe generalised bacterial sepsis, serum PCT values can reach 1,000 µg/L. With great success, PCT determination is used for the detection of early neonatal sepsis (note - the PCT level in newborns is physiologically higher and normalises within 72h of birth). Elevation of PCT compared to CRP and IL6 better differentiates bacterial sepsis from non-infectious SIRS.

Question 3.3.4 Which other inflammatory markers do you know? Compare them to one another.

Answer 3.3.4 In addition to PCT, commonly measured inflammatory markers include C-reactive protein (CRP), presepsin, and interleukin 6 (IL6); the dynamics of changes in their serum concentrations during inflammation are shown in Table 4.

Table 4 Time-dependence of changes in serum levels of inflammatory markers

Marker	Start of elevation	Maximum level	Biological half-life
IL6	1 hr	3 – 5 hrs	< 1 hr
Presepsin	2 hrs	3 hrs	4 – 5 hrs
Procalcitonin	6 hrs	6 – 12 hrs	12 – 24 hrs
CRP	6 – 9 hrs	24 – 72 hrs	19 hrs

Interleukin 6 is a pro-inflammatory cytokine that activates the synthesis of acute phase proteins, including PCT and CRP. It is widely used as a marker of neonatal infection and sepsis. The higher the initial IL6 level, the higher the severity of sepsis, as well as the probability of septic shock and organ dysfunction. A serum IL6 level of more than 1,000 ng/L persisting for several days after the onset of sepsis is associated with a very high mortality. In contrast, in patients with a good prognosis, there is a decrease of almost 90% of the IL6 level within the first 3 days. The concentration can also be increased after surgery, trauma and in critically ill patients (brain death, neoplasia) and is proportional to the extent of damage.

C-reactive protein is formed in hepatocytes and its name was derived from its ability to precipitate the C-polysaccharide of pneumococci. CRP is structurally homologous to the constant region of IgG heavy chains and is used in the biochemical differential diagnosis of infection by bacteria (>50 mg/L) and viruses (5-35 mg/L). Levels > 200 mg/L are usually present in patients with severe sepsis, however the increase is slower when compared with other inflammatory markers. Because of its low specificity, CRP measurement is more suitable for monitoring the efficacy of antibiotic treatment than for diagnosis. In recent years, rapid "POCT" CRP tests are commonly used in practice by outpatient clinicians when considering the appropriateness of indicated antibiotic treatment. However, a normal CRP level during severe clinical signs of a serious bacterial infection is not a contraindication for antibiotic treatment, and may still be present for some "blind" period of time after the disease onset (see Table 4). For these reasons, the assessment of dynamics of serum levels changes of CRP and other laboratory markers, such as differential leukocyte count and urinary sediment, is of particular importance.

Presepsin is produced by the proteolytic cleavage of a serum CD14-ST fragment from a complex of bacterial lipopolysaccharide and leukocyte membrane glycoprotein receptor. It is an inflammatory marker that is released from lysosomes after the phagocytosis of bacteria, and is useful in the early diagnosis of sepsis and in the stratification of the prognosis and risk of mortality in septic patients. The serum level of presepsin increases earlier than that of other inflammatory markers and is not affected by liver function (see Fig. 36). The physiological serum level is < 337 ng/L. A level of < 200 ng/L excludes sepsis, a level of < 300 ng/L indicates an unlikely systemic infection, a level of 300–500 ng/L is associated with possible sepsis, 500–1,000 ng/L correlates with an increased risk of sepsis progression and a poor "outcome", a level ≥ 1,000 ng/L is associated with a serious risk of severe sepsis and septic shock, and also a very high 30-day mortality rate. In individuals with sepsis, a serum presepsin level > 1,900 ng/L has been associated with 60% mortality.

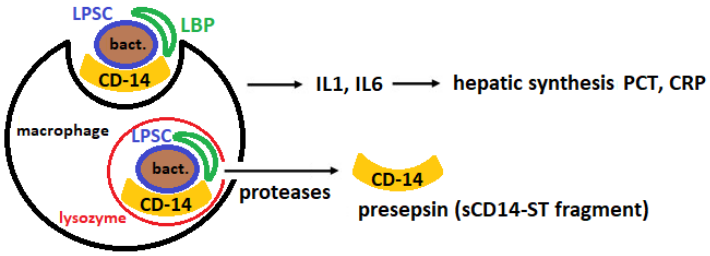

Figure 36 The formation of inflammatory markers in bacterial infections and sepsis (according to Chenevier-Gobeaux et al. 2015) Lipopolysaccharide (LPSC), which is present in the bacterial wall, binds with lipopolysaccharide-binding protein (LBP). The LPSC-LBP complex is coupled to the glycoprotein receptor CD14, which is expressed on the surface of macrophages. After the internalisation of the complex and its cleavage by proteases, a soluble fragment (sCD14-ST), also called presepsin, is released into the blood. As we can see, its synthesis is faster compared to PCT and CRP and is not dependent on proteosynthesis in the liver.

3.4 Case 4 Acid-base balance, serum minerals, kidney function

A 38-year-old female patient who had not been previously treated for any serious disease came to the hospital because of vomiting lasting for 4 days, a feeling of weakness and dizziness present from the previous evening. Objectively, the patient had decreased skin turgor, a tachycardia of 120/min, a blood pressure of 100/60 mm Hg and shallow breathing.

Question 3.4.1 Which biochemical markers would you indicate to be measured?

Answer 3.4.1 On admission, the following biochemical tests were carried out:

Laboratory marker	Result	Reference range	Units
Acid-base balance capillary			
pO_2	12.5	9.8 – 13.3	kPa
pCO_2	6.91	4.64 – 6.00	kPa
pH	7.474	7.36 – 7.43	-
BE	11.4	-2.5 – 2.5	
HCO_3^-	37.2	22 – 26	mmol/L
O_2 sat	95.6	95 – 99	%
Minerals in serum			
Sodium	149	136 – 146	mmol/L
Potassium	2.4	3.5 – 5.1	mmol/L
Calcium	2.32	2.20 – 2.65	mmol/L
Chlorides	84	101 – 109	mmol/L
Serum			
Glucose	5.4	4.1 – 5.9	mmol/L
Creatinine	162	58 – 96	µmol/L
Urea	19.9	2.8 – 7.2	mmol/L
Uric acid	286	155 – 357	µmol/L
Total protein	75	66 – 83	g/L
Albumin	43	35 – 52	g/L
Bilirubin - total	8.8	5.0 – 21.0	µmol/L
Bilirubin - conjugated	2.1	0.1 – 3.4	µmol/L
AST	0.40	< 0.6	µkat/L
ALT	0.22	< 0.6	µkat/L
ALP	1.12	0.50 – 2.15	µkat/L
GMT	0.45	< 0.63	µkat/L
AMS	1.08	0.46 – 1.66	µkat/L
LPS	0.89	0.22 – 1.00	µkat/L
CRP	14	< 5	mg/L
Osmolality	324	275 – 295	mmol/kg

Urine chemistry			
pH of urine	4.0	4.8 – 7.4	
Specific gravity	1.022	1.001 – 1.035	kg/m^3
Protein	negat		
Glucose	negat		
Acetone	negat		
Bilirubine	negat		
Urobilinogen	negat		

Leucocytes chemically	negat		
Eyrthrocytes chemically	negat		
Nitrites	negat		
Urine microscopy			
Leukocytes	10	0 – 15	/ µL
Erythrocytes	4	0 – 10	/ µL
Squamous epithelial cells	9	0 – 15	/ µL
Amoprhous crystals	present		
Mucus threads	present		

Question 3.4.2 Explain what acid-base balance disorder it is. Why is the partial pressure of CO_2 elevated?

Answer 3.4.2 Since there is an increase in pH and bicarbonate concentration (correlating with BE increase) combined with a decrease in serum chlorides, we are talking about hypochloremic metabolic alkalosis. The partial pressure of CO_2 is elevated due to its retention - compensation of metabolic alkalosis by the lungs.

Question 3.4.3 Are changes in serum minerals and nitrogen waste products concentrations in correlation with the medical history and clinical symptoms? What is the Cockroft-Gault eGFR if the patient's weight is 59 kg?

Answer 3.4.3 The patient has hypochloremia, hypokalemia and mild hypernatremia. The above findings can be explained by the fact that during vomiting, the patient's organism lost mainly chlorides (110 mmol/L content in gastric juice) and potassium (10 mmol/L content in gastric juice, i.e. about 2 – 3 times the serum concentration). The hypernatremia resulted from the dehydration of the patient, who was losing more water than sodium by vomiting (Na^+ content in gastric juice is 70 mmol/L, i.e. about half of the serum concentration), and because she was unable to compensate this fluid loss by per os hydration. Thus, the ratio of solute to solvent (sodium to serum water) changed in favour of sodium (hypernatremia). Changes in the concentration of nitrogen waste products (creatinine, urea) are suggestive of acute renal failure due to dehydration. eGFR according to Cockroft-Gault (currently obsolent method, see chap. 3.5) is:

$$\textbf{eGFR}_{\textbf{Cockroft-Gault}} = \frac{(140 - \text{age}) \times \text{weight}}{49 \times \text{serum creatinine}} \times 0.85 \text{ in women} = \frac{(140 - 38) \times 59}{49 \times 168} \times 0.85 = 0.73 \text{ mL/s} = \underline{\textbf{44 mL/min}}$$

Question 3.4.4 Which hormonal systems are activated during hypertonic dehydration and a reduction of effective circulating volume?

Answer 3.4.4 A decreased perfusion of the renal *vas afferens* results in the activation of the renin-angiotensin-aldosterone system (RAAS), which causes the stimulation of aldosterone production and the subsequent expansion of the effective circulating volume by retention of sodium (along with water), but at the cost of increased potassium losses in the distal tubule. Serum hyperosmolality stimulates the production of antidiuretic hormone (ADH), which causes water retention in the distal tubule and renal collecting ducts.

Question 3.4.5 What urine pH would you expect in metabolic alkalosis? Why does the patient have a decreased urine pH?

Answer 3.4.5 In metabolic alkalosis, the organism tries to excrete excess HCO_3^- into the urine and thus to alkalise it. The formation of so-called "paradoxically acidic urine" is conditioned by hypokalemia, since K^+ is saved by the kidneys' resorbtion, and in its place, H^+ is excreted into the urine, making it paradoxically acid. The lack of potassium is also aggravated by the already described hyperaldosteronism (increasing potassium losses).

Question 3.4.6 What simple urine test could we perform to determine renal concentrating ability? Could a urine specific gravity measurement also be helpful?

Answer 3.4.6 One of the markers of renal concentrating ability is the urine osmolality test, which was administered to the patient with the following result:

Laboratory marker	Result	Reference range	Units
Urine			
Osmolality	560		mmol/kg

Urine osmolality (mainly composed of Na^+, K^+, NH_4^+ cations and urea) can serve as a simple diagnostic tool for the biochemical differential diagnosis of renal failure. In prerenal failure it is usually > 400 mmol/kg; in case of renal failure the urine osmolality is similar to plasma. In our patient, the urine osmolality was 560 mmol/kg, indicating a partially preserved renal concentrating ability (water retention, urine concentration). Urine osmolality above approximately 600 mmol/kg (or specific gravity above approximately 1,018 kg/m^3) in adult subjects suggests intact tubular function.

