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2 PATHOPHYSIOLOGY LAB NOTES

FOR MEDICAL STUDENTS

GHIDURI ȘI ÎNDRUMĂTOARE DE LABORATOR

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FOREWORD

In recent years, the curriculum of medical schools has undergone a series of innovative changes, the main objective being that of forming competent doctors capable of scientific reasoning based on permanently updated information.

Thus, the study of Pathophysiology provides the knowledge necessary for understanding the causes and mechanisms underlying the occurrence and evolution of the pathological processes, as well as the production of the functional imbalances induced by disease.

The "*Pathophysiology Lab Notes for Medical Students II"* book is structured in such a way as to allow the learning of the basic principles of disease etiopathogenesis as well as the main current methods of their investigation. The present body of work corresponds to the didactic concept of our team to structure, in a systematic and updated way, the laboratory analyses and paraclinical investigations used in current clinical practice.

Each chapter specifies the learning objectives proposed in order to obtain a unitary vision on the concept of disease and to develop an analytical medical reasoning, essential in preparing students for clinical practice. The multiple choice questions and the representative case studies that conclude the chapters aim to evaluate the degree of understanding of the significance of investigations corresponding to the studied pathology and of their pathological changes interpretation in order to formulate the paraclinical diagnosis. Last but not least, the manner in which the information systematized in this handbook is presented during the practical laboratory aims to stimulate the interactive aspect of communication with students, in order to correlate the mechanisms of disease production (presented in the lecture) with the laboratory and paraclinical changes that they induce (exemplified by case studies).

We hope that this teaching material will be regarded as useful in shaping medical reasoning and will be of considerable help to students during university studies.

Sincere thanks to all who will use the learning instrument that we propose, being open to any suggestions that could lead to the continuous improvement of the material and having the belief that a scientific field becomes truly fertile when readers and authors engage in a constructive dialogue.

The authors

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1. INVESTIGATION OF RED BLOOD CELL DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. *Request the common tests used in the laboratory diagnosis of anemias*
2. *Interpret the laboratory changes characteristic for the main types of anemia encountered in practice*
3. *List the changes of erythrocyte morphology present on the peripheral blood smear in the main types of anemias encountered in practice*
4. *List the indications for the bone marrow smear and the common pathological changes it reveals in the most common types of anemia*
5. *Request special investigations necessary for the etiological diagnosis of anemia.*

Red blood cell disorders include anemias and polycythemias. In clinical practice anemias are more frequently encountered.

ANEMIAS

- **Definition:** anemias are pathological conditions defined by the decrease of hemoglobin (Hb) concentration, hematocrit (Ht) and/or of the number of erythrocytes below reference values.
- **Positive diagnosis:** is mainly based on the laboratory investigations
- **Stages of the diagnosis:**
  - The study of the red blood cell count and of the peripheral blood smear for morphological classification
  - Interpreting the reticulocyte count for the functional classification
  - The study of the bone marrow smear and special investigations for the etiopathogenic classification (described in the lecture)

I. COMPLETE BLOOD CELL (CBC) COUNT

The complete blood cell count is an important screening test, being the first step in establishing the hematological status.

The test is performed from the venous blood (collected on anticoagulant after fasting) using automated analyzers.

The **complete blood count** includes the following parameters:

- **Hemoglobin (Hb)**
- **Number of erythrocytes (Nr. E)**
- **Hematocrit (Ht)**
- **Red blood cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)**
- **Red Cell Distribution Width (RDW)**
- Number of leukocytes and the leukocyte formula
- Platelet count and platelet indices: mean platelet volume (MPV) and the platelet Distribution Width (PDW)

(The characterization of the last two parameters does not represent the objective of this chapter).

A. Hemoglobin concentration (Hb)

Hb is the **main parameter used for the diagnosis and severity assessement of anemias**. Together with the red blood cell indices and the analysis of the peripheral blood smear it allows the **MORPHOLOGICAL classification** of anemias.

Normal values:

- Men: 15,5 ± 2 g/dL
- Women: 13,5 ± 2 g/dL

Depending on the Hb value, the following degrees of anemia severity are defined:

- ***mild***: Hb = 10 – 11,5 g/dL for women, 10-13,5 g/dL for men, respectively
- ***moderate***: Hb = 8 – 10 g/dL
- ***severe***: Hb < 8 g/dL

B. Number of erythrocytes

The erythrocyte count is the basic test for the evaluation of erythropoiesis in the bone marrow (BM). The correct evaluation of the body's number

of erythrocytes can only be obtained in correlation with the hematocrit.

The number of erythrocytes is influenced by changes in plasma volume, hydrosaline retention leading to hemodilution while dehydration leads to hemoconcentration.

Normal values:

- Men: 4,5 – 6 millions/mm^3
- Women: 3,9 – 5 millions/mm^3

C. Hematocrit (Ht)

Ht or the **red blood cell to plasma ratio** measures the ratio between the volume occupied by erythrocytes and the total blood volume. The hematocrit depends on the mass of red blood cells, the mean corpuscular volume and plasma volume. Usually, when the red blood cells are of normal size, changes in hematocrit are consistent with those in erythrocyte number.

Normal values:

- Men: 45 ± 5 %
- Women: 42 ± 5 %

Observation!

Fast Ht evaluation follows "the rule of 3": (Hb (g/dL) x 3 = Ht (%). Ex., if Hb =10 g/dL x 3, then Ht = 30%.

Remember!

- In *acute post-haemorrhagic anemias*, the number of erythrocytes and hemoglobin concentration remain unchanged in the first hours due to concomitant plasma loss; they begin to decrease as the correction of the volemia deficit occurs.
- In *chronic anemia*, blood volume is almost normal due to the compensatory increase in plasma volume and the number of erythrocytes and the hematocrit are usually low.
- *Relative anemia* is a condition characterized by a normal number of red blood cells but increased blood volume due to increased plasma volume, which may be: i) *physiological* (eg in pregnancy - when the Hb concentration drops by 2-3 g/dL and may be associated with a relative hypoproteinemia due to hemodilution) or ii) *pathological* (eg, after excessive fluid infusion, cardiac failure, cirrhosis).

D. Red blood cell indices

They are used to assess the size and hemoglobin load of erythrocytes, which allows the morphological classification of anemias. In practice, the following erythrocyte indices are determined:

1. Mean corpuscular volume (MCV) - refers to the *average volume of an individual red blood cell*. The MCV is expressed in femtoliters (fl) or cubic micrometers (μm^3).

MCV = Ht (%) x 10 / Nr. erythrocytes (mill./mm^3)

***Normal values*:** 80 – 96 μm^3 (fl)

***Pathological changes**:*

- ***Decreased MCV*** (< 80 μm^3) defines **microcytic anemia**: e.g., iron deficiency anemia, sideroblastic anemia, chronic disease anemia, thalassemia
- ***Increased MCV*** (> 96 μm^3) defines **macrocytic anemia** which can be: **megaloblastic** (vitamin B12 or folic acid deficiency anemia) or **normoblastic** (severe hemolytic and posthemorrhagic anemias - due to reticulocytosis; anemia in chronic liver disease - !alcoholism; hypothyroidism).

Observation!

A normal MCV (80 – 96 μm^3) in the presence of anemia defines **normocytic anemia:** e.g. posthemorrhagic anemias, anemia due to chronic inflammation, myelodysplastic sdr..

2. Mean corpuscular hemoglobin (MCH) - refers to the *mean hemoglobin content per red blood cell*. It is expressed in picograms (pg).

MCH = Hb(g/dl) x 10/Nr. erythrocytes(mill./mm^3)

Normal values: 27 – 32 pg/E

3. Mean corpuscular hemoglobin concentration (MCHC) - measures the mean Hb concentration from a given volume of erythrocytes. It is calculated as the ratio between the amount of Hb and the volume of red blood cells.

MCHC = Hb (g/dL) x 100 / Ht (%)

Normal values: 32 – 36 g/dL

***Pathological changes**:*

- ***The decrease of MCH*** (< 27 pg/E) and of ***MCHC*** (< 32g/dL) defines **hypochromic**

anemia and is induced by the causes listed for microcytic anemia, being determined by the decrease in Hb synthesis.