3.5 Case 5 Chronic renal failure, vitamin D, proteinuria and urine collection

A 51-year-old patient suffers from chronic kidney disease and is regularly monitored in a nephrology outpatient clinic. The patient has a long-lasting dysfunctional urination of psychogenic origin, he also occassionally inserts an urinary catether by himself. He suffers from recurrent urinary tract infections that are manifested by a painful urge to urinate and a burning sensation during urination. Ultrasonography of the kidney showed the presence of small cysts and local hypertrophy of the parenchyma. The results of the examination at the last routine check-up, currently without subjective signs of urinary tract infection, are:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	139	136 - 146	mmol/L
Potassium	5.5	3.5 – 5.1	mmol/L
Calcium	2.24	2.20 – 2.65	mmol/L
Chlorides	106	101 - 109	mmol/L
Phosphates	1.52	0.81 – 1.45	mmol/L
Serum			
Glucose	5.6	4.1 – 5.9	mmol/L
Creatinine	215	74 – 110	µmol/L
Urea	10.6	2.8 – 7.2	mmol/L
Uric acid	466	208 – 428	µmol/L
Total protein	70.8	66 – 83	g/L
Albumin	41.6	35 – 52	g/L
Total cholesterol	3.53	< 5.17	mmol/L
Triacylglycerols	1.21	0.4 – 1.7	mmol/L
AST	0.40	< 0.85	µkat/L
ALT	0.24	< 0.85	µkat/L
ALP	1.68	0.50 – 2.15	µkat/L
GMT	0.28	< 0.92	µkat/L
CRP	1.7	< 5	mg/L
Vitamin D total	33	30 – 100	µg/L
Parathormone	176.9	1.6 – 6.9	pmol/L
Urine chemistry			
pH of urine	6.2	4.8 – 7.4	
Specific gravity	1012	1.001 – 1.035	kg/m^3
Protein	posit		
Glucose	negat		
Acetone	negat		
Bilirubine	negat		
Urobilinogen	negat		
Leucocytes chemically	negat		
Eyrthrocytes chemically	negat		
Nitrites	negat		
Urine microscopy			
Leukocytes	7	0 – 15	/ µL
Erythrocytes	3	0 – 10	/ µL
Squamous epithelial cells	8	0 – 15	/ µL
Tubular epithelial cells	rare		

Question 3.5.1 Interpret and explain the findings of the serum and urine examinations. Is there any correlation between osmolality and urine specific gravity?

Answer 3.5.1 A chronic kidney disease in this patient typically manifests itself by the retention of nitrogen waste products. In chronic compared to acute renal failure, there is a larger increase in serum creatinine than urea, also including a common increase in uric acid serum level. In addition, hyperkalemia is present in the patient due to the decreased renal excretion of potassium. The finding of renal tubular epithelial cells in the urinary sediment is a sign of tubular damage; renal function impairment is also indicated by increased urinary protein loss (proteinuria). The patient's urine density is 1,012 kg/m^3 and approaches 1,010 kg/m^3 (isosthenuria), suggesting impaired renal concentrating capacity. A urine osmolality of 50-1,000 mmol/kg corresponds to a urine specific gravity of approximately 1,001-1,035 kg/m^3.

Question 3.5.2 Do you know the most recent eGFR calculation that is routinely used in clinical practice? Using the above mentioned creatinine serum level, calculate the eGFR and determine what stage of chronic kidney disease the patient has.

Answer 3.5.2 Currently, the Chronic Kidney Disease - Epidemiology Collaboration (CKD-EPI) calculation of eGFR per standard body surface area of 1.73 m^2 is used, and the value is determined using serum creatinine level, age, sex, and race. According to eGFR (CKD-EPI), chronic kidney disease is divided into the following stages (KDIGO, *Kidney Disease Improving Global Outcomes*): stage 1 – eGFR $\geq$ 90 mL/min/1.73 m^2 (normal or increased), stage 2 – eGFR = 60–89 mL/min/1.73 m^2, stage 3A – eGFR = 45-59 mL/min/1.73 m^2, stage 3B – eGFR = 30–44 mL/min/1.73 m^2, stage 4 – eGFR = 15–29 mL/min/1.73 m^2, stage 5 – eGFR <15 mL/min/1.73 m^2. Our patient's glomerular filtration rate according to the CKD-EPI is 30 mL/min/1.73 m^2, which corresponds to stage 3 of chronic kidney disease (calculated using an online calculator according to Levey et al. 2009; currently, in the UHM, eGFR CKD-EPI is automatically calculated in each patient with a measured serum creatinine level).

Question 3.5.3 How do serum calcium and parathyroid hormone concentrations change in patients with chronic kidney failure?

Answer 3.5.3 Hypocalcemia develops in patients with chronic renal failure due to the decreased intestinal resorption of calcium resulting from an inadequate production of the

active vitamin D metabolite (calcitriol). Typically present hyperphosphatemia is a result of decreased glomerular filtration rate. As prolonged hypocalcemia continuously stimulates the parathyroid glands, their hyperplasia leads to the development of secondary hyperparathyroidism (rationale for higher parathyroid hormone reference limits in patients with eGFR values <30 ml/min). Renal osteodystrophy is a consequence of elevated serum parathormone levels that can normalise calcemia at the cost of bone resorption.

Question 3.5.4 Which vitamin D metabolite do we measure in serum when assessing its status in the body? Describe vitamin D metabolism and its reference range.

Answer 3.5.4 The optimal "mirror" of vitamin D metabolism in human body is the serum level of calcidiol (25-hydroxycholecalciferol). Calcidiol has a biological half-life of approximately 19 days, and it is a major circulating form of vitamin D, formed in the liver by the hydroxylation of cholecalciferol and beeing very stable in the pre-analytical phase. Cholecalciferol is produced in the skin spontaneously from 7-dehydrocholesterol after the exposure to UV-B radiation, or is taken into the human body by diet. Calcitriol (1,25-dihydroxycholecalciferol) is the biologically active form of vitamin D that is formed by the action of 1-α hydroxylase in target tissues, including also the kidney. However, its serum level is measured only rarely, more in research than in routine practice. The optimal serum calcidiol level is 30–100 ng/mL (or mg/L), a level of 20–30 ng/mL is considered as insufficiency. The serum calcidiol of less than 20 ng/mL indicates vitamin D deficiency that is rather frequent also in the healthy population living in our latitude, especially during the non-sunny season. For this reason, in the last decades, the worldwide recommendations on the peroral substitution of vitamin D have been elaborated and published.

Question 3.5.5 The patient's 24-hour urine was collected, and then the creatinine and protein concentrations were determined. Calculate and evaluate the creatinine clearance (C_{creat}) and daily proteins waste if we know that the total urine volume was 2,900 mL.

Laboratory marker	Result	Reference range	Units
Urine – collected			
Creatinine	4.8	-	mmol/L
Total protein	0.7	-	g/L
Diuréza	2900	-	ml/day

Answer 3.5.5 The waste of proteins is 2.03 g/day, which markedly exceeds the physiological proteinuria of 150 mg/24 h. Creatinine clearance (C_{creat}, the volume of plasma completely cleared of creatinine per time) is 45 mL/min, which is about one-third of the physiological clearance of 120 mL/min.

24hrs urine protein waste = $[\text{protein}]_{urine}$ × 24hrs urine volume = 0.7 g/L × 2.9 L/d = **2 g/day**

$$\mathbf{C_{creat}} = \frac{[\text{U creat}] \times \text{Vurine}}{[\text{P creat}]} = \frac{4.8\ \text{mmol/L} \times 2900\ \text{ml/day}}{0.215\frac{\text{mmol}}{\text{L}} \times 24\ \text{hr} \times 60\ \text{min/hr}} = \mathbf{45\ mL/min}$$

Question 3.5.6 How would the result of the C_{creat} and daily urine protein waste change if we found that the above urine volume was collected for only 16 hours instead of 24 hours? Is there another marker that would give us information about daily proteinuria without the need to collect urine?

Answer 3.5.6 Considering this pre-analytical error and using the correct time value (16 hrs) in the calculation would increase the creatinine clearance value to 67 mL/min.

$$\mathbf{C_{creat}} = \frac{[\text{U creat}] \times \text{Vurine}}{[\text{P creat}]} = \frac{4.8\ \text{mmol/L} \times 2900\ \text{ml/day}}{0.215\frac{\text{mmol}}{\text{L}} \times \mathbf{16\ hrs} \times 60\ \text{min/hr}} = \mathbf{67\ mL/min}$$

We cannot comment on the 24hr proteinuria, because we do not know the concentration of protein in the total 24hr urine volume. The laboratory has to use the values available from the ordering hospital department (outpatient clinic), therefore their correctness is very important. For these practical reasons, the albumin/creatinine ratio [mg/mmol] calculation from a single urine sample (ACR, albumin-to-creatinine ratio) is used in clinical routine. This parameter strongly correlates with proteinuria measured from a 24hr urine collection sample, however, ACR assessment minimises the errors resulting from the urine collection procedure. The results of the determination in a single urine sample were:

Laboratory marker	Result	Reference range	Units
Urine – single sample			
Creatinine	7.1	-	mmol/L
Albumin	645.4	-	mg/L
Albumin to creatinine ratio	90.9	< 3	mg/mmol

Albumin to creatinine ratio > 30 mg/mmol indicative of severe protein loss or even nephrotic syndrome; 3-30 mg/mmol is moderately elevated and correlates with clinically relevant proteinuria. A value of 100 mg/mmol corresponds to a quantitative proteinuria of 1 g/24 hrs.

Question 3.5.7 Which test would you recommend to further specify the type of proteinuria? What subtypes of glomerular proteinuria do you know regarding its severity and the patient's prognosis?

Answer 3.5.7 For the evaluation of proteinuria, we use the electrophoretic separation of urine proteins; the laboratory report of the electropherogram is illustrated by Fig. 37. The ordering physician receives a written description of the electropherogram, also specifying the abnormal proteins.

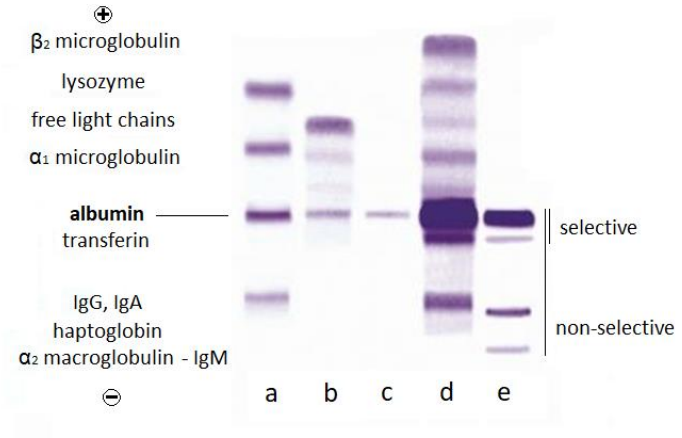

Figure 37 Results of urine protein electrophoresis analysis The urine sample is concentrated, then the negatively charged proteins are separated according to their molecular weight from cathode to anode. The "low molecular weight" tubular proteins are moving the fastest, whereas the "high molecular weight" glomerular proteins are found close to the start: a - molecular weight marker, b - tubular proteinuria with free light chains, c - albumin, physiological proteinuria (< 150 mg/day), d - mixed proteinuria, e - glomerular proteinuria selective and non-selective.

Glomerular proteinuria can be selective or non-selective (Fig. 37 e). In **selective** proteinuria, proteins with molecular weights of up to approximately 100 kDa (albumin, transferrin) are found in the urine because of a "mild" glomerular membrane disruption with loss of its negative charge; these patients respond well to immunosuppressant treatment. **Non-selective** glomerular proteinuria is characterised by the presence of immunoglobulins with a high molecular weight in urine. The damage to the glomerular membrane is more severe and the prognosis, as well as the treatment response, is poorer.

3.6 Case 6 Liver failure, osmolality, nitrogen waste products, septic state

A 73-year-old polymorbid female patient with type II diabetes mellitus, chronic coronary artery disease, chronic obstructive pulmonary disease, and long-term alcohol addiction was hospitalised for vascular and metabolic decompensation of liver cirrhosis. Objectively, the patient is pale, lethargic, has lower extremity oedemas and ascites, and auscultatory pulmonary basal attenuated vesicular breathing, wheezing and crepitations. The biochemical findings on admission were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	140	136 – 146	mmol/L
Potassium	3.9	3.5 – 5.1	mmol/L
Chlorides	109	101 – 109	mmol/L
Serum			
Glucose	6.9	4.1 – 5.9	mmol/L
Creatinine	422	58 – 96	µmol/L
Urea	39.8	2.8 – 7.2	mmol/L
Uric acid	651	155 – 357	µmol/L
Total protein	63.2	66 – 83	g/L
Albumin	27.5	35 – 52	g/L
Bilirubin - total	22.3	5.0 – 21.0	µmol/L
Bilirubin - conjugated	5.4	0.1 – 3.4	µmol/L
AST	0.51	< 0.6	µkat/L
ALT	0.21	< 0.6	µkat/L
ALP	2.62	0.50 – 2.15	µkat/L
CRP	11.1	< 5	mg/L
Osmolality	339	280 – 300	mmol/kg (> 60 y)
Ammonia	98.4	16 – 53	µmol/L
Ethanol	0.46		g/L

Question 3.6.1 Calculate the serum osmolality and compare it with the measured osmolality. What caused the increase in serum osmolality? Define the osmolal gap.