Observation!
MCHC increase occurs when erythrocytes become spherocytes, e.g. in hereditary microspherocytosis (the spherocytes do not have the pale central area).

4. The Red Cell Distribution Width (RDW) - is an erythrocyte index that quantifies the variation in erythrocyte size (volume), namely the degree of **anisocytosis**. It is calculated as a variation coefficient using the MCV and expressed as a percentage.

Normal values: 11 – 15%

Pathological changes*:*

- ***RDW increase*** means that the red blood cells vary a lot in size. To determine what the possible cause of a high RDW level is, a comparison is made to the MCV:
  - **RDW increased with decreased MCV** (microcytosis) suggests: iron deficiency anemia, thalassemia, hemolytic anemia (due to the presence of schizocytes)
  - **RDW increased with increased MCV** (macrocytosis) suggests: folate deficiency or vitamin B12 deficiency, liver disease, haemolytic or posthemorrhagic anemia (due to increase in reticulocytes which have a large volume)
  - **RDW increased with a normal MCV** suggests: deficiency of iron, B12 or folate in the early stages.

Observations!
An increased RDW may occur early in iron deficiency.
A decrease of RDW below 10.2% indicates a *minimal* variation in erythrocyte size and may occur both in iron deficiency anemia (most of the erythrocytes are small) as well as in macrocytic anemia (most erythrocytes are large).

II. THE PERIPHERAL BLOOD SMEAR

If Hb drops below **8 g/dL**, it is recommended to perform the peripheral blood smear, stained May-Grünwald-Giemsa, which allows highlighting changes in the size, shape and color of erythrocytes as well as the presence of erythrocyte inclusions (Tab.1.1).

Table 1.1. Pathological changes of erythrocytes on the peripheral blood smear.

Erythrocyte modification	Description	Causes
Changes in SIZE or ANISOCYTOSIS		
Microcytosis	Mature erythrocytes of a lower than normal size (MCV < 80 fl). Physiological microcytosis does not exceed 20% of the total number of erythrocytes.	Iron deficiency anemia Sideroblastic anemia Thalassemias
Macrocytosis	Mature erythrocytes of a higher than normal size (MCV > 96 fl). Physiological macrocytosis does not exceed 20% of the total number of erythrocytes.	Folic acid deficiency anemia (anemia from alcoholic liver disease) Macrocytic/megalocytic anemias Hemolytic anemias (due to reticulocytosis) Vitamin B12 deficiency megaloblastic anemia (Addison-Biermer pernicious anemia)
Changes in SHAPE or POIKILOCYTOSIS (from Gr., poikilos = different)		
Spherocytes	Spherical erythrocytes	Hereditary microspherocytosis
Drepanocytes/sickle cells	Sickle shaped erythrocytes	Drepanocytosis (sickle cell anemia)
Acanthocytes („spur cells")	Erythrocytes with an irregular surface (3-5 large spicules)	Chronic liver disease (cirrhosis) Hepatorenal syndrome
Echinocytes („burr cells")	Erythrocytes that present on their surface numerous regular small growths	Chronic liver disease (cirrhosis) Chronic kidney disease/Uremia

Erythrocyte modification	Description	Causes
	(numerous spicules)	Hemolytic anemias
"Target cell" erythrocytes/ codocytes	Erythrocytes with a particular Hb distribution in the center and the periphery, between which there is a clear area	Severe thalassemia
Schizocytes/schistocytes	Fragments of erythrocytes (triangle or helmet-shaped)	Hemolytic anemias, DIC
Changes in COLOUR		
Hypochromia	Presence of pale erythrocytes with a low Hb load on the peripheral blood smear	Iron deficiency anemia Sideroblastic anemia Thalassemias
Anisochromia	Presence on the same blood smear of hypochromic as well as normochromic erythrocytes	
Polychromatophilia/ polychromasia	The presence on the peripheral blood smear of young, immature (reticulocyte) erythrocytes that appear as blue-gray colored macrocytes because they contain residual nuclear material.	Their presence in large numbers is found in: – haemolytic anemias (where reticulocytosis is the rule!) – fibrosis or tumor infiltration of the BM (normal reticulocyte count)
Erythrocyte INCLUSION BODIES		
Jolly bodies	Round, dense, dark blue/purple granulations representing nuclear chromatin condensations pathologically remaining in erythrocytes	Megaloblastic anemias Thalassemias
Basophilic granules	Small, blue or purple granules spread over the entire surface of erythrocytes, consisting of ribosomal aggregates	Lead poisoning (saturnism) Thalassemias
Cabot rings	Purple red filament-like formations representing debris of the mitotic spindle that have not been reabsorbed	Megaloblastic anemias Severe hemolytic anemias
Heinz bodies	Intraerythrocytic aggregates of Hb that has been subjected to oxidative denaturation	Hemoglobinopathies Glucose-6-phosphate dehydrogenase deficiency anemia

DIC = disseminated intravascular coagulation

MORPHOLOGICAL classification of anemias

Depending on the values of the erythrocyte indices and the morphological aspect of the erythrocytes on the peripheral blood smear three types of anemias can be described (Tab.1.2).

Table 1.2. Morphological classification of anemias.

Morphology	Anemia type	Etiological mechanism
Microcytic hypocromic anemia – low MCV, MCH, MCHC – small and pale erythrocytes on the blood smear	Iron-deficiency anemia Sideroblastic anemia Thalassemias	Iron deficiency in the body Deficient heme synthesis Deficient synthesis of α/β globin chains
Macrocytic normochromic anemia – increased MCV – normal MCH and MCHC – large erythrocytes with no central pale area (uniform Hb distribution)	Vitamin B12 and folic acid deficiency anemia Hemolytic anemias accompanied by reticulocytosis Anemia of chronic liver disease (alcoholism)	Vitamin B12 and folic acid deficiency

Normocytic normochromic anemia – normal MCV, MCH, and MCHC – normal erythrocytes on the blood smear, with classical central pale area (Hb distribution at the periphery)	Acute post-hemorrhagic anemia Anemia of chronic inflammation Anemia in chronic kidney disease Hemolytic anemias Aplastic/hypoplastic anemia	Acute bleeding Iron sequestration in macrophages Decreased erythropoietin synthesis Early destruction of erythrocytes Deficit of medullary erythropoiesis

III. THE RETICULOCYTE COUNT

Reticulocytes (Rt) are circulating erythrocyte precursors that mature in the spleen and their evaluation provides information on the ability of the bone marrow to produce erythrocytes in response to anemia. Determining the number of reticulocytes allows:

- **The functional classification** of anemias into: *regenerative* and *hypo-/ aregenerative.*
- **The monitoring of the response to substitution treatment** with *iron, folic acid* or *vitamin B12*.
- **Erythropoiesis evaluation after bone marrow transplantation** or after erythropoietin treatment.
- **Normal values:** 0,5 – 2,5% (of the number of erythrocytes)

Remember!
In the presence of **anemia**, the % of Rt should be corrected to the patient's low red blood cell count:

% Rt corrected **(Rt_c)= %Rt x Ht / 45**
(where 45 = normal Ht)

Interpretation:

- $\%Rt_c \geq 3\%$ = **regenerative** anemia (efficient erythropoiesis)
- $\%Rt_c < 3\%$ = **hypo-/aregenerative** anemia (inefficient erythropoiesis)

<u>FUNCTIONAL</u> classification of anemias: Depending on the ***number of reticulocytes***, that represent the *most accurate indicator of bone marrow hematogenic production*, anemias can be:

- **Regenerative**, accompanied by **reticulocytosis** (increased reticulocyte count):
  - hemolytic anemias
    ! Reticulocytosis is *the main difference* between anemias due to increased hemolysis and anemias due to decreased erythropoesis
  - anemias due to acute blood loss (7 days after an acute hemorrhage)
  - pernicious anemia (7 - 14 days after receiving vit. B12 treatment)
  - iron deficiency anemia (7 - 10 days after starting iron therapy)
  - chronic kidney disease after erythropoietin treatment
- **Hypo-/aregenerative**, accompanied by **reticulocytopenia** (low reticulocyte count):
  - hypo-/aplastic anemias
  - anemia in chronic inflammatory diseases (rheumatoid arthritis, Crohn's disease)
  - anemia in chronic kidney disease prior to the introduction of recombinant erythropoietin treatment
  - deficiency anemias (iron, vitamin B12, folic acid deficiency) before the initiation of substitution treatment.

IV. THE BONE MARROW SMEAR

The bone marrow smear is obtained by sternum or posterior iliac crest puncture with aspiration (suction) of the medullary content.

A. INDICATIONS FOR BONE MARROW SMEAR EVALUATION

- Assessment of bone marrow cellular status:
  - global cellular hypoplasia: hypo-/aplastic anemia
  - erythrocyte precursors hyperplasia: haemolytic and posthemorrhagic anemia
- Evaluation of the granulocyte-erythroblastic ratio:
  - *normal*: the ratio between granulocyte and erythrocyte precursors = 3/1

- *an equal or reversed ratio*: erythrocyte hyperplasia (haemolytic and post-hemorrhagic anemias)
- *increased ratio*: erythrocyte hypoplasia (aplastic anemia) *or* granulocytic hyperplasia (myeloid leukemias, leukemoid reactions)

- Examination of bone marrow iron deposits in sideroblasts and macrophages by the Perls stain (with Prussian blue):
  - normal: 20-60% sideroblasts
  - low deposits: iron deficiency anemia
  - increased deposits: sideroblastic anemia
- Highlighting bone marrow infiltration with neoplastic cells (leukemias and bone marrow metastases) or with plasma cells (multiple myeloma).

B. PATHOLOGICAL FINDINGS

- **Bone marrow hypocellularity***:* occurs in hypoplastic/aplastic anemias due to dysfunction, suppression or destruction of stem cells, resulting in decreased production of one or all cell lines (pancytopenia). The etiology of bone marrow hypocellularity is in 2/3 of cases idiopathic, in the remaining cases the etiological factors incriminated being: radiation, chemical agents (benzene, insecticides), drugs (chloramphenicol, cytostatics, etc.).

- **Bone marrow hypercellularity** is recognized as an increased cellularity and it is due either to hyperplasia or neoplasia:
  - **Bone marrow hyperplasia** can be:
    - an erythrocyte hyperplasia under conditions of erythropoiesis stimulation (e.g. due to chronic hypoxia) or increased peripheral destruction (in hemolytic anemias)
    - a granulocytic hyperplasia in severe, persistent infections
    - a megakaryocyte hyperplasia in peripheral platelets destruction
  - **Bone marrow neoplasia** occurs in the case of leukemias and bone marrow metastases of other cancers.

V. SPECIAL LABORATORY TESTS IN THE MAIN TYPES OF ANEMIAS

A. IRON METABOLISM

1. Serum iron

- **Normal values:**
  - Men: 60 – 160 µg/dL
  - Women: 50 – 150 µg/dL
- **Low values:** iron deficiency anemia
- **High values:** sideroblastic anemia

2. Serum Total Iron Binding Capacity (TIBC) - the amount of circulating transferrin

- **Normal values:** 250 - 400 µg/dL
- **Low values:** sideroblastic anemia
- **High values:** iron deficiency anemia

3. Transferrin saturation: ratio between serum iron / TIBC.

- **Normal values:** 20 - 45%
- **Low values:** iron deficiency anemia
- **High values:** sideroblastic anemia

4. Serum ferritin: the *readily mobilizable* form of iron storage in the body and correlates with the **iron deposits** of the body. Ferritin is the **main indicator** used in assessing them - *in the absence of hepatic parenchymal diseases* (eg, cirrhosis, hepatocellular carcinoma, liver metastases) that are consistently accompanied by elevated levels of serum ferritin (correlated with disorder severity).

- **Normal values:**
  - Men: 30 – 300 ng/mL
  - Women: 15 – 200 ng/mL
- **Low values:** iron deficiency anemia
- **High values*:*** sideroblastic anemia

***Remember*!**

- A ferritin decrease **below 15 ng/mL** indicates **iron deficiency** in the body.

The differential diagnosis of microcytic anemias is presented in Tab.1.3.

Table 1.3. Differential diagnosis of microcytic anemias

Test	Iron deficiency anemia	Sideroblastic anemia	Thalassemia	Anemia in chronic disease
Serum iron	↓	↑	Normal	↓
TIBC	↑	↓	Normal	↓
Transferrin saturation	↓	↑	Normal	Normal or ↑
Serum ferritin	↓	↑	Normal	Normal or ↑
Bone marrow iron	Absent	Present	Present	Present
Iron in erythroblasts	Absent	Present Ringed sideroblasts	Present	Low or absent

B. VITAMIN B_{12} AND FOLIC ACID

1. Serum vitamin B_{12} (cobalamin) measurement

- Normal values: 160 - 925 ng/L

Serum levels are reduced below 160 ng/L in pernicious anemia.

Holotranscobalamin is the "active" fraction of cobalamin, and its measurement may be a more relevant marker for vitamin B_{12} deficiency than B_{12} serum dosage.

2. Serum folic acid measurement

- Normal values: 4 - 18 µg/L

In folate deficiency (deficient diet in the elderly, chronic alcoholism), the serum level is below 3 µg/L. Chronic alcohol-related anemia is macrocytic or megaloblastic, as alcohol has a toxic effect on erythropoiesis. In ethanolic liver diseases, macrocytosis may be present in the absence of anemia.

3. Serum level of metabolites: homo-cysteine (HCY) and **methyl-malonic acid (MMA)** both increase parallel to the severity of ***vitamin B_{12} deficiency*****.** In ***folic acid deficiency <u>only</u>*** *the* ***serum homocysteine*** *level is increased*.

4. Anti-intrinsic factor (IF) and anti-parietal cell antibodies

Anti-IF antibodies are present in the serum in 50% of patients with pernicious anemia (Addison-Biermer) caused by IF deficiency, being **specific for the diagnosis.**

Anti-gastric parietal cells antibodies are present in 90% of patients with pernicious anemia (but also in 10% of normal individuals).

5. Increase in bilirubin and LDH - reflects an increase in inefficient erythropoiesis (increase in the number of destroyed erythroblasts).

Remember!

In megaloblastic anemias, the peripheral blood smear shows macrocytes/macro-ovalocytes in association with **nuclear hypersegmentation of neutrophils** (≥ 6 lobes).

C. PATHOLOGICAL HEMOLYSIS EVALUATION

Hemolytic anemias are characterized by **increased rate of red blood cells destruction** (shortened survival in the circulation), responsible for **compensatory bone marrow hyperplasia** in the erythrocyte line and **reticulocytosis** (Tab. 1.4).

- **Extravascular hemolysis** is *frequent* and occurs in **tissue macrophages** of the **spleen, liver** (previously called the reticuloendothelial system - RES), **BM** and has the following characteristics:
  - Increased level of **indirect (unconjugated) bilirubin** that does not appear in the urine (acholuric jaundice)
  - Increased serum lactate dehydrogenase (LDH) from lysed erythrocytes
- **Intravascular hemolysis** is *rare* and is characterized by:
  - presence of **free Hb** in plasma = **hemoglobinemia**
  - *decreased* level of serum **haptoglobin** (globulin synthetized by the liver that binds free Hb) due to phagocytosis of the haptoglobin-Hb complex by RES macrophages
  - part of the free Hb is oxidized into **methemoglobin,** which will dissociate into heme and globin
  - decrease of serum **hemopexin**, a protein that binds heme
  - **methemalbuminemia** formed by binding of the oxidized heme to albumin (in conditions of decreased hemopexin). In plasma

spectrophotometry, methem-albumin forms a characteristic band, evidenced by the Schumm test.
- significant increase in serum **lactate dehydrogenase (LDH)** due to erythrocyte lysis
- presence of hemosiderin in urine = **hemosiderinuria**, identified in urinary sediment by Perls reaction
- the presence of free Hb in the urine = **hemoglobinuria** (in severe forms).