Answer 3.6.1 It is posible to calculate the osmolality using serum concentrations of sodium, glucose and urea (all in mmol/L):

Serum osmolality = $2 \times [Na^+] + [\text{glucose}] + [\text{urea}] = 2 \times 140 + 6.9 + 39.8 =$ **<u>327 mmol/kg</u>**

The main cause of the increase in serum osmolality is the elevation of serum urea to 39.8 mmol/L, which exceeds several-fold the upper reference limit in adult subjects (7.2 mmol/L).

The patient's measured serum osmolality was 339 mmol/kg. The osmolal gap (OG) is defined as the difference between the measured and calculated osmolality.

Osmolal gap (OG) = measured osmolality – calculated osmolality = 339 – 327 = **12 mmol/kg**

The patient's osmolal gap is elevated to 12.3 mmol/kg (physiologically < 10 mmol/kg). Normally, osmolality is predominantly generated by the major osmotically active compounds of plasma (sodium, glucose and urea). Calculation of the osmolal gap is important in suspected intoxication, its increase being a sign of the presence of additional osmotically active substances in the serum. These may include most commonly alcohols (ethanol, methanol, ethylene glycol, mannitol, sorbitol), acetone, or a pathologically increased concentration of triacylglycerols or proteins. The presence of 1‰ ethanol in the blood causes an osmolality increase of more than 20 mmol/kg. This correlates with our finding - an alcoholemia of 0.46 g/L corresponds to 0.46‰ of alcohol in serum (0.23 mg/L in breath) and should increase osmolality by approximately 10 mmol/kg (1‰ by 23 mmol/kg).

Question 3.6.2 Why does the patient have a decreased concentration of total protein and albumin?

Answer 3.6.2 In liver failure (cirrhosis), there is a decrease in proteosynthesis; in addition, a significant amount of albumin is lost into the ascitic fluid (approximately 5 - 20 g per day).

Question 3.6.3 Characterise the liver transaminases AST and ALT. How can you explain their physiological serum activity in a patient with liver failure?

Answer 3.6.3 Aspartate aminotransferase (AST) is an enzyme that catalyses the transfer of an amino group from amino acids to oxaloacetate with the formation of aspartate. It is found in the liver, skeletal muscle, myocardium, erythrocytes, and other tissues. The biological half-life of AST in serum is 17 ± 5 hrs. In the liver, AST is predominantly composed of the mitochondrial isoform, and its tissue activity is about 7000× higher than that of serum. **Alanine aminotransferase (ALT)** catalyses the transfer of an amino group to pyruvate forming alanine. It is found primarily in the cytosol of hepatocytes, having an activity 3,000× higher than that in serum. The biological half-life of ALT is 47 ± 10 hrs.

The activities of both serum hepatic transaminases are typically elevated in various forms of liver damage; AST activity is also elevated in patients with muscle damage (myocardial or skeletal) and hematological diseases. The normal activity of AST (0.51 μkat/L) and ALT (0.21 μkat/L) in the serum of the above-mentioned patient with cirrhosis can be explained by the terminal stage of liver failure. In this situation, such a portion of the functional liver parenchyma is destroyed that the activity of hepatic enzymes in liver tissue is already too low and insufficient to increase its serum activity. This example illustrates the situation when a biochemical result within a reference range does not automatically guarantee the absence of disease in the patient.

Question 3.6.4 The patient's serum creatinine over the previous year tended to be steady with concentrations of around 200 μmol/L, and 205 μmol/L at the previous check-up 14 days ago. Could you interpret the results of the serum nitrogen waste product measurements. Does the current increase in creatinineemia suggest any specific syndrome?

Answer 3.6.4 The long-term increase in the serum concentration of nitrogen waste products (creatinine, urea, uric acid) can be explained by their retention in chronic renal failure resulting from diabetic nephropathy. The glomerular filtration rate calculated from the latest steady-state creatinine value of 205 μmol/L was 20 ml/min/1.73 m^2 according to the CKD-EPI (stage 4 of chronic kidney disease).

Currently, the patient's creatininemia increased to 422 μmol/L, which, considering the presence of decompensated hepatic failure and ascites, could be explained by acute renal failure associated with hepatorenal sydrome. The renal vascular changes and adrenal cortical hypoperfusion are a consequence of systemic circulatory changes occurring in portal hypertension. The patient has rapidly progressive hepatorenal syndrome type I, that is characterised by a twofold or more increase in serum creatinine within the last 2 weeks and a poor prognosis.

Question 3.6.5 The increase of which serum biochemical marker may be a major contributor to the current impairment of the patient's consciousness? Explain the mechanism.

Answer 3.6.5 Ammonia in the human body comes mostly from the metabolism of amino acids. The patient's hyperammonemia (98.4 µmol/L), was caused by the high ammonium content in portal blood entering the systemic circulation, coupled with impaired ammonia detoxification in the liver (ureagenesis). Measuring the serum ammonia is an excellent marker of the hepatic capacity to metabolise endogenously produced nitrogenous substances. Once the ammonia crosses the blood-brain barrier, it is itrathecally detoxified by the formation of glutamine through reaction with glutamate. Deficiency of this excitatory neurotransmitter in the brain is one of the main causes of the patient's impaired consciousness (so called hepatic encephalopathy).

The patient was given intravenous diuretics and albumin. For portosystemic encephalopathy, she was given lactulose. The patient developed diarrhoea and hyponatremia (132 mmol/L), which was corrected. After a surgical palliative treatment of the portal hypertension (transjugular intrahepatic portosystemic shunt), bacterial peritonitis and urinary tract infection developed, and the patient's consciousness has deteriorated, being in a coma.

Question 3.6.6 Could you interpret the biochemical markers results in relation to the patient's prognosis?

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	142	136 – 146	mmol/L
Potassium	4.9	3.5 – 5.1	mmol/L
Chlorides	102	101 – 109	mmol/L
Serum			
Glucose	6.7	4.1 – 5.9	mmol/L
Creatinine	234	58 – 96	µmol/L
Urea	39.9	2.8 – 7.2	mmol/L
Uric acid	382	155 – 357	µmol/L
Total protein	44.6	66 – 83	g/L
Albumin	19.2	35 – 52	g/L
CRP	98.1	< 5	mg/L
Presepsin	2850	< 337	ng/L

Answer 3.6.6 The patient's serum total protein and albumin concentrations decreased by more than 30%, even with intravenous albumin replacement. The serum urea level remained critically elevated, however, a significant decrease in creatininemia indicated an improvement in glomerular filtration in response to the administered treatment. In patients with end-stage renal disease, serum urea concentration more consistently correlates with symptoms of

uremia than serum creatinine. The development of a septic state is suggested by an almost 10-fold increase in serum CRP and in particular by an increase in presepsin up to 2,850 ng/L. Prospectively, a serum presepsin level of more than 1,000 ng/L correlates with a significant risk of severe sepsis and increased mortality. A presepsin level above 1,900 ng/L is associated with mortality rates of 60%. This fact was unfortunately confirmed also in this patient, as she died after the progression of septic shock and multi-organ failure. However, the levels of inflammatory markers in relation to the patient's prognosis should always be interpreted with a caution, taking into consideration the overall status of the patient and other additional factors.

Question 3.6.7 Do you know any other serum marker of glomerular filtration rate (in addition to nitrogen waste products) that is useful in practice? What are the advantages of its determination and reference ranges?

Answer 3.6.7 A plausible serum marker of glomerular filtration rate is cystatin C, a serine protease-inhibiting polypeptide being produced in all nuclear cells, which passes freely through the glomerular membrane and is uptaken and metabolised in the tubules. Serum cystatin C concentration shows a hyperbolic dependence on glomerular filtration rate, reflects GFR changes more sensitively and more rapidly than serum creatinine, shows no diurnal variation, and is independent of sex, body weight, muscle mass, and other factors. The reference ranges are 0.7–1.21 mg/L.

Question 3.6.8 The patient was examined for serum protein electrophoresis. Which finding is typically present in chronic hepatopathies? Could you describe other pathognomic abnormal serum electrophoretic patterns as well?

Laboratory marker	Result	Reference range	Units
Elpho of serum proteins			
Elpho albumin	37.3	54.3 – 65.5	%
Elpho alpha 1	4.2	1.2 – 3.3	%
Elpho alpha 2	9.9	8.3 – 15	%
Elpho beta 1	6.3	6.5 – 11.5	%
Elpho beta 2	12.5	2.5 – 7.2	%
Elpho gamma	29.8	7.1 – 19.5	%

Answer 3.6.8 The typical finding of serum protein electrophoresis in patients with liver fibrosis and cirrhosis is a decrease in albumin, alpha and beta1 globulins, and an increase in gamma globulins due to impaired interstitial cell function and polyclonal immunoglobulin synthesis. The increase in the IgA concentration, which is located between the beta and gamma fractions, is so-called "beta-gamma (β-γ) bridging".

Other electrophoretic patterns characterised by a typically altered proportion of serum protein fractions are: acute inflammation (acute phase reaction), chronic inflammation, nephrotic syndrome (protein loss), malnutrition, and monoclonal hyperimmunoglobulinemia.

3.7 Case 7 Icterus, minerals, cholestasis

A 44-year-old male patient was treated for the past 6 years for primary sclerosing cholangitis, which had a mild course presented with occasional pruritus of the skin and abdominal pain. The patient came to the hospital because of weakness, nausea, a sudden onset of colic-like pain in the right side of the abdomen irradiating to the back; he became jaundiced and has pruritus as well. He had diarrhoea several times during the last two days, did not take any food and drank only pure water or tea. He has lost considerable weight over the last six months. Objectively the patient is icteric; on physical examination the abdomen is painless during palpation, the liver is enlarged with positive Courvoisier's sign, blood pressure is 130/80 mm Hg, pulse 95/min, temperature 36.7 °C.

Question 3.7.1 What laboratory tests would you recommend to be performed?

Answer 3.7.1 Due to suspected cholestatic icterus, the patient's serum was examined for glycemia, mineralogram, basic parameters of renal and liver functions, CRP, and single urine "chemistry + sediment".

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	133	136 – 146	mmol/L
Potassium	3.3	3.5 – 5.1	mmol/L
Chlorides	98	101 – 109	mmol/L
Serum			
Glucose	5.0	4.1 – 5.9	mmol/L
Creatinine	101	74 – 110	µmol/L
Urea	6.9	2.8 – 7.2	mmol/L
Uric acid	icter.	208 – 428	µmol/L
Total protein	75	66 – 83	g/L
Albumin	44	35 – 52	g/L
Bilirubin - total	490.1	5.0 – 21.0	µmol/L
Bilirubin - conjugated	259.2	0.1 – 3.4	µmol/L
AST	5.42	< 0.85	µkat/L
ALT	7.85	< 0.85	µkat/L
GMT	27.83	< 0.92	
ALP	14.48	0.50 – 2.15	µkat/L
CRP	94.8	< 5	mg/L

Urine chemistry			
pH of urine	6.7	4.8 – 7.4	
Specific gravity	1023	1.001 – 1.035	kg/m^3
Protein	negat		
Glucose	negat		
Acetone	negat		
Bilirubine	posit		
Urobilinogen	negat		
Leucocytes chemically	negat		
Eyrthrocytes chemically	negat		
Nitrites	negat		
Urine microscopy			
Leukocytes	4	0 – 15	/ µL
Erythrocytes	7	0 – 10	/ µL
Squamous epithelial cells	10	0 – 15	/ µL
Oxalates	present		

Question 3.7.2 Explain the cause of the changes in the mineralogram. Do the results of the biochemical examination correlate with the clinical findings and the suspected diagnosis of cholestasis? What does the result "icter." mean?

Answer 3.7.2 The patient has hyponatremia, hypochloremia and hypokalemia, which can be explained by the loss of minerals due to diarrhoea (intestinal fluid contains 50 mmol/L Na^+ and Cl^-, and 30 mmol/L K^+). The minerals were not replenished since the patient did not take any food and drank only pure hypotonic water. In the urine, the typical finding is conjugated bilirubine (darker colour), because it is water soluble and easily passes into urine. Since the urobilinogen can not be produced in the intestine due to bile duct obstruction (pale to acholic stool), urobilinogen in the urine was negative. Due to severe hyperbilirubinemia, the serum was icteric ("icter.") and uric acid level could not be reliably measured (analytical interference in the assay).

Question 3.7.3 Could you describe the physiological functions of cholestatic enzymes and the mechanisms of increase in their activity during cholestasis. In which other diseases do these enzymes have a diagnostic utility?

Answer 3.7.3 Gamma-glutamyltransferase (GMT) is a membrane enzyme located in various tissues that catalyses the transfer of γ-glutamyl peptide residues. The biological half-life of GMT is 7–10 days. Serum GMT activity increases in response to the toxic or inflammatory damage of hepatocytes. The very high activity of GMT is present in the epithelial cells of hepatic bile ductuli, from where it is released into blood as a sensitive marker of the

hepatobiliary system diseases. In obstructive icterus, GMT is released from membranes by the detergent action of bile acids, reaching a 12-fold or more serum activity increase. Inducible microsomal GMT isoform is typically elevated in the serum of alcoholics and, since the biological half-life is 26 days from the cessation of alcohol intake, it provides information about chronic alcohol intake prior to the testing. In alcoholics, the GMT/AST activity ratio is typically around 6, also together with elevated serum levels of carbohydrate-deficient transferrin (CDT) with a biological half-life of approximately 2 weeks. **Alkaline phosphatase (ALP)** is a ubiquitous membrane enzyme that catalyses the hydrolysis of phosphorylated monoesters in an alkaline environment, with a biological half-life of 3–5 days. The most important isoforms of ALP are the hepatic and bone ones, in addition to the intestinal and placental ones. ALP activity tends to be increased in tumour, inflammatory, toxic and other liver damage, and in patients with increased formation or turnover of bone tissue. ALP activity is also physiologically increased in children. Regan's ALP isoenzyme is described in the case of ectopic enzyme production by malignant tumours. During bile duct obstruction, biliary α1-fraction synthesis of ALP is induced about one day after colic, with an approximately 5-fold increase in its activity. The hepatobiliary origin of ALP is supported by the finding of increased GMT activity.

The patient underwent an ERCP procedure (endoscopic retrograde cholangiopancreatography), which showed a severe malignant biliary stenosis in the *ductus hepaticus communis*. The patient was surgically treated by the insertion of a duodenobiliary stent, as well as the drainage of right *ductus hepaticus*. A CT scan of the abdomen revealed a malignant bile duct tumour, metastases in the liver and metastatically enlarged hepatic hilar lymph nodes. The pancreas and *ductus pancreaticus* were intact. Hemoculture confirmed the presence of *Escherichia coli*; intravenous antibiotic therapy was administered.

Question 3.7.4 Do the results of the biochemical testing correlate with the findings? Assess the dynamics of biochemical markers changes on days 3 and 6 after the surgery. How can the increase in AMS and LPS activity be explained?

Laboratory marker	**Result**		**Reference range**	**Units**
Minerals in serum	**3rd day**	**6th day**		
Sodium	136	141	136 – 146	mmol/L
Potassium	3.6	4.1	3.5 – 5.1	mmol/L
Chlorides	104	102	101 – 109	mmol/L

Serum				
Glucose	5.4	4.6	4.1 – 5.9	mmol/L
Creatinine	89	97	74 – 110	µmol/L
Bilirubin - total	114.3	85.1	5.0 – 21.0	µmol/L
Bilirubin - conjugated	48.5	32.5	0.1 – 3.4	µmol/L
AST	3.63	1.73	< 0.85	µkat/L
ALT	4.28	2.05	< 0.85	µkat/L
GMT	14.39	9.17	< 0.92	µkat/L
ALP	6.87	5.47	0.50 – 2.15	µkat/L
CRP	169.2	73.9	< 5	mg/L
AMS	5.02	0.92	0.46 – 1.66	µkat/L
LPS	1.47	0.67	0.22 – 1.00	µkat/L

Answer 3.7.4 The finding of an intrahepatic bile duct tumour is consistent with the previously described obstructive icterus and clinical manifestations of cholestasis. The generalisation of the malignant process with metastatic liver damage correlates with the elevation of hepatic transaminases activities. The occuring destruction of hepatocytes explains the increase of not only conjugated but also unconjugated bilirubin in the serum. After the onset of antibiotic therapy, there was a gradual decrease in CRP, the level of which, however, may also be affected by the presence of the malignant process. After surgical treatment of acute cholestasis, the serum concentrations of total and conjugated bilirubin gradually decreased, as well as the activities of cholestatic enzymes. Due to the underlying malignant disease, a complete normalisation of the activities of transaminases, ALP, GMT, and bilirubine concentrations could not be expected. Amylase and lipase are pancreatic enzymes. Their activities were elevated after pancreatic irritation during ERCP procedure, and gradually normalised. Typically after ERCP, their activities elevate and normally persist for up to 3 days, with lipase reaching values up to 12-times and amylase 2–4 times the upper reference limit.

Question 3.7.5 Which other two types of icterus besides posthepatic do you know? What biochemical findings in serum and urine would you expect to be found in them?

Answer 3.7.5 Prehepatic (hemolytic) "flavin" icterus is characterised by an increase in the concentration of unconjugated bilirubin in the serum, which does not pass into the urine. Urobilinogen in the urine is present. Serum cholestatic enzymes and hepatic transaminases are usually normal. Stool is dark because more conjugated bilirubin is passing in; urine has a normal colour. **Hepatocellular "ruby" icterus** is characterised by an elevation of both conjugated and unconjugated bilirubins in serum, both bilirubin and urobilinogen in urine are

positive; urine is dark and stool has a normal color. Serum ALP and GMT activities tend to be normal or elevated, hepatic transaminases are elevated.

Question 3.7.6 Which types of crystals can be detected by the microscopic examination of urine? Is the presence of oxalates in urine a pathological finding?

Answer 3.7.6 Crystals can be normally present in the urinary sediment, except cystine ones, which are always a sign of a pathological process (cystinuria, a disorder of amino acid metabolism). In the urine, we can find oxalates (CaC_2O_4) and uric acid, which are mostly formed at an acidic pH. In an alkaline pH, the crystallisation of ammonium urates and triphosphates (NH_4MgPO_4) is facilitated. These are often formed during uroinfections because bacteria alkalinise urine by the action of urease (forming ammonia). Amorphous crystals are composed of urates or phosphates. The crystals in urinary sediment are shown in Fig. 38.

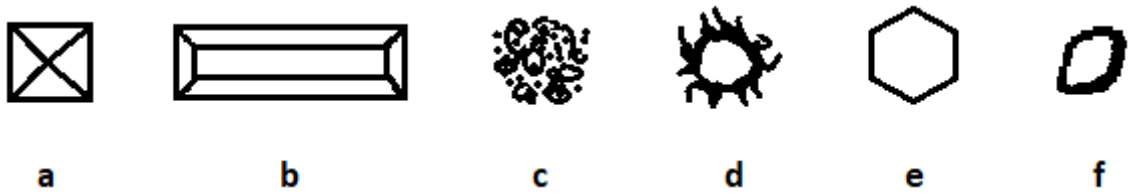

Figure 38 Schematic illustration of crystal shapes in urinary sediment a - oxalates (envelope shape), b - triphosphates (casket shape), c - amorphous crystals, d - ammoniumurates, e - cystine crystals (hexagonal shape), f - uric acid

3.8 Case 8 Acid-base balance, *diabetes mellitus*, sepsis

A 12-year-old boy was brought to the hospital by an emergency ambulance called by his parents after he fell unconscious. The patient had not yet been attending any specialised outpatient clinic. In the ambulance, a glycemia of 30 mmol/L was detected and a bolus of saline was given. On admission, the patient was soporous, hypotrophic (weight 36 kg), has decreased skin turgor, oliguria, spontaneous respiration - Kussmaul's breathing (39 breaths/min) with oxygen support, a heart rate of 135/min, a blood pressure of 75/40 mm Hg, acetone foetor ex ore, anisocoria, no signs of meningeal irritation. Biochemical results on admission were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	127	136 – 146	mmol/L
Potassium	3.7	3.5 – 5.1	mmol/L
Chlorides	102	101 – 109	mmol/L
Calcium	2.37	2.20 – 2.75	mmol/L (2 – 15 y)
Phosphates	0.91	1.29 – 2.26	mmol/L (0 – 15 y)
Serum			
Glucose	73.6	3.3 – 5.6	mmol/L (0 – 15 y)
Creatinine	219	23 – 68	µmol/L (1 – 15 y)
Urea	15.1	1.8 – 6.4	mmol/L (6 wk. – 15 y)
Bilirubin - total	6.4	5.0 – 21.0	µmol/L
Albumin	32.8	35 – 52	g/L
CRP	26.7	< 5.0	mg/L
Acid-base balance			
pO_2	13.96	9.3 – 12.0	kPa (1 – 14 y)
pCO_2	1.33	4.40 – 5.60	kPa (1 – 14 y)
pH	7.051	7.36 – 7.44	-
BE	-25.5	-2.5 – 2.5	
HCO_3^-	2.7	22 – 26	mmol/L
O_2 sat	95.3	95 – 99	%

Question 3.8.1 Which acid-base balance balance disorder is this? Assess its severity. Does the BE correlate with the disorder? Explain the changes in the partial pressures of CO_2 and O_2.

Answer 3.8.1 Considering the critically low pH (< 7.1) and bicarbonate, this is a severe metabolic acidosis (diabetic ketoacidosis). The BE value is decreased and correlates with the metabolic "component" of the acid-base balance disorder. CO_2 partial pressure is reduced due to compensatory hyperventilation and CO_2 excretion (Kussmaul's deep breathing), oxygen partial pressure is increased, because the patient is inhaling oxygen.

Question 3.8.2 Does the patient have signs of impaired kidney function? If yes, explain why?

Answer 3.8.2 An increase in the serum concentration of nitrogen waste products (creatinine 219 µmol/L, urea 15.1 mmol/L) and medical history findings (osmotic diuresis in critical hyperglycemia and subsequent dehydration - reduced skin turgor, hypotension, oliguria) suggest prerenal acute renal failure, caused by hypoperfusion and reduction of kidney functional blood supply.

The patient was given therapy for ketoacidosis (i.v. insulin, rehydration); diuresis was potentiated by furosemide application. He gradually developed subfebrilia, abdominal pain and signs of peritoneal irritation. Imaging examinations (CT, USG) detected hepatomegaly, ascites, fluidothorax and paralytic ileus. Biochemical findings on the second day of hospitalization were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	139	136 – 146	mmol/L
Potassium	2.7	3.5 – 5.1	mmol/L
Chlorides	116	101 – 109	mmol/L
Calcium	2.23	2.20 – 2.75	mmol/L (2 – 15 y)
Serum			
Glucose	13.5	3.3 – 5.6	mmol/L (0 – 15 y)
Creatinine	206	23 – 68	µmol/L (1 – 15 y)
Urea	17.8	1.8 – 6.4	mmol/L (6 wk. – 15 y)
Uric acid	884	190 – 440	µmol/L
Bilirubin - total	6.7	5.0 – 21.0	µmol/L
Bilirubin - conjugated	2.6	0.1 – 3.4	µmol/L
Total protein	45.1	57 – 80	g/L (0 – 18 y)
Albumin	26.5	35 – 52	g/L
GMT	0.26	< 0.70	µkat/L
AST	2.55	< 0.85	µkat/L
ALT	0.67	< 0.85	µkat/L
ALP	1.28	1.25 – 6.5	µkat/L (12 – 15 y)
Cholinesterase	58.22	77 – 192	µkat/L
AMS	9.22	0.46 – 1.66	µkat/L
Lipase	1.90	0.22 – 1.00	µkat/L
CK	93.43	< 4.50	µkat/L
CK-MB	3.82	< 0.40	µkat/L
Myoglobin	2435	< 70.0	µg/L
hs Troponin I	436.20	< 11.6	ng/L
NT-proBNP	28413	< 125	ng/L
Inflammatory markers			
CRP	182.3	< 5.0	mg/L
Interleukin 6	> 1554	< 6.40	ng/L
Procalcitonin	> 100.47	< 0.50	µg/L
Presepsin	430	< 337	ng/L

Question 3.8.3 Interpret the results of the examination of hepatocyte damage markers. Describe the enzyme cholinesterase. Why do we measure its serum activity?