D. Hb ELECTROPHORESIS (ELFO)

Is used in the diagnosis of hemoglobinopathies to identify hemoglobins with abnormal structure/ function or present in increased amounts:
- HbS identified in sickle cell anemia
- HbF and HbA2 increased in beta-thalassemia

Table 1.4. Laboratory diagnosis of hemolysis.

	Extravascular	Intravascular
HEMATOLOGIC PARAMETERS		
Peripheral blood smear	Polychromatophilia	Polychromatophilia
Reticulocyte count	↑	↑
Bone marrow evaluation	Erythrocyte hyperplasia	Erythrocyte hyperplasia
PLASMA/SERUM		
Indirect bilirubin	↑	↑
Haptoglobin	↓	Absent
Hemoglobin	Normal / ↑	↑↑↑
Lactate-dehydrogenase (LDH)	↑	↑↑↑
URINE		
Urobilinogen	↑	↑
Bilirubin	Absent	Absent
Hemosiderin	Absent	Present
Hemoglobin	Absent	Present in severe cases

E. THE COOMBS TEST

It is used in the diagnosis of **immune-hemolytic anemias** in which premature hemolysis occurs, caused by ***anti-erythrocyte antibodies***. This test is based on the property of the antiglobulinic serum (which contains antibodies obtained from the rabbit after human globulin injection) to agglutinate erythrocytes on the surface of which anti-erythrocyte antibodies are present.
There are **2 types:**

- **Direct Coombs test:** identifies anti-erythrocyte antibodies **bound to erythrocytes**. It is based on the property of a polyvalent serum (anti-IgG, anti-IgM) to agglutinate the patient's erythrocytes if they are coated with antibodies.
- **Indirect Coombs test:** identifies **free serum anti-erythrocyte antibodies** that react against erythrocytes.

CHECKPOINT!

1. Which of the following belong to the microcytic hypochromic anemias category?
A. Iron-deficiency anemia
B. Sideroblastic anemia
C. Aplastic anemia
D. Thalassemia
E. Pernicious anemia

***2. The term that indicates an unequal size of erythrocytes is:**
A. Anisocytosis
B. Hypochromia
C. Anisochromia
D. Polychromatophilia
E. Poikilocytosis

3. In which of the following anemias does the Hb electrophoresis become modified?
A. Pernicious anemia
B. Thalassemia
C. Aplastic anemia
D. Hereditary microspherocytosis
E. Sickle-cell anemia

4. In which of the following does reticulocytosis occur?
A. Iron treatment in iron-deficiency anemia
B. Vitamin B12 treatment in pernicious anemia
C. Aplastic anemia
D. Anemia of chronic inflammation
E. Anemia of chronic kidney disease

***5. In which type of anemia are the anti-intrinsic factor antibodies positive?**
A. Folic acid deficiency anemia
B. Aplastic anemia
C. Iron deficiency anemia
D. Pernicious anemia
E. Thalassemia

6. Which of the following anemias belong to the normochromic normocytic morphological type:
A. Aplastic anemia
B. Folic acid deficiency anemia
C. Acute post-hemorrhagic anemia
D. Hemolytic anemia
E. Sideroblastic anemia

***7. The Coombs test is used for the diagnosis of:**
A. Myelophthisic anemia
B. Pernicious anemia
C. Aplastic anemia
D. Sickle cell anemia
E. Autoimmune hemolytic anemia

***8. The term defining erythrocyte shape irregularities is:**
A. Anisocytosis
B. Anisochromia
C. Poikilocytosis
D. Microcytosis
E. Polychromatophilia

***9. Which of the following investigations is used to monitor the response to treatment with iron, folic acid or vitamin B12 in deficiency anemias?**
A. Hematocrit
B. Serum ferritin
C. Homocysteine (HCY)
D. Reticulocyte count
E. Bone marrow smear

***10. What characteristic does the bone marrow smear present in haemolytic anemias?**
A. Hyperplasia of the granulocyte line
B. Hyperplasia of the erythrocyte line
C. Megakaryocyte hyperplasia
D. Erythrocyte line hypocellularity
E. Pancytopenia

CASE STUDIES

1. A 28-year-old woman presented to her medical examination accusing dyspnea, palpitations and chronic fatigue, symptoms accentuated in the past 3 weeks. In the past 4 months she has also presented heavy menstrual bleeding (menorrhagia).

Complete blood count:

Leukocyte count = 6500 /mm^3
RBC = 3 mil./mm^3
Hemoglobin = 9 g/dl
Ht = 30%
MCV = 68 fl
MCH = 25 pg
MCHC = 30 g/dl
Platelets = 160000/mm^3 (N.V. = 150,000-400,000/mm^3)

What diagnosis do you suspect?
What additional investigations would be justified?
Provide arguments for your answer.

……

……

……

……

2. A 59-year-old female patient refers to the primary care physician for lethargy, loss of appetite and difficulties walking. The peripheral blood smear reveals macrocytosis and hypersegmented neutrophils.

Complete blood count:

Leukocyte count = 3500/mm^3
RBC count = 2,12 mil/mm^3
Hb = 7.5 g/dl
Ht = 22%
MCV = 120 fl
MCH = 33 pg
MCHC = 35 g/dl
Platelets = 105000/mm^3

What diagnosis do you suspect?
What additional investigations would be justified?
Provide arguments for your answer.

……

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NOTES

2. INVESTIGATION OF WHITE BLOOD CELL DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. *Know and interpret the quantitative disorders of the leukocyte line*
2. *Ask for the investigations necessary for the diagnosis of acute and chronic leukemias*
3. *Discuss the differential diagnosis of acute and chronic leukemias*
4. *Ask for the investigations necessary for the diagnosis of lymphomas and lympho-plasmocytic neoplasms*

The quantitative (number) and qualitative (function) changes of the different leucocyte line elements are found in a variety of haematological disorders (mainly malignant haemopathies), but also in infections, inflammatory conditions (acute and chronic) and metabolic diseases. Due to the fact that leukocytes can be affected by a significant number of diseases, routine patient investigation begins with the *leukocyte count* followed by the study of the *peripheral blood smear*.

I. THE LEUKOCYTE COUNT

The leukocyte count is performed during the complete blood count using automatic flow cytometry analyzers (dependent on cell volume, nucleus segmentation and cytoplasmic granules' density). Normal values are presented in Tab.2.1.

Table 2.1. Normal values for the leukocyte count

	Normal values (/mm^3 or /μL)	**Normal values (x 10^9/L)**	**Normal values (%)**
Total no. of leukocytes	**4 000 – 11 000**	**4-11**	
Neutrophils (Ne): Segmented (NeS) Non-segmented (NeNS)	2 000 – 7 500 10 – 300	2 – 7,5 0,01 – 0,03	50– 70 1 – 3
Eosinophils (Eo)	40 – 400	0,04 – 0,4	0,4 – 4
Basophils (Ba)	10 – 100	0,01 – 0,1	0,1 – 1
Lymphocytes (Ly)	1 500 – 4 000	1,5 – 4	15 – 40
Monocytes (Mo)	200 – 800	0,2 –0,8	2 – 8

II. THE PERIPHERAL BLOOD SMEAR

- Peripheral blood smear examination provides information on leukocyte morphology. Depending on their morphology, five types of circulating leukocytes can be identified on the peripheral blood smear:
  - *neutrophil granulocytes (polymorpho-nuclear)*
  - *eosinophilic granulocytes*
  - *basophilic granulocytes*
  - *lymphocytes*
  - *monocytes*
- The presence of immature cells on the peripheral blood smear (normally present exclusively in the bone marrow) is considered pathological (Tab.2.2)

Table 2.2. Leukocyte abnormalities on the peripheral blood smear.

Abnormality	Description	Pathological conditions
Hypersegmented neutrophils	The nucleus has more than 5 lobes	*Megaloblastic anemia*, chronic infections
Blasts (myeloblasts, lymphoblasts, monoblasts, atypical promyelocytes)	Variable aspect *! Their presence is always a pathological finding on the peripheral blood smear*	Acute *granulocytic (myeloid)* and *lymphocytic (lymphoid)* leukemias
Auer rods	Needle-shaped, azurophilic inclusion bodies in the cytoplasm of myeloblasts and promyelocytes (that result from an abnormal fusion of primary azurophilic granules) that indicate a *poor prognosis*	Acute myeloid leukemia (!promyelocytic) - **pathognomonic** *! They are absent in acute lymphoid leukemia*
Gumprecht's nuclear shadows	Lymphocytes injured after smearing the blood on the microscope slide that appear in high numbers in leukemias (sign of lymphocytic fragility)	Chronic *lymphoid* leukemia
Plasma cells (plasmocytes)	Cells with a low nucleus-to-cytoplasm ratio, with an intensely basophilic, vacuolar cytoplasm and an eccentric nucleus with chromatin placed in a "cartwheel" pattern	*Multiple myeloma (plasmacytoma)* Infectious mononucleosis Collagen diseases, sarcoidosis Bacterial and viral chronic infections (eg, pulmonary) Allergies, hypersensitivity, immunizations (conditions characterized by elevated serum gamma globulins)
Atypical lymphocytes (reactive T lymphocytes, Downey cells)	Large cells resembling monocytes or immature cells, with an oval nucleus and abundant cytoplasm with multiple azurophilic granules – pathologically, **over 10%** of the total number of lymphocytes *! They may be present up to 10% in healthy individuals!*	*Infectious mononucleosis* Viral infections (hepatitis A, rubella, measles, mumps, HIV) or post-vaccination After multiple transfusions Allergic reactions, autoimmune diseases Chronic infections (tuberculosis, syphilis, toxoplasmosis)
"Hairy" cells	Gray/light blue mononuclear cells with irregular edges (laced), large nucleus with fine chromatin (they are malignant B lymphocytes)	Hairy cell leukemia (a subtype of chronic lymphoid leukemia)
Shift to the *left* of the leukocyte count	Excessive presence of granulocyte precursors (especially intermediate forms: myelocytes and metamielocytes) in the peripheral blood	Leukemoid reaction Chronic myeloid leukemia

III. CLASSIFICATION OF THE LEUKOCYTE LINE DISORDERS

- **Non-malignant disorders**
  - **Quantitative disorders:** cell number changes
    - **Leukocytosis**
    - **Leukopenia**
  - **Qualitative disorders:** changes in cellular function (mainly chemotaxis and phagocytosis)
- **Malignant disorders**
  - **Myeloproliferative disorders**
  - **Lymphroproliferative disorders**

IV. NON-MALIGNANT DISORDERS

A. LEUKOCYTOSIS

- **Definition:** increased number of leukocytes **> 11 000/mm^3**

1. Neutrophilia

- **Physiological** – by ***shifting the neutrophils from the peripheral vascular compartment*** to the ***circulating*** compartment
  - Pregnancy (IIIrd trimester)
  - Extreme temperatures
  - Increased release of catecholamines (acute stress, intense exercise, after surgery)
- **Pathological:**
  - By ***increasing the release of cells from the bone marrow reserves*** with an increase in the number of non-segmented neutrophils (NeNS) ≥ 10% and the *deviation to the left of the leukocyte formula* in:
    - Burns, after surgery
    - Acute bleeding
    - **Acute** inflammation: acute appendicitis, acute myocardial infarction, ischemic stroke, gout attack, acute glomerulonephritis
    - **Acute bacterial infections (cocci, bacilli)**: lobar pneumonia
    - Corticotherapy
    - Cushing's syndrome
  - By ***bone marrow hyperplasia on the granulocyte line*** in:
    - An acute flare-up of a **chronic** inflammation: collagen diseases, arthritis
    - **Chronic bacterial** infections
    - **Decompensated endocrine or metabolic** diseases: hyperthyroidism, ketoacidosis, gout, uremia, cirrhosis
    - **Myeloproliferative disorders**: chronic myeloid leukemia (CML), polycythemia vera
    - Post-splenectomy
    - Preeclampsia

LEUKEMOID REACTION

- **Definition:** persistently high neutrophilia with leukocyte counts between 30,000 to 50,000/mm^3 (this degree of leukocytosis usually suggests leukemia) but which is *reversible* after treatment of the underlying condition
- **Causes:** it is a **benign exaggerated leukocyte response** that occurs as a defense reaction to:
  - *severe bacterial infections:* pneumonias, meningitis, tuberculosis
  - *organ necrosis:* liver, colon
  - *side effect of drugs:* growth factors, corticosteroids (in high doses)
- **Characteristics:**
  - the increase is due to the **mature neutrophils** and the detection in the peripheral blood of **intermediate** (*myelocytes, metamielocytes*) and **young elements** (*promyelocytes,* rarely *myeloblasts*) of the granulocyte line (normally, present exclusively in the bone marrow)
  - *leukocyte alkaline phosphatase* (LAP) is **increased** (normally present in mature neutrophils)
  - *serum vit. B12* is **normal/slightly increased**
  - ***absence*** *of the Philadelphia chromosome* (Ph^1, short chromosome 22)

These criteria are used to differentiate the leukemoid reaction from **chronic myeloid leukemia** where:

- there is a **malignant** proliferation of myeloid lineage precursors (granulocytes) at the level of the bone marrow
- LAP is very ***low*** **or** ***absent***
- serum vit. B12 is **greatly** ***increased***
- Philadelphia chromosome is ***present*** in 95% of cases

Observation!

Assessement of the LAP activity (calculating an LAP index/ score) is also used for the positive and differential diagnosis of other malignant hematopoietic diseases.

Low values: myelodysplastic sdr., secondary polycythemias

Increased values: polycythemia vera, myelofibrosis, hairy cell leukemia, as well as in CML remission (where LAP may be normal or increased).

2. Eosinophilia

- **Causes:**
  - **Parasitic infections**: infections with helminths, giardiasis, trichinosis, echinococcosis
  - **Allergic diseases**: allergic rhinitis, asthma, eczema, allergic vasculitis, as well as drug *reactions - the latter is the main cause in hospitalized patients!*
  - **Malignant disorders**: **CML**, ulcerated/ metastatic tumors, Hodgkin's lymphoma
  - **Hypereosinophilic syndromes**: Loeffler's syndrome, eosinophilic leukemia.
  - **Endocrine disorders**: Addison's disease with hypocorticism (by eosinophils' release from the lymph nodes due to decreased cortisol)

3. Basophilia

- **Causes:**
  - **Allergic** diseases: allergic asthma, eczema, anaphylactic shock
  - **Chronic** inflammations: rheumatoid arthritis, ulcerative colitis
  - **Malignant** disorders: **CML**, polycythemia vera, myelofibrosis (basophilia helps to distinguish these diseases from leukemoid reactions)

4. Lymphocytosis

- **Causes:**
  - **Acute VIRAL infections**: infectious mononucleosis, viral hepatitis, mumps
  - **Chronic BACTERIAL infections**: tuberculosis, syphilis
  - **Endocrine disorders**: thyrotoxicosis, adrenal gland failure
  - **Malignant disorders**: chronic lymphocytic leukemia (CLL), lymphomas with cell 'spill-over' into the bloodstream

5. Monocytosis

- **Causes:**
  - **Infections:**
    - *viral* – infectious mononucleosis
    - *bacterial* – subacute bacterial endocarditis, brucellosis, tuberculosis
  - **Granulomatous chronic inflammations**: sarcoidosis, Crohn's disease (regional ileitis)
  - **Malignant disorders**: acute monocytic and myelomonocytic leukemias, CML, breast, kidney, ovary, digestive neoplasms.