Answer 3.8.3 The finding is not suggestive of hepatocellular damage with a massive release of transaminases into the circulation, because ALT activity is within normal limits and the 3-fold increase of AST activity may also be caused by myocardial or muscle damage. Since GMT and ALP activity are also within the normal range, the disorder is probably not present at the bile duct and canalicular pole of the hepatocyte. This is supported by the finding of normal serum levels of total and conjugated bilirubin. Hypoproteinemia and hypoalbuminemia are suggestive of impaired liver proteosynthetic function, however, these may also be related to protein loss into the ascitic fluid. For this reason, we examined the serum cholinesterase activity (decreased in this patient), as well as other markers of hepatic proteosynthetic function such as Ig levels and hematological markers (coagulation factors, prothrombin time - Quick).

Cholinesterase (CHS, acylcholinacyltransferase) is an enzyme originating in the ribosomes of hepatocytes that catalyses the hydrolysis of organic acids choline esters with relatively broad substrate specificity (it is not a specific acetylcholinesterase from synapses). The synthesis of cholinesterase does not depend on the synthesis of albumin; the biological half-life is 12-14 days. The increase or decrease of its activity in the serum reflects changes in the liver parenchyma synthetic function. A diagnostically significant decrease in serum CHS activity is associated with liver damage (inflammatory, toxic, tumour, blood congestion in heart failure...), protein malnutrition, severe hypercatabolic states and intoxications.

Question 3.8.4 Could you interpret the results of cardiac markers testing? In spite of their elevation, a cardiological examination did not confirm myocardial infarction, but revealed the presence of tricuspid regurgitation and reduced myocardial contractility.

Answer 3.8.4 The patient was found to have an increased serum creatine kinase (CK) activity and myoglobin levels, which may also be elevated after damage to other organs, especially skeletal muscle. Myoglobin levels may also increase in renal failure. Serum cardiac-specific markers include creatine kinase muscle-brain isoform (CK-MB) and troponin I, both of which were elevated in this patient. In addition to the latter, an increase in NT-proBNP, a marker of the myocardial volume overload, was observed. The findings presented above are indicative

of cardiac damage and failure in sepsis (elevation of inflammatory markers) and the development of multiorgan dysfunction syndrome. It is important to realise that the elevation of cardiac markers can also be caused by conditions other than myocardial infarction itself (more detailed information is provided in the next case).

Question 3.8.5 Why did we get a result "> 100.47 µg/L" when testing procalcitonin in this case?

Answer 3.8.5 Generally, a result "> value" indicates the level of marker in sample to be so high that it cannot be measured with the required precision, because it exceeds the upper limit of the method's detectability. In this region, the dependence of variable change (absorbance, luminescence...) on the marker concentration is linear.

Question 3.8.6 Which patient's condition is evidenced by the elevation of inflammatory markers in correlation with other biochemical findings and clinical picture (subfebris, signs of peritoneal irritation)?

Answer 3.8.6 The elevation of inflammatory markers supports the suspicion of a systemic inflammatory response syndrome (SIRS). The probable presence of infection and sepsis is supported by the finding of a more than 200-fold increase in IL6 levels, presepsin levels varying between 300 and 500 ng/L, and elevated CRP levels (182.3 mg/L). Also, a detected procalcitonin level of 100 µg/L is suggestive of severe sepsis (>10 µg/L). However, inflammatory markers are unable to differentiate between infectious and non-infectious causes of inflammation. In our patient, sepsis was found to be caused by intestinal necrosis, which was managed surgically. The progression of the septic state is indicated by the assessment of kinetics of inflammatory marker levels over time.

Question 3.8.7 The patient was given empirical intravenous antibiotic therapy, and samples were collected for microbiological culture and sensitivity testing. Below, we can see the consecutive changes in the levels of inflammatory markers (dynamics) from the day of the patient’s admission. Try to comment on the effectiveness of the antibiotic treatment.

Laboratory marker	Result							Reference range	Units
Inflammatory markers									
Serum	1. day	2.day	3.day	4. day	5. day	6. day	7. day		
CRP	182.3	322.7	359.4	157.0	98.2	63.0	10.6	< 5.0	mg/L
Procalcitonin	> 100.47	45.43	17.10	10.18	5.04	1.91	0.07	< 0.50	µg/L

Answer 3.8.7 Antibiotic therapy was effective since serum procalcitonin levels decreased by approximately 50% in 24 hours following its biological half-life. Similarly, after the initial increase, there was also a decrease in CRP levels. Besides the levels of inflammatory biochemical markers, it is very important to monitor body temperature, leukocyte counts, other clinical signs of infection and organ dysfunction. Antibiotic therapy is usually withdrawn when the PCT level falls below 0.5 µg/L or decreases by more than 80% of its maximum level. A decrease in PCT levels in septic states means that the generalisation of infection has been successfully managed.

Question 3.8.8 Which other serum marker should be monitored regularly for the correct interpretation of PCT and CRP levels changes in septic patients with liver failure?

Answer 3.8.8 A false decrease in CRP and PCT levels mimicking effective antibiotic treatment may be present when liver proteosynthetic function is impaired. Therefore, for a proper interpretation, the regular monitoring of serum cholinesterase activity, or total protein and albumin levels, is necessary.

Question 3.8.9 Describe the physiological functions of amylase and lipase. How do we explain the increase in their serum activities if the pancreas ultrasonography did not detect any pathological changes?

Answer 3.8.9 Alpha-amylase (AMS) is an enzyme that catalyses the hydrolysis of starch into oligosaccharides, maltose and dextrins. The biological half-life of AMS is 6–12 hours. The total activity of AMS in serum is made up of salivary isoform (S-AMS) and pancreatic isoform (P-AMS). P-AMS is found primarily in the pancreas, as well as in other organs of the gastrointestinal tract. An increase in serum AMS activity is observed in acute and chronic pancreatitis (release from pancreatic ducts and acini), in cholecystitis, peptic ulcer perforation, mesenteric ischemia, ileus and biliary colic.

Lipase (LPS) is an enzyme mainly produced in the pancreas that catalyses the hydrolysis of triacylglycerols to fatty acids and monoacylglycerols. A pathological release to serum occurs in acute and chronic pancreatitis and other "acute abdomen" events, where an increase of up to 5 times the upper reference limit is typical (cholecystitis, intestinal perforation). In acute pancreatitis, the serum amylase activity exceeds 5-fold or more the upper reference limit, increases within 3–12 hours and normalises in about 3 days; the lipase activity increases up to 80-fold in about 5–6 hours and remains elevated for 3–6 days. In our patient, the approximately 5-fold elevated AMS activity and the 2-fold elevated LPS activity were probably caused by intestinal ischemia.

Question 3.8.10 The patient's glycemia was monitored à 1 h after insulin injection and gradually decreased. Which biochemical marker do we use to monitor the accuracy of treatment and long-term compensation of diabetes mellitus? What collection tubes would you use for the examination and what are the reference ranges?

Answer 3.8.10 In addition to glycemia and glycemic profile testing, in diabetic patients, we regularly determine a **glycated hemoglobin (HbA1c)** that is produced by the non-enzymatic glycation of the N-terminal amino groups of hemoglobin A (DOF, β1-N-deoxyfuranosylhemoglobin). Blood must be taken into tubes containing an anti-coagulant agent (EDTA), since the test is a chromatographic assay from whole blood. The blood glycated hemoglobin level gives us information about glycemia of the last few months, since the lifetime of erythrocytes is 120 days. The reference range according to the IFCC (International Federation of Clinical Chemistry) is 2.8–4.8%, which corresponds to 28–48 mmol DOF per 1 mol of hemoglobin. Albuminuria examination provides information about the progression of diabetic nephropathy.

3.9 Case 9 Cardiomarkers

A 68-year-old male patient, a habitual smoker (30 years, 20 cig/day), who is being treated for arterial hypertension comes to the hospital in the morning with resting chest pain that appeared about 5 hours ago. The patient has been taking long-term hypolipidemic treatment - statins. The patient is anxious and he is sweating. An electrocardiogram (ECG) showed the ST-segment elevations. Biochemical results on admission were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	138	136 – 146	mmol/L
Potassium	3.9	3.5 – 5.1	mmol/L
Chlorides	107	101 – 109	mmol/L
Serum			
Glucose	8.5	4.1 – 5.9	mmol/L
Creatinine	122	74 – 110	µmol/L
Urea	10.3	2.8 – 7.2	mmol/L
Uric acid	412	208 – 428	µmol/L
Total protein	68	66 – 83	g/L
Bilirubin - total	9.6	5.0 – 21.0	µmol/L
Bilirubin - conjugated	2.3	0.1 – 3.4	µmol/L
Cholesterol total	2.67	< 5.17	mmol/L
Triacylglycerols	0.50	0.4 – 1.7	mmol/L
Cholesterol HDL	0.76	1.20 – 2.20	mmol/L
Cholesterol LDL	1.71	1.00 – 3.30	mmol/L
AST	0.50	< 0.85	µkat/L
ALT	0.48	< 0.85	µkat/L
GMT	0.34	< 0.92	µkat/L
ALP	1.12	0.50 – 2.15	µkat/L
CK	3.62	< 2.85	µkat/L
CK-MB	0.36	< 0.4	µkat/L
Myoglobin	482.3	< 70.0	µg/L
hs Troponin I	1329.8	< 19.8	ng/L

Question 3.9.1 Do the biochemical results of cardiomarkers testing correlate with the clinical picture and ECG findings? Which diseases can cause their elevation in serum?

Answer 3.9.1 The biochemical examination shows an increase in creatine kinase activity, which is typically present within approximately 4 to 6 hours of myocardial ischemia. Total CK activity may also be elevated in patients with skeletal muscle diseases (dystrophies, myositis, trauma, intramuscular injection, physical activity) or other conditions - e.g., malignant hyperthermia, shock, pulmonary embolism, intoxication, ethylism, therapy with statins, beta-blockers, etc. For this reason, we routinely determine the cardiac-specific isoenzyme CK-MB,

which normally comprises about 5% of skeletal muscle CK and 40% of myocardial CK. In addition to myocardial infarction, CK-MB activity may also be elevated in myocarditis, pericarditis, myocardial contusion or after cardiovascular surgery. In addition to the assessment of CK-MB activity, it is also possible to determine the mass concentration "CK-MB mass". This method is more sensitive and also measures the degraded and inactive enzyme. In our patient, the CK-MB activity was within normal range, which can be explained by the relatively early time of sample collection. An increase in myoglobin concentration is typical already between 0.5 and 2 hours after cardiac ischemia; the troponin I increases between 3 and 6 hours. In our patient, both markers were already elevated on admission. In biochemical diagnostics of myocardial infarction in patients with acute chest pain, the repeated evaluation of cardiomarkers and the dynamics of their changes in serum are of major importance.

After admission, the patient underwent urgent coronary angiography, showing acute transmural anterior wall myocardial infarction, followed by percutaneous coronary intervention (PCI) in the *ramus interventricularis anterior* and stent implantation.

Question 3.9.2 Describe the physiological functions of cardiomarkers. Are serum levels changes in our patient consistent with theoretical knowledge about cardiomarker dynamics over time?

Laboratory marker	**Result (time from ischemia)**			**Reference range**	**Units**
Serum	admission (5 h)	11 h	30 h		
CK	3.62	78.15	27.69	< 2.85	µkat/L
CK-MB	0.36	7.04	1.26	< 0.4	µkat/L
Myoglobin	482.3	502.4	55.8	< 70.0	µg/L
hs Troponin I	1329.8	> 26553.0	> 26553.0	< 19.8	ng/L

Answer 3.9.2 Creatine kinase catalyses the reversible reaction of phosphorylation of creatine to creatine phosphate. The biological half-life of CK in serum is approximately 18 hours, depending on the predominant isoform and being shorter for CK-MB. The activities of CK and CK-MB during myocardial necrosis persist elevated in the serum for approximately 24–36 hours, and normalise within 2–5 days. The higher the proportion of CK-MB from total serum CK activity (>6%), the more likely it is to be of cardiac origin. In our patient, the CK maximal values (approximately 20-fold increase from the first sampling) were observed at the 11th hour

after the onset of ischemia symptoms. By the 30th hour, there was a significant decrease correlating with the biological half-life of CK. The normalisation of values was also accelerated by reperfusion.