INFECTIOUS MONONUCLEOSIS

- **Definition:** benign lymphoproliferative disorder caused by the **Epstein-Barr (EB) virus**, with oral transmission via infected saliva ("kissing disease") that is particularly common among *teenagers* and *young adults.*
- **Viral infection consequences:**
  - *Infection of B lymphocytes* (in the oropharyngeal tissue) with **2 consequences***:*
    - destruction of the infected B lymphocytes and local inflammation (pharyngitis)
    - virus incorporation into the cellular genome (persistence of infection), B lymphocyte proliferation and production of **heterophilic antibodies** with diagnostic role
  - *Activation of cytotoxic T lymphocytes and NK cells* by the antigen (in the lymph nodes and spleen) with the occurrence of **atypical lymphocytes** (large reactive lymphocytes with abundant basophilic cytoplasm - "ballerina skirt") in the peripheral blood - over **10% of the total lymphocyte number** is suggestive for the diagnosis.
- **Clinical manifestations**: fever, pharyngitis, *adenopathies*, hepato- / splenomegaly
- **Paraclinical diagnosis:**
  - *lympho-monocytosis* on the CBC
  - presence of *atypical lymphocytes* (Downey cells) on the peripheral blood smear
  - tests meant at highlighting the *heterophile antibodies* (Anti-EB virus antibodies capable of agglutinating erythrocytes from another species), the maximum titer being reached 2-5 weeks after the onset of symptomatology (Paul-Bunell test, Monospot test)
  - presence of *serum antibodies directed against viral antigens*: VCA-IgG, VCA-IgM (VCA-Viral Capsid Antigen) and EBNA-IgG (EBNA-Ebstein-Barr Nuclear Antigen) – determined when the heterophile antibodies are absent
  - possibly, *hepatocytolysis with increased transaminases* (AST, ALT)

B. LEUKOPENIA

- **Definition:** decreased number of leukocytes **< 4 000/mm^3**

1. Neutropenia

- **Definition**: decreased number of neutrophils **< 1.500/mm^3**; it is always *pathological*. **Agranulocytosis** is a decrease in granulocytes

<500/mm³ in the peripheral blood and is associated with an increased risk of death through **severe bacterial and fungal infections.**

- **Causes:**
  - **Decreased bone marrow production***:*
    - **Hereditary** conditions: familial or cyclic neutropenia (rare)
    - **Acquired** diseases: bone marrow hypo/aplasia, bone marrow infiltration (leukemias, metastatic tumors, granulomatous diseases), post-radiation therapy
    - **Drug induced**: anticancer chemo-therapy with alkylating agents and antimetabolites inducing bone marrow suppression → neutropenia is associated with anemia and thrombocytopenia.
    - **Ineffective hematopoiesis**: megaloblastic anemias in which the pathological precursors of all cell lines are destroyed at the bone marrow level
  - **Increased peripheral destruction and/or use:**
    - antibody production in *autoimmune diseases* (systemic lupus erythematosus)
    - disorders associated with splenomegaly (splenic sequestration & neutrophil destruction): sarcoidosis, Felty syndrome (characterized by the triad: rheumathoid arthritis + splenomegaly + neutropenia)
    - increased consumption in: *severe bacterial* (sepsis, typhoid fever, severe tuberculosis) and *fungal infections*.
  - **Increased adhesion and movement of circulating neutrophils towards the periphery in septic shock** (because bacterial endotoxins activate adhesion molecules)
- **Clinical manifestations:** caused by decreased phagocytosis (infections, mucosal ulceration, septicemia risk)

2. Lymphopenia

- **Causes:**

a) Acute: acute myocardial infarction, severe pneumonia, sepsis, stress

b) Chronic:

- *decreased production:*
  - B cell, T cell or combined immune deficiencies
  - post-radiation therapy, chemo-therapy, cortisone therapy
  - malignancies: bone marrow aplasia
- *increased destruction:* AIDS, autoimmune diseases (eg, SLE)
- *sequestration at the level of lymph nodes*: excess corticosteroid hormones

3. Eosinopenia

- **Causes:**
  - Excess of corticosteroid hormones (therapeutic use or Cushing's syndrome - by eosinophil sequestration at the level of lymph nodes)
  - Stress states: surgery, shock, burns, trauma

4. Monocytopenia

- **Causes:**
  - Excess of corticosteroid hormones (therapeutic use or Cushing's syndrome)
  - Aplastic anemias
  - Acute leukemias

V. MALIGNANT DISORDERS

A. MYELOPROLIFERATIVE DISORDERS

- **Definition**: acquired clonal disorders of the *pluripotent stem cell* characterized by various quantitative and qualitative changes of all leukocyte, erythrocyte and thrombocyte line elements.

- **Classification:**
  - **Acute myeloid leukemia (AML)**
  - **Myelodysplastic syndromes**
  - **Myeloproliferative neoplasms:**
    - Chronic myeloid leukemia (CML)
    - Polycythemia vera
    - Essential thrombocythaemia/thrombocytosis
    - Primary myelofibrosis

The laboratory diagnosis of myeloproliferative neoplasms is presented in Tab. 2.3.

Table 2.3. Differential diagnosis of myeloproliferative neoplasms

	Nr. L	Nr. E/Ht	Nr. Tr.
CML	↑↑	N	N or ↑
Polycythemia vera	N or ↑	↑↑	N or ↑
Essential thrombocytosis	N or ↑	N	↑↑
Primary myelofibrosis	N, ↓, ↑	↓	↓, N,↑

Observation!
Both myelodysplastic syndromes as well as myeloproliferative neoplasms can evolve towards acute myeloid leukemia (AML).

B. LYMPHOPROLIFERATIVE DISORDERS

1. **Acute and Chronic lymphocytic leukemia (ALL and CLL, respectively)**
2. **Lymphomas**
3. **Lympho-plasmacytic neoplasms**

In the following section, the most common malignant hemopathies (leukemias and lymphomas) will be presented distinctly, although their separation is sometimes difficult in clinical practice (leading to a new cumulative WHO classification for both pathologies) and finally, lympho-plasmacytic neoplasms will be discussed.

1. LEUKEMIAS

- **Definition**: chaotic proliferation of *leukocyte precursors* (granulocytes and lymphocytes) *in the bone marrow with their discharge into the peripheral circulation* and *variable infiltration into tissues/organs*.
- **Classification:**
  - According to the *onset* and *clinical course*:
    - **Acute** leukemias (AL)
    - **Chronic** leukemias (CL)
  - According to the *cell type* that proliferates:
    - **Lymphocytic (lymphoid)** leukemias
    - **Myeloid (granulocytic)** leukemias
  - According to the *number of cells discharged into the peripheral blood*:
    - **Hyperleukemic** forms: leukocytosis > 50.000/mm^3 and presence of blasts (atypical cells) in the peripheral blood
    - **Leukemic** forms: leukocytosis > 20.000/mm^3 and presence of blasts (atypical cells) in the peripheral blood
    - **Subleukemic** forms: leukocytosis between 7.000 – 20.000/mm^3 and presence of blasts (atypical cells) in the peripheral blood
    - **Aleukemic** forms: normal number of leukocytes and no abnormal cells detectable in the peripheral blood.
- **Paraclinical diagnosis:**
  - *Complete blood count:* leukocytosis (except for the aleukemic forms)
  - *Morphological diagnosis:*
    - *Peripheral blood smear:* shows an increased number of *immature cells in* ***acute*** *leukemias* or *mature leukocytes in* ***chronic*** *leukemias*
    - *Bone marrow smear:* highlights the bone marrow hypercellularity and the morphological characteristics of malignant cells.
  - *Molecular diagnosis:*
    - *Flow-cytometry:* allows the immuno-phenotyping of malignant cells and the detection of the expression of surface antigens („cluster of differentiation", CD) characteristic for malignant leukocytes by using monoclonal antibodies. Currently, it is a **mandatory** investigation both for **diagnostic** as well as **therapeutic purposes**.
  - *Genotypic diagnosis:*
    - *Cytogenetic tests:* **mandatory** because they reveal anomalies of the karyotype (deletions, translocations) with both **diagnostic** and **prognostic** roles.

a) ACUTE leukemias

- **General characteristics:**
  - Sudden onset and **rapid** progression
  - Increased proliferation of ***immature, blastic cellular forms*** - precursors of the myeloid or lymphoid lines at bone marrow level with ***blocked cell differentiation and maturation***.
  - ***Rapid*** infiltration of the bone marrow with suppression of hematopoiesis and the occurrence of bone marrow insufficiency manifested in the peripheral blood by **pancitopenia** along with the **classical triad** of acute leukemias:
    - Rapid onset and progressive *anemia* responsible for *severe asthenia*
    - *Granulocytopenia* leading to the *infectious syndrome* (recurrent infections, especially Gram (-) bacteria, mucosal ulceration, fever)

- *Thrombocytopenia* responsible for *haemorrhagic syndrome* (skin purpura, bleeding gums, epistaxis).

- **Classification:**

The **current WHO** classification of acute leukemias mainly takes into account ***cytogenetic abnormalities*** and has replaced in clinical practice the initial **French-American-British (FAB)** classification based on the ***morphological characteristics*** of leukemic cells. However, reference is still being made in the literature to the subtypes described by the FAB classification for acute leukemias, for which reason they will be briefly presented below.

i) Acute Myeloid Leukemia (AML)

- **Definition**: form of acute leukemia characterized by ***malignant proliferation of myeloid (granulocyte) precursors*** in the bone marrow.
- **WHO classification** – identifies a single class: **Acute myeloid leukemia with genetic abnormalities** that play a decisive role in AML prognosis:
  - t(8;21) translocation has a good prognosis
  - t(15;17) translocation is characteristic for **acute promyelocytic leukemia** that has two main characteristics:
    - it is often complicated by DIC
    - responds favorably to non-cytostatic therapy with high doses of vitamin A (retinoic acid)
- **FAB classification** identifies 8 AML subtypes:
  - **M0 -** acute myeloblastic leukemia with minimal differentiation
  - **M1 -** acute myeloblastic leukemia without maturation
  - **M2** - acute myeloblastic leukemia with maturation
  - **M3** - promyelocytic acute leukemia
  - **M4** - myelomonocytic acute leukemia
  - **M5** - monocytic acute leukemia
  - **M6** - erythroleukemia
  - **M7** - megakaryocytic acute leukemia
- **Laboratory diagnosis:**
  - *Peripheral blood smear* reveals the ***leukemic hiatus*** - excessive presence in the peripheral blood of blasts along with a progressively reduced number of mature elements **without** intermediate elements (myelocytes and metamielocytes) that are constantly present in the periphery in chronic myeloid leukemia
  - *Bone marrow smear* shows an **increased number (> 20%) of myeloblasts** and **a low number of mature cells**

ii) Acute Lymphoid Leukemia (ALL)

- **Definition:** the most frequent form of childhood malignancy, characterized by ***malignant proliferation of lymphoid precursors*** in the bone marrow and lymphoid tissues.
- **WHO classification:**
  - **B-cell lymphoblastic leukemia** (80% of cases have pre-pre-B cells and have the best prognosis, healing being estimated in 2/3 of cases)
  - **T-cell lymphoblastic leukemia**
- **FAB classification** identifies 3 ALL subtypes:
  - **L1**: small lymphoblasts without surface markers for B or T lines (null cells); common in children and has the best prognosis
  - **L2**: large lymphoblasts with T-lymphocytes surface markers; frequent in adults and has a reserved prognosis
  - **L3**: lymphoblasts with surface markers of B lymphocytes; rare, it is the leukemic form of Burkitt's lymphoma, with the most severe prognosis.
- **Laboratory diagnosis:**
  - *Bone marrow smear* shows an **increased number (> 20%) of lymphoblasts**

Observation!

In addition to the investigations necessary to confirm the diagnosis of acute leukemia, it is recommended that supplemental tests be performed to further guide treatment:

- *Uric acid*
- *Assessment of renal and hepatic function*
- *Evaluation of cardiac function* - in case of treatment with chemotherapeutic drugs that have cardiotoxic potential - ex. doxorubicin
- *Identification of possible concomitant infection with HIV, hepatitis B or C virus*
- *Analysis of cerebrospinal fluid* in all patients with ALL due to the high risk of damage to the central nervous system
- *Investigation of coagulation*, especially in the case of acute promyelocytic leukemia, to assess the presence of DIC
- HLA typing - for situations in which bone marrow transplantation is considered.

b) CHRONIC leukemias

- **General characteristics:**
  - ***Insidious*** onset with sweating, fever and weight loss (as a result of increased metabolic rate) and splenomegaly manifested by

abdominal discomfort (early satiety through compression of the stomach)
- ***Slow evolution*** due to progressive infiltration of the bone marrow with malignant cells; variable prognosis, depending on cell type
- Increased proliferation of ***mature, well-differentiated leukocytes*** (cell type can be identified).

i) Chronic lymphoid leukemia (CLL)

- **Definition:** *proliferation of well-differentiated, "mature" lymphocytes.*
- **Laboratory diagnosis:**
  - *Complete blood count*: leukocytosis 15,000 - 200,000/mm3 with **lymphocytosis** and **neutropenia**
  - *Peripheral blood smear* reveals **well-differentiated lymphocytes** with **mature B lymphocytes** morphological characteristics – cells which proliferate in **95%** of cases (in only 5% of cases T lymphocytes proliferate) and **Gumprecht nuclear shadows** (fragile leukocytes that get torn after smearing the blood on the microscope slide)
  - The *bone marrow smear*, shows marrow infiltration with **small lymphocytes** (monomorphic metaplasia), **lymphoblasts being < 20%**

ii) Chronic Myeloid Leukemia (CML)

- **Definition:** *proliferation of well-differentiated, "mature" leukocytes*.
- **Laboratory diagnosis** – depends on the phase of evolution**:**
  - ***The chronic phase (proliferation of mature elements)***:
    - Complete blood count:
      - leukocytosis (15000-500000/mm^3) with basophylia and eosinophylia
      - platelet count may be normal or increased (thrombocytosis)
    - The peripheral blood smear shows marked increase in granulocytes with the presence of all elements of the myeloid line in the periphery, mainly of the intermediate forms (myelocytes and metamielocytes)
    - The bone marrow smear reveals:
      - marked hypercellularity with proliferation of granulocyte and megakaryocyte precursors (myeloproliferative disorder)
      - presence of the Philadelphia chromosome (in over 95% of cases)
      - myeloblasts < 10%
    - Leukocyte alkaline phosphatase decrease/ absence (in malignant granulocytes)
    - Increased serum level of vitamin B12
  - ***The accelerated phase***:
    - *new cytogenetic abnormalities occur*
    - *aggravated leukocytosis and basophilia*
    - *progressive anemia and thrombocytopenia*
    - *hyperuricaemia* and risk of gout and renal lithiasis
    - in the BM, *myeloblasts 10-19%*
  - ***The acute phase (proliferation of immature elements)*:**
    - increase in the number of blasts in the bone marrow and peripheral blood > 20% ("blast crisis")

2. LYMPHOMAS

- **Definition:** lymphomas are *solid tumors of the lymphoreticular tissue* (neoplastic proliferation of cells residing in lymphatic tissues), usually *without the presence of malignant cells in the peripheral blood* at the onset of the disease.
- Traditionally, lymphomas have been **classified** into:
  - **Hodgkin lymphoma (HL)**
  - **Non-Hodgkin lymphoma (NHL)**

  Currently, the distinction between leukemias-lymphomas has become less obvious because many lymphomas are associated with peripheral discharge of cells (the leukemic phase), which has led to both of them being collectively known as **lymphoid neoplasms**.
- **Current WHO classification** of lymphoid neoplasms based on the elements of morphological, immunophenotypic, cytogenetic and clinical diagnosis, includes 5 major groups:
  - **Neoplasms with immature B-cells**
  - **Neoplasms with mature B-cells**
  - **Neoplasms with immature T-cells**
  - **Neoplasms with mature T cells and natural killer cells (NK)**
  - **Hodgkin lymphomas**

  The first 4 entities correspond to the previous classification of *non-Hodgkin lymphomas*. This classification aims at grouping lymphomas according to the *neoplastic cell type of origin* and comprises for each group several subtypes according to the phenotypic, morphological and cytogenetic characteristics.
- **Positive diagnosis in lymphomas**
  - **Peripheral blood analysis**
    - **Aim: positive and differential diagnosis** (exclusion of a reactive proliferation and other disorders).

- **Includes:** complete blood count (CBC), assessment of kidney and liver function, uric acid, calcium and phosphate levels, ESR, LDH.