Myoglobin binds and carries oxygen in all muscle cells, which accounts for its low organ specificity. Myoglobin is quickly released into the blood after myocardial necrosis (peak in 6–12 hours). Due to its relatively small molecular weight, it is easily eliminated by the kidneys, having a biological half-life of 10–20 minutes. In the diagnostics of myocardial infarction, myoglobin testing has an excellent negative predictive value: if the concentration is within a normal range 4-6 hours after the onset of symptoms, we can exclude myocardial damage. A normalisation of serum myoglobin level appears in 12–24 hours, as confirmed by the findings in our patient.

Troponin I (TnI) inhibits the actin-myosin interaction that is normally necessary for cardiomyocyte contraction. As it is not found in skeletal muscle, TnI is a cardiospecific marker. Release from the myocardium in 3–6 hours after ischemia also occurs in microinfarcts, with the maximum increase by up to 24 hours. Due to the long-term persistence of elevated serum concentrations (7–10 days), TnI is not suitable for reinfarction detection. On the other hand, it is excellent for the diagnosis of myocardial infarction delayed from the onset of clinical symptoms. A normal TnI value in the first hours the after onset of symptoms does not exclude myocardial infarction (diagnostic window). The TnI testing 6 hours after the last clinical symptoms has a high negative predictive value. The TnI serum level in our patient was very high at the second and subsequent assessments (exceeding the upper limit of quantification). Compared with the first TnI measurement, there was a dynamic increase in 24 hours, indicating a very massive necrosis of myocardial tissue, also confirmed by imaging.

Question 3.9.3 Which other cardiomarkers were previously investigated in the serum of suspected myocardial infarction patients, but are now being withdrawn from clinical use? Describe the time dependence of their serum increase.

Answer 3.9.3 The **AST** activity tends to be elevated up to 25-fold in myocardial infarction within approximately 4-6 hours of ischemia, with peak levels on day 1–2 and normalisation within 5 days. **Lactate dehydrogenase (LD)** activity typically increases between 12–18 hours

after necrosis and remains elevated for 10 days. A limitation of AST and LD determinations is their low organ specificity.

Question 3.9.4 The patient was previously not treated for diabetes mellitus. The glycemia was monitored at regular check-ups, tending to be within normal limits. How can we explain the hyperglycemia on admission? We know that the patient had not eaten 10 hours before and the next day after the surgery, glycemia was already within reference limits.

Answer 3.9.4 Hyperglycemia in myocardial infarction is commonly caused by the stress response and the release of the insulin counterregulatory hormones.

Question 3.9.5 How can we explain the normal lipid panel findings (apart from the HDL cholesterol decrease) if we know that hyperlipidemia is a major risk factor for atherosclerosis? Is it possible for a patient with normocholesterolemia to have an atherogenic lipid profile?

Answer 3.9.5 The result of cholesterolemia measurement is valid when the sample is taken within 24 hours of the onset of angina pectoris. After this period, the serum concentration of both total and LDL cholesterol (low-density lipoprotein) decreases. The determination of real lipid concentration can be optimally performed only approximately 3 months or later after ischemia. In addition, the patient is taking long-term hypolipidemic treatment (statins), which significantly lower cholesterol levels.

Atherogenic dyslipidemia is characterised by hypertriacylglycerolemia (TAG > 2.3 mmol/L), low HDL cholesterol (high-density lipoproteins, HDL < 0.9 mmol/L) and elevated LDL cholesterol (LDL 3.5–4.1 mmol/L) with an increase in small dense LDL (sdLDL). SdLDL have significant atherogenic properties and may also be detected in patients with normocholesterolemia. **Atherogenic lipid phenotype** comprises atherogenic dyslipidemia in combination with other pathological factors (hypertension, basal hyperinsulinemia, hyperuricemia, elevation of apoprotein B-100 concentration, abdominal obesity).

3.10 Case 10 Life-threatening hyponatremia

A 22-year-old female patient, followed over a long period for anemic syndrome, was hospitalised due to a severe acute headache lasting about 2 hours. The patient has no other significant pre-existing condition. She regularly drinks 4-6 litres of fluids per day, always feels thirsty, and is on a diet due to a feeling of stomach fullness. She has frequent diarrhoea, has no abdominal pain, has not lost weight in the last months, has not been unconscious, feels tired, and has not had any fever. Objectively, the patient has an asthenic habitus (BMI 15.1), is bradypsychic, has pale skin, anxious and panic moods, polydipsia, polyuria, blood pressure of 110/75 mm Hg, a heart rate of 85/min, a body temperature of 36.4 °C, saturation 97%. On admission, the chest X-ray was normal, the 12-lead ECG was without signs of acute ischemia. Biochemical results on admission were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	105	136 – 146	mmol/L
Potassium	3.7	3.5 – 5.1	mmol/L
Chlorides	74	101 – 109	mmol/L
Serum			
Glucose	5.1	4.1 – 5.9	mmol/L
Creatinine	37	58 – 96	µmol/L
Urea	1.8	2.8 – 7.2	mmol/L
Bilirubin - total	18.3	5.0 – 21.0	µmol/L
Bilirubin - conjugated	2.5	0.1 – 3.4	µmol/l
AST	0.47	< 0.6	µkat/L
ALT	0.18	< 0.6	µkat/L
ALP	2.04	0.50 – 2.15	µkat/L
GMT	0.16	< 0.63	µkat/L
AMS	1.13	0.46 – 1.66	µkat/L
LPS	0.49	0.22 – 1.00	µkat/L
Osmolality	226	275 – 295	mmol/kg
CK	2.61	< 2.42	µkat/L
CK-MB	0.42	< 0.40	µkat/L
hs Troponin I	0.02	< 11.6	ng/L
CRP	0.3	< 5	mg/L

Urine chemistry			
pH of urine	6.4	4.8 – 7.4	
Specific gravity	1.000	1.001 – 1.035	kg/m^3
Protein	negat		
Glucose	negat		
Acetone	traces		
Bilirubine	negat		
Urobilinogen	negat		
Nitrites	negat		

Urine microscopy			
Leukocytes	5	0 – 15	/ µL
Erythrocytes	7	0 – 10	/ µL
Squamous epit. cells	4	0 – 15	/ µL
Urine			
Osmolality	47		mmol/kg
Minerals in urine			
Sodium	4		mmol/L
Potassium	1		mmol/L
Acid-base balance (capillary)			
pO_2	8.84	9.8 – 13.3	kPa
pCO_2	4.90	4.64 – 6.00	kPa
pH	7.412	7.36 – 7.44	-
BE	- 0.3	-2.5 – 2.5	
HCO_3^-	23.7	22 – 26	mmol/L
O_2 sat	96	95 – 99	%

Question 3.10.1 Evaluate the mineralogram and serum osmolality. What is the cause and what are the mechanisms of the patient's problems?

Answer 3.10.1 The patient has critical symptomatic hyponatremia (105 mmol/kg) and serum hypoosmolality (226 mmol/kg). The calculated osmolality is 216.9 mmol/kg; the osmolality gap is normal (9.1 mmol/kg). Hyponatremia and hypochloridemia are caused by the excessive loss of minerals from the body during prolonged polydipsia, polyuria and also losses from the gastrointestinal system by diarrhoea. The mechanism of central nervous system symptoms is the brain hyperhydration due to the passage of hypotonic fluid from the circulation along the osmotic gradient.

Question 3.10.2 What degrees of hyponatremia severity do you know? How can it threaten the patient's life?

Answer 3.10.2 Hyponatremia is classified according to the serum sodium concentration into: mild/moderate hyponatremia (125–134 mmol/L), severe hyponatremia (121–125 mmol/L) and severe/critical hyponatremia (≤120 mmol/L). The patient's life is threatened by the development of cerebral edema, herniation and the compression of vital midbrain centers. In addition, a mortality rate of 50% due to central demyelination has been described when hyponatremia progresses over several days to values of <120 mmol/L (and serum osmolality <250 mmol/kg).

Question 3.10.3 To demonstrate, imagine that in an attempt to rapidly treat the disorder, hypertonic saline would have been given intravenously. After five hours, the hyponatremia would be corrected from 105 mmol/L to 126 mmol/L. Would this kind of therapeutic intervention be appropriate?

Answer 3.10.3 In hyponatremia, brain cells compensatorily deplete ions and osmotically active organic compounds, thereby adapting to the hypoosmolality of serum. With a rapid correction of natremia, the patient is at high risk of myelinolysis syndrome due to dehydration and cell shrinkage caused by the quick increase in effective extracellular fluid osmolality. The risk of a shear effect at the interface of axons and myelin sheaths increases with the duration and severity of hyponatremia, as well as resulting compensatory loss of intracellular solutes.

Question 3.10.4 How should hyponatremia be treated to minimise the risk of brain damage or threatening the patient's life?

Answer 3.10.4 If the disorder is acute (within 2 days of the onset of hyponatremia), it should be increased by a maximum of 1-2 mmol/hr, in chronic hyponatremia by 0.5 mmol/hr. The ideal daily correction of natremia should not exceed 10 mmol/L/24 h, 18 mmol/L/48 h and 20 mmol/L/72 h.

Question 3.10.5 On admission, elevated CK (2.61 µkat/L) and CK-MB (0.42 µkat/L) activities were detected in the patient's serum; however, the troponin I level was within normal range. In correlation with the clinical picture and examination findings, how would you explain this observation?

Answer 3.10.5 An increase in CK activity could indicate the presence of muscle or myocardial damage, but the values only slightly exceed the upper reference limit (< 2.42 µkat/L). An increase in CK-MB activity is specific for myocardial damage, but in this case the activity is only slightly elevated (upper ref. limit < 0.40 µkat/L). Clinical symptoms of acute coronary syndrome were not present in our patient, serum troponin I level was normal, similarly, ECG did not detect ischemic or other pathological findings. As we have already mentioned, the elevation of cardiomarkers occurs several hours after ischemia. To make sure that we are not in the "diagnostic window", the examination of the above-mentioned cardiomarkers,

especially troponin I, should be repeated and possibly myoglobin testing could be added. Slight increases in CK and CK-MB activities without further dynamics, clinical and ECG correlate may represent the individual patient norm (5% of healthy individuals are outside reference ranges). Eventually, the markers may be elevated for a long time in individuals with muscular diseases or in sportsmen, or transiently after physical exercise.

Question 3.10.6 How can we explain the decreased serum creatinine and urea levels? What do the traces of ketone bodies in the urine indicate?

Answer 3.10.6 Creatinine is formed in muscle; urea is a waste product of amino acid metabolism. Decreased values may be related to the impaired production of these substances due to asthenic habitus, reduced muscle mass in the patient's body, and a lower protein intake on a special diet. In addition, increased renal losses of creatinine and urea due to long-lasting polydipsia and polyuria could also be accounted. Detected urinary ketone bodies may indicate ketogenesis while on a special diet.

Question 3.10.7 Which syndrome should we consider when critical hyponatremia and serum hypoosmolality are detected? Can the testing of urine osmolality and urine sodium concentration be helpful in the diagnostics?

Answer 3.10.7 Such findings may be present in the syndrome of inappropriate antidiuretic hormone secretion (SIADH), in which we typically observe: sodium < 134 mmol/L, serum osmolality < 280 mmol/kg, urine osmolality > 100 mmol/kg, or urine sodium concentration > 30 mmol/L, without dehydration or oedema. In our patient, urine osmolality was 47 mmol/kg and urine sodium was 4 mmol/L, correlating with increased water loss and thus not suggesting SIADH. However, it is certainly necessary to recommend an endocrinological examination.

3.11 Case 11 Examination of urine, kidney functions

A 40-year-old man with no significant pre-condition was admitted to hospital because of weakness, fatigue, swelling of both eyelids and lower extremities. Two days previously, he had observed dark blood-colored urine and urinated very little. Three weeks previously, he had scratched his leg with a wire, the wound had become inflamed, he did not pay attention to the injury and later he started taking antibiotics. Objectively, there is bilateral periorbital and pretibial oedema, a purulent laceration on the right calf (approximately 10 cm long, surrounding circular red flushing), a body temperature of 37.4 °C, a blood pressure of 155/95 mm Hg, tapotement bilaterally negative. The results of biochemical examination were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	133	136 – 146	mmol/L
Potassium	5.3	3.5 – 5.1	mmol/L
Chlorides	104	101 – 109	mmol/L
Serum			
Glucose	4.7	4.1 – 5.9	mmol/L
Creatinine	132	74 – 110	µmol/L
Urea	11.5	2.8 – 7.2	mmol/L
Uric acid	389	208 – 428	µmol/L
Total protein	72	66 – 83	g/L
Bilirubin - total	15	5.0 – 21.0	µmol/L
Bilirubin - conjugated	2.1	0.1 – 3.4	µmol/L
AST	0.50	< 0.85	µkat/L
ALT	0.25	< 0.85	µkat/L
GMT	0.30	< 0.92	µkat/L
Osmolality	280	275 – 295	mmol/kg
CRP	193.2	< 5.0	mg/L
Urine chemistry			
pH of urine	5.6	4.8 – 7.4	
Specific gravity	1.011	1.001 – 1.035	kg/m^3
Protein	posit		
Glucose	negat		
Acetone	negat		
Bilirubine	negat		
Urobilinogen	negat		
Nitrites	negat		
Leukocytes	posit		
Erythrocytes	posit		
Urine microscopy			
Leukocytes	160	0 – 15	/ µL
Erythrocytes	8700	0 – 10	/ µL
Squamous epithelial cells	4	0 – 15	/ µL
Tubular epithelial cells	rare		
Hyaline casts	19		/ µL
Granular casts	7		/ µL
Erythrocyte casts	28		/ µL

Question 3.11.1 Interpret the serum and urine biochemical findings in relation to the medical history and clinical picture. Which organ damage is suspected?

Answer 3.11.1 The suspected acute nephritic syndrome is indicated by signs of renal failure (oliguria, increased concentration of nitrogen waste products - creatinine and urea, hyperkalemia, dilutional hyponatremia from water retention), oedema, hypertension in combination with findings of proteinuria and macroscopic hematuria, and non-specific symptoms (fatigue, weakness). The ongoing inflammatory process is characterised by leukocyturia and elevated serum CRP.

Question 3.11.2 The patient was examined for proteinuria in a single urine sample (Albumin to Creatinine Ratio). What is the approximate daily protein loss corresponding to the result below?

Laboratory marker	Result	Reference range	Units
Urine			
Creatinine	3.1		mmol/L
Albumin	300.7		mg/L
Albumin to Creatinine Ratio	97	< 3	mg/mmol

Answer 3.11.2 The Albumin to Creatinine Ratio of 97 mg/mmol corresponds to protein losses of 0.97 g/24 h (100 mg/mmol correlates with losses of 1 g/24 h). Proteinuria in nephritic syndrome is usually around 0.5 – 2 g/24 h, but it may also be of a nephrotic character.

Question 3.11.3 Considering the above results, what is the probable etiology of acute renal failure? Explain. Do you know the marker called "ASLO"?

Laboratory marker	Result	Reference range	Units
Serum			
ASLO	503.2	< 200.0	IU/mL
C3-complement	0.16	0.9 – 1.8	g/L

Answer 3.11.3 Untreated streptococcal bacterial infection of the skin caused the patient to develop acute endocapillary (poststreptococcal) glomerulonephritis with a clinical picture of nephritic syndrome in a few weeks. A decrease in serum C3 complement concentration is typical of this diagnosis. **Antistreptolysin O (ASLO)** is produced as an organism's antibody response to a Streptococcus pyogenes infection. In 75–80% of cases with upper respiratory

tract infections, the production starts the first week after infection, with a peak at 3-6 weeks, and the increase can persist for up to 6 months. In cutaneous streptococcal infections, ASLO serum concentration increases in fewer than half of patients. As the extent of the antibody response against streptococci increases, so does the likelihood of complications. The predictive value of biochemical testing is greatly enhanced by determining the marker dynamics (before and one month after treatment). In the case of ASLO negativity, it is possible to test for anti-DNase B, the serum concentration of which increases about 6-8 weeks after infection and persists longer. It is an bacterial exoprotein that cleaves nucleic acids and proteins, thereby facilitating the spread of infection.

Question 3.11.4 The finding of macroscopic hematuria was also confirmed by microscopic examination of the urinary sediment. Which other specific urine test can give us more precise information about the origin of the erythrocytes?

Answer 3.11.4 The examination of **erythrocyte dysmorphism** (evaluation of hematuria) is performed by a polarised light microscope (phase contrast) and allows us to recognise the origin of erythrocytes based on changes in their shape. **Renal hematuria** is microscopically characterised by dysmorphic erythrocytes, because when passing through the damaged glomerular membrane, they change in shape, size, gaining membrane defects and vesicle-like protrusions (acanthocytes). Simultaneously a proteinuria is also formed. **Postrenal hematuria** is characterised by microscopically eumorphic erythrocytes of normal shape that originate from the injured urinary tract.

The result of phase-contrast erythrocytes microscopy in urine was received from the laboratory:

Laboratory marker	Result	Reference range	Units
Typing of hematuria			
Eyrthrocytes dysmorphic	98		%
Erythrocytes isomorphic	2		%

Question 3.11.5 What percentage of dysmorphic erythrocytes found by phase-contrast microscopy differentiates between renal and postrenal hematuria?

Answer 3.11.5 The finding of more than 60% of dysmorphic erythrocytes suggests reno-parenchymal hematuria.

Question 3.11.6 Which cells and structures can be detected by the microscopic examination of urinary sediment? Describe their pathognomonic and diagnostic significance.

Answer 3.11.6 Leukocyturia is a sign of infectious or non-infectious inflammation; the majority of leukocytes are neutrophilic granulocytes. **Erythrocytes** are present in the urine when there is microscopic or macroscopic hematuria. The chemical assay detects heme and it is also positive when hemoglobinuria or myoglobinuria is present. **Squamous epithelial cells** originate from the distal part of urethra and are found physiologically in urine. **Urothelial cells** come from the urinary tract, and if they are normal in shape, they are a physiological finding. **Renal tubular epithelial cells** are always a pathological sign of tubular damage or acute tubular necrosis. In addition to the cells, we can also detect the microscopic cylindrical structures called **urinary casts**, which are formed in the distal tubule and the collecting duct. **Hyaline casts** are formed by the precipitation of Tamm-Horsfall protein, which is secreted by tubular cells during the water resorption process. Their formation is facilitated by dehydration, decreased pH and increased urinary protein concentration. Hyaline casts are physiologically present in urine, providing the basis for other types of urinary casts, the presence of which is always pathological. **Cellular casts** contain tubular cells and typically appear in acute tubular necrosis. **Granular casts** arise from cellular cylinders through the degeneration of adherent tubular epithelial cells and proteins (coarse granulation). Their progressive disintegration through **fine granular casts** results in the formation of **waxy casts** that are typical of glomerulonephritis, interstitial nephritis or acute tubular necrosis. **Erythrocyte casts** are formed during renal glomerular hematuria. **Leukocyte casts** tend to be present in pyelonephritis and glomerulonephritis.

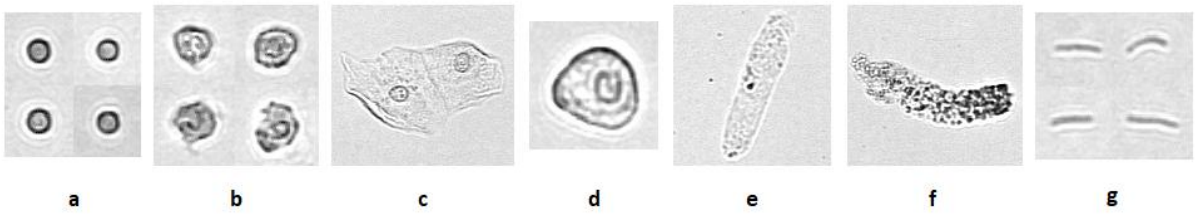

Figure 39 Selected findings of microscopic urinary examination a - erythrocytes, b - leukocytes, c - squamous epithelial cells, d - tubular epithelia, e - hyaline casts, f - granular casts, g - bacteria (rod-shaped)

Question 3.11.7 What serum levels of nitrogen waste products and minerals in acute renal failure are an indication for urgent hemodialysis?

Answer 3.11.7 When considering the patient's overall clinical condition and the possibilities of conservative treatment, the biochemical indications for hemodialysis are: creatininemia > 500 – 800 µmol/L, serum urea > 30 – 35 mmol/L, hyperuricemia > 1,000 µmol/L, hyperkalemia > 6.5 mmol/L, hypercalcemia > 4.5 mmol/L, severe metabolic acidosis.

3.12 Case 12 Basic examination of cerebrospinal fluid

A 4-year-old non-vaccinated boy was admitted to hospital due to fever and headache. The previous night he had woken up crying, vomited, did not want to eat, yesterday he was agitated, had a body temperature of up to 37 °C, in the evening, he vomited again. Yesterday he started to have a fever of approximately 38 °C, a strong headache, body rigidity and pain in the left lower limb. On admission, the child was dehydrated, had positive meningeal signs and neck opposition, a temperature of 37.5 °C, a pulse of 169/min, a blood pressure of 100/55 mm Hg and a respiratory rate of 28/min. The chest X-ray was without pathological findings; the CT scan of the head showed changes in the white matter and *pia mater* suspicious for meningitis, and maxillary sinus filling typical for sinusitis. A hematological examination detected significant leukocytosis and neutrophilia; the biochemical results were:

Laboratory marker	Result	Reference range	Units
Minerals in serum			
Sodium	137	132 – 144	mmol/L (1 m. – 15 y)
Potassium	3.9	3.5 – 5.1	mmol/L
Calcium	2.29	2.20 – 2.75	mmol/L (2 – 15 y)
Chlorides	106	101 – 109	mmol/L
Phosphates	1.45	1.29 – 2.26	mmol/L (0 – 15 y)
Horčík	0.85	0.77 – 1.03	mmol/L
Calcium ionised	1.192	1.050 – 1.450	mmol/L (0 – 15 y)
Serum			
Glucose	5.8	3.3 – 5.6	mmol/L (0 – 15 y)
Creatinine	26	23 – 68	µmol/L (1 – 15 y)
Urea	4.2	1.8 – 6.4	mmol/L (6 w – 15 y)
Total protein	68.0	57.0 – 80.0	g/L (1 m – 18 y)
Albumin	34.4	35.0 – 52.0	g/L
Bilirubin - total	9.3	5.0 – 21.0	µmol/L
Bilirubin - conjugated	2.5	0.1 – 3.4	µmol/L
AST	0.10	< 0.85	µkat/L
ALT	0.18	< 0.85	µkat/L
GMT	0.12	< 0.37	µkat/L (1 – 12 y)
Inflammatory markers			
CRP	327.2	< 5.0	mg/L
Procalcitonin	55.07	< 0.50	µg/L
Imunochemical testing			
IgG	8.25	5.40 – 18.22	g/L
IgA	1.46	0.21 – 2.91	g/L (1 – 12 y)
IgM	1.55	0.41 – 1.83	g/L (1 – 12 y)

Question 3.12.1 Interpret the results of serum biochemical testing. Which further examinations would you recommend?

Answer 3.12.1 We detected increased serum levels of inflammatory markers, which, together with fever, leukocytosis and neutrophilia, are indicative of an ongoing severe bacterial infection (CRP > 50 mg/L, PCT > 10 µg/L). Hemoculture was positive and confirmed the presence of *Haemophilus species*.

Question 3.12.2 Which further investigations should be carried out for definitive proof of meningitis?

Answer 3.12.2 It is necessary to perform a lumbar puncture and examination of the cerebrospinal fluid (biochemical and microscopical).