– **Lymph Node Biopsy**
  - **Aim:** allows placing the ***definitive diagnosis***.
  - The biopsy consists of harvesting a fragment of a modified lymph node (incisional biopsy) or the sampling of the whole node (excisional biopsy). The histopathological examination allows the identification of the *morfological type* (according to the degree of architectural distorsion of the lymph node) and of the *cytological type* (according to the malignant cell type that proliferates). Immunehistochemistry tests performed on the biopsy are useful for the identification of the type of lymphoma.

– **Flow-cytometry:** allows the *immuno-phenotyping* of leukocytes from the biopsy fragment and is mandatory for establishing *the lymphoma subtype* and the therapeutic approach, respectively.

– **Cytogenetic tests** complete the molecular diagnosis by highlighting chromosome anomalies.

– **Bone Marrow Aspiration and Biopsy**
  - **Aim:** it is routinely performed in order to assess disease progression.
  - The bone marrow smear may determine the extent to which the bone marrow is affected. In addition, the data obtained from this investigation will also establish if the pathology is a lymphoma or an acute leukemia. If there are **more than 20% blasts** in the marrow, the most likely diagnosis is **acute leukemia**. Morphological features may allow the diagnosis of the type of leukemia.

– **Imaging Techniques**
  - **Chest X-ray:** allows the identification of the enlarged lymph nodes in the mediastinum for HL and for NHL with mediastinum onset.
  - **Computed tomography:** it is the **imaging investigation of choice** that allows the diagnosis and staging of Hodgkin's disease.
  - **Other investigations**: Positron emission tomography (PET), nuclear magnetic resonance (MRI), lymphangiography.

3. LYMPHO-PLASMACYTIC NEOPLASMS

- **Definition**: diseases characterized by the appearance in the serum of pathological, non-functional proteins - *paraproteins* (hence their name of paraproteinemias), absent in the normal serum and produced by a *malignant plasma cell clone* causing the appearance on the ELFO of a single band called the *M (monoclonal) band*, hence their name of monoclonal gammopathies.

MULTIPLE MYELOMA

Multiple myeloma is a plasma cell neoplasm **(plasmacytoma)** in which paraproteins are most common from the **IgG** or **IgA** classes.

- The paraclinical diagnosis of ***multiple myeloma*** comprises:

– *Complete Blood Count (CBC)* in order to assess the severity of anemia, leucopenia and thrombocytopenia.

– *Total serum proteins: normal or increased*.

– *Measurement of serum Ig free light chains:* kappa and lambda (also comprising the assessement of their ratio).

– *Electrophoresis (ELFO) of serum proteins:* highlights the pathological protein (paraprotein) in the form of the M band

– *ELFO of serum proteins with immunofixation* highlights the subtype of pathological Ig - IgG (55%), IgA (20%) and rarely, IgM or IgD and the decrease of normal Ig ("immune paralysis", with increased risk of infections).

– *Urinary protein electrophoresis* is rarely used nowadays (given the identification of light chains in plasma). It allowed the identification of the *presence of Bence-Jones paraproteinuria* consisting in the urinary elimination of *light chains of immunoglobulins* (kappa or lambda) with tubular nephropathy. This protein is thermolabile: it forms a precipitate when the urine is heated to 50-60°C which disappears totally or partially when the temperature approaches boiling point and re-emerges upon cooling.

– *Serum beta-2 microglobulin* is evaluated for prognostic purposes, being a marker of disease activity (indicates increased cell turnover) and renal impairment and high serum values were associated with a low survival rate.

– *Serum LDH:* prognostic role, increased level at the time of diagnosis is associated with decreased survival rate

– *Creatinine clearance* is useful to define the severity of the patient's renal impairment.

– *Hypercalcemia* due to osteolysis

– *ESR*: increased due to blood hyperviscosity

Examination of the bone marrow (bone marrow biopsy):

- *The bone marrow biopsy* is **mandatory for diagnosis** and allows the detection on the bone marrow smear of myelomatous plasma cells (in immunohistochemistry) as well as the calculation of the percentage of plasma cells (flow-cytometry) from the aspirate (normally below 10%) - in myeloma, usually over 30% of cellularity (10 → 60%).
- *Cytogenetic analysis of the bone marrow* (FISH technique) - required for staging and prognosis.

Imaging techniques:

- *CT, PET/CT or MRI* highlights the presence of *osteolysis* areas (> 5 mm) in the spine, long bones, skull, ribs - secondary to spinal infiltration and increased osteoclastic activity.

Observation!

The production of paraproteins is also found in lymphoproliferative disorders such as: **CLL, non-Hodgkin's B-cell lymphomas**, as well as in a particular form of lympho-plasmacytic lymphoma called **Waldenstrom macroglobulinemia** (in the latter the paraproteins secreted belong to the IgM class).

CHECKPOINT!

1. Which of the following may cause lymphocytosis?
A. Acute bacterial infections
B. Chronic bacterial infections
C. Acute viral infections
D. ALL
E. CLL

2. Which of the following changes are present in allergic reactions?
A. Neutrophilia
B. Neutropenia
C. Lymfocytosis
D. Eosinophilia
E. Basophilia

***3. The Philadelphia chromosome is specific for which type of leukemia?**
A. CML
B. CLL
C. AML
D. ALL
E. Burkitt's lymphoma

***4. The leukemic hiatus is specific for:**
A. AML
B. ALL
C. CML
D. CLL
E. None of the options

***5. The definitive diagnosis in lymphomas is placed based on the:**
A. Peripheral blood smear
B. Bone marrow smear
C. Cytochemical tests
D. Computed tomography
E. Lymph node biopsy

6. The classical clinical triad of acute leukemias includes:
A. Weight loss
B. Anemia
C. Profuse sweating
D. Infectious syndrome
E. Haemorrhagic syndrome

7. Which of the following are characteristics of chronic leukemia?
A. Insidious onset
B. Prognosis dependent on cell-type
C. Increased proliferation of immature, blast forms
D. Rapid infiltration of the bone marrow
E. Increased blast count (> 20%) on the peripheral blood smear

8. What can serum ELFO with immunofixation detect, *most frequently,* in the case of a plasmacytoma?
A. IgM class paraproteins
B. IgG class paraproteins
C. IgA class paraproteins
D. The presence of κ and λ heavy chains
E. The presence of Bence Jones proteinuria

9. Which of the following are characteristics of the leukemoid reaction?
A. LAP very low
B. Reactive leukocytosis (30,000-50,000 /mm^3)
C. Malignant proliferation of granulocyte precursors
D. Absence of the Philadelphia chromosome
E. Highly increased vitamin B12 serum levels

10. Which of the following changes in the leukocyte formula appear in chronic bacterial infections?
A. Lymphocytosis
B. Lymphopenia
C. Neutrophilia
D. Eosinophilia
E. Basophilia

CASE STUDIES

1. A 14-year-old boy is taken to the family doctor for progressive asthenia and weight loss.

Complete blood count

RBC count = 2,5 mil /mm^3
Leukocyte count = 60,000 /mm^3
Platelet count = 50,000 /mm^3

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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2. A 60-year-old female presents for lumbar pain. The X-ray shows a compression fracture at the level of L2-L3 lumbar vertebrae.

Complete blood count:

Leukocyte count = 4.500 /mm^3
RBC = 3,2 mil/mm^3
Hb = 9,1 g/dl
Ht = 29%
MCV = 87 fl
MCH = 28 pg
MCHC = 33 g/dl
Platelet count = 135.000 /mm^3

Serum calcium = 13,2 mg/dl (NV = 8,5-10,5 mg/dL)
Serum creatinine = 1,6 mg/dL (NV: 0,6 -1,2 mg/dL)
ELFO proteins (NV: Alb. = 50-60%, α1 = 3-6%, α2 = 7-10%, β = 11-14%, γ = 15-23%):

albumins= 36%
α1-globulins = 6%
α2-globulins = 10%
β-globulins = 13%
γ-globulins = 35%

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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NOTES

3. INVESTIGATION OF HEMOSTASIS DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. *Ask for the main laboratory tests used for the assessment of primary hemostasis and interpret their changes*
2. *Ask