When cerebrospinal fluid was withdrawn by lumbar puncture, the physician observed its cloudy appearance. The results of biochemical and microscopic examination of the CSF were:

Laboratory marker	Result	Reference range	Units
Cerebrospinal fluid chemically			
Glucose	1.8	1.8 – 4.6	mmol/L (0 – 16 y)
Total protein	1.94	0.100 – 0.450	g/L (0 – 15 y)
Chlorides	117	113 – 131	mmol/L
Albumin	0.95	0.09 – 0.21	g/L
IgA	9.350	< 5.000	mg/L
IgG	82.520	< 34.000	mg/L
IgM	16.547	< 1.300	mg/L
Lactate	4.89	1.10 – 1.80	mmol/L (0 – 15 y)
Cerebrospinal fluid microscopy			
Erythrocytes	present	< 5	/3
Granulocytes	9024	0 – 1	/3
Lymphocytes	112	< 5	/3

Question 3.12.3 Does the result of CSF examination correlate with the clinical and other findings? Can this examination be helpful to differentiate between bacterial and other types of meningitis?

Answer 3.12.3 Yes, the patient's CSF findings - marginally low glucose concentration, elevated albumin, protein, lactate, and increased number of segmented leukocytes are typical for bacterial meningitis. The finding was also confirmed by the microbiological examination of liquor, which confirmed the positivity of Haemophilus influenzae type b. Virological examinations for EBV, CMV, HSV, mycoplasma and chlamydia were negative.

The examination of CSF in patients with meningitis is inevitable to differentiate bacterial and viral etiology. In bacterial meningitis, **CSF glucose concentration** (glycorrhachia) tends to be decreased due to its consumption by bacteria and leukocytes. In the assessment of glycorrhachia, the comparison with serum glycemia has an outstanding diagnostic value. The glucose quotient (Q_{glu}), calculated as the ratio of serum and CSF glucose, is normally approximately 0.6, while in bacterial meningitis, it is typically decreased to < 0.4 (our patient: $\mathbf{Q_{glu}} = \frac{\text{Glucose CSF}}{\text{Glucose serum}} = \frac{1.6\ \text{mmol/L}}{5.8\ \text{mmol/L}} = \underline{\mathbf{0.28}}$). Increased **total CSF protein** (hyperproteinorhachia) is typical of the inflammatory process. In our case, it suggests a bacterial etiology (discriminatory value for distinguishing bacterial from serous meningitis is 1 g/L). Similarly, a **CSF lactate** level > 4.2 mmol/L differentiates bacterial meningitis from viral meningitis. The microscopic finding of a greatly increased number of **neutrophilic granulocytes** (also called "segmented" because of the segmented nucleus) is a sign of bacterial purulent meningitis. A significant increase in the number of **CSF lymphocytes** is usually associated with a viral or aseptic etiology of inflammation. The extent of the blood-brain barrier disruption is reflected by an increased **concentration of albumin**, which penetrates the damaged barrier. In meningitis with severe barrier disruption, we usually find a decrease in the **concentration of CSF chlorides**. The presence of an increased concentration of **immunoglobulins** correlates with the ongoing inflammatory process.

Question 3.12.4 In what other pathological conditions do we detect hyperproteinorhachia, hypoglycorhachia, and increased lactate concentration in a patient's CSF?

Answer 3.12.4 Normally, about 80% of total CSF protein is of serum origin (reference range in adults: 0.1–0.49 g/L). **Hyperproteinorhachia** is present in blood-brain barrier disruption, release from disintegrated CNS cells, neoplastic processes and so-called intrathecal protein synthesis in pathological immune system activation. An artificial increase of protein concentration also appears with bleeding into the CNS ventricles or subarachnoid space. To ensure the correct interpretation of the results, for every 1,000 CSF erythrocytes, we need to subtract 10 mg/L from the measured total protein level.

Due to the diffusion from serum, the CSF level of glucose normally accounts for approximately 60% of glycemia (2.7–4.4 mmol/L in adults; Q_{glu} = 0.6). The CSF level of lactate is independent of plasma concentration, and is exclusively a product of anaerobic glycolysis in

CNS (1.5–2.1 mmol/L in adults). **Hypoglycorrhachia and elevated CSF lactate** are typically associated with increased metabolic turnover in bacterial meningitis, impaired cerebral oxygen supply, tumours, epilepsy and subarachnoideal hemorrhage.

Question 3.12.5 What is the physiological finding of CSF cytology? Why are the reference ranges for cell counts reported as the numeral/3 ?

Answer 3.12.5 Under normal conditions, a total of less than 10/3 cells are present in CSF, commonly termed as oligocytosis. From those, the mononuclear cells are predominant, 65–80% of which are lymphocytes. The counting of the cells is carried out in a Fuchs-Rosenthal chamber after staining the CSF using Türk's solution (gentian violet) or fuchsin in a conventional optical microscope at 400-fold magnification. Cells are counted in 16 large square fields of a total volume of 3 µl, therefore the number of cells/3 is given in the result. If necessary, this can be converted to cells/1 microliter. However, it is important to note that this is an ''orientative'' cytological examination. The cells present cannot be much detailed, since the staining and methodology used do not show the cytoplasmic granulations or the cell nuclear structures. For a precise classification of the CSF cellular content, we can use a centrifugation cytology, when the permanent slide is prepared by the cytosedimentation method. This provides a more detailed picture of the cellular abnormalities, leukocyte cell lineages, etc.

Question 3.12.6 The child was given intravenous antibiotic, analgesic and supportive therapy. In the table below, we can see the gradual dynamics of changes in serum inflammatory markers levels. Was the therapy effective?

Inflammatory markers									
Laboratory marker	**Result (time of hospitalisation)**							**Reference range**	**Units**
Serum	1. day	2. day	4. day	8. day	12. day	15. day	20. day		
CRP	327.2	176.8	43.9	52.0	50.1	30.3	10.0	< 5.0	mg/L
Procalcitonin	55.07	34.02	7.84	0.75	0.16	0.14		< 0.50	µg/L

Answer 3.12.6 Yes, due to the gradual decrease in both CRP and PCT levels and the remission of clinical symptoms, the therapy was effective.

3.13 Case 13 Examination of pleural punctate

A 45-year-old male patient with hypertension, type 2 diabetes mellitus and heart failure, a long-term smoker, was hospitalised for fluidothorax l.dx. A cardiac origin of the fluidothorax was excluded. Due to the observed elevation of inflammatory markers (leukocytosis, neutrophilia, CRP 198.4 mg/L), the patient was given empirical antibiotic treatment. Due to the progression of fluidothorax (CT scan), the patient underwent pleural puncture. The punctate of cloudy appearance and unpleasant odour was sent for microbiological analysis (culture + sensitivity), and was also examined in the clinical-biochemical laboratory.

Laboratory marker	**Result**	**Reference range**		**Units**
Punctate		***Exudate***	***Transudate***	
pH	**6.8**	< 7.4	7.4	
Glucose	**0.6**	< 1.7	like plasma	mmol/L
Total protein	**38.4**	> 30	< 30	g/L
Cholesterol	**7.32**	> 1.15	< 1.15	mmol/L
LD	**109.6**	> 5.3	< 5.3	µkat/L
AMS	0.01	< 1.66	< 1.66	µkat/L
Rheumatoid factor	4.0	0.0 – 5.4	0.0 – 5.4	kU/L
Erythrocytes	massive	< 1	< 1	/µL
Leukocytes	**28 000**	> 100	< 100	/µL

Question 3.13.1 Using the results of biochemical and microscopical examination, are we able to discriminate whether the punctate is exudate or transudate? Explain the causes of differences in the composition of these body fluids.

Answer 3.13.1 According to the low pH, low glucose level, high concentration of total protein and cholesterol, high lactate dehydrogenase activity and greatly increased number of leukocytes, we can conclude that this punctate is an exudate. In our case, the patient had a chest empyema when glucose levels are typically approaching zero, pH is < 7, and total protein and cholesterol levels can even reach the plasma levels. **Transudate** is produced by the ultrafiltration of plasma, but the concentration of proteins, as well as lipids and enzymes bound to them, which are more difficult to penetrate from plasma, is lower. **Exudate** has an inflammatory origin (infection, tumour), and it is formed by the passage of substances through a damaged and thus more permeable capillary wall. Therefore the exudate composition resembles plasma, but through the activity of inflammatory or tumour cells, the concentration of certain substances is further reduced (pH, glucose) or increased (lactate).

The patient subsequently underwent thoracoscopy, exudate evacuation, pleural cavity lavage and drainage followed by targeted antibiotic treatment.

Question 3.13.2 Which other biochemical markers can be used to differentiate exudate from transudate?

Answer 3.13.2 The exudate is typically yellow, cloudy, with high concentrations of albumin (> 12 g/L), triacylglycerols (> 0.5 mmol/L), lactate (> 1.85 mmol/L), and fibrinogen positivity. In a bacterial infection, numerous neutrophilic granulocytes are typically present. In malignant processes, high erythrocyte counts and abnormal tumour cells are often detected, and these can be further specified by cytologic examination. If the inflammatory process is not extensive, the permeability of the capillary wall is increased or the transudate becomes infected, the biochemical examination can identify intermediate forms between the transudate and exudate, with no reliable differentiation between them.

For equivocal findings, in addition to the above differentiation markers, we can use the **Light's criteria for exudate**: the ratio of $\frac{\text{Total protein in exudate}}{\text{Total protein in serum}} > 0.5$ and the LD activities ratio $\frac{\text{LD exudate}}{\text{LD serum}} > 0.6$. **Albumin gradient** is calculated as the difference of albumin concentration in exudate and serum, with the exudate value > 12 g/L.

Question 3.13.3 Why do we need to determine the amylase activity in the punctate ?

Answer 3.13.3 Amylase activity in ascitic fluid is significantly increased in cases with pancreatitis, when it is many fold higher than physiological AMS activity in serum.

3.14 Case 14 Differentiation of body fluids

A 55-year-old male patient came for an outpatient examination in the springtime due to his watery nasal secretion that had lasted for about 2 – 3 days. It was a clear, odourless fluid dripping from his nose; he collected the fluid in a quantity of about 2 ml and brought it to the hospital, claiming that it was "fluid from the brain". The patient came on his own, was not dizzy, did not have any injuries and operations on the head and face, did not report any allergies, and had been taking medication containing levocetirizine a couple of years ago. Objectively, the patient was neurologically normal, oriented; the physical examination, including the head, was without any pathological findings, his body temperature was 36.4 °C. A dripping, clear nasal secretion was obviously present. The treating physician consulted the laboratory regarding the indication of biochemical markers which should be examined from the fluid to differentiate between rhinorrhea vs. liquorrhea. Based on the history and physical examination, the physician had rather suspected allergic rhinorrhea, and the biochemical examination should have confirmed his decision.

Question 3.14.1 Evaluate the results of the fluid examination and comment on its biochemical composition and origin. When the examination was indicated, the sample was submitted to the laboratory system as CSF, to be able to check the proper reference values.

Laboratory marker	Result	Reference range		Units
		Cerebrospinal fluid	***Nasal secretion***	
Glucose	**0.2**	2.7 – 4.4	< 0.05	mmol/L
Total protein	**4.08**	0.1 – 0.49	3.0 – 40.0	g/L
Chlorides	226	113 – 131		mmol/L
Albumin	1.27	0.09 – 0.21		g/L
Lactate	0.83	1.5 – 2.1		mmol/L
Potassium	**30**	< 3.0	> 17	mmol/L

Answer 3.14.1 According to the results of the biochemical examination, this is certainly a secretion from the nasal mucosa (rhinorrhoea), as evidenced by significantly higher levels of total protein and potassium compared to the CSF, as well as significantly low to almost zero glucose levels. Also, a chloride level of 226 mmol is highly unlike for CSF, in which they are normally present in approximately halved concentrations. Moreover, such CSF chlorides elevation would be accompanied by a severe blood-brain barrier disruption, with obvious clinical manifestation of CNS disease.

Question 3.14.2 Which other specific markers could help differentiate rhinorrhoea and liquorrhoea in this case?

Answer 3.14.2 Through the electrophoretic separation of CSF proteins, we can typically detect carbohydrate-deficient transferrin (CDT), formed by the cleavage of transferrin sialic acid using brain neuraminidase. In addition, the CSF can also be confirmed by the presence of the so-called β-trace protein (prostaglandin D synthase), which is synthesised in the brain leptomeninges and choroid plexus, and its CSF concentration is much higher than in serum.

Question 3.14.3 The examination of which markers allows us to distinguish urine from peritoneal or ascitic fluid?

Answer 3.14.3 In particular, urine contains many-fold higher concentrations of urea and potassium, which in ascitic fluid are very similar to serum concentrations.

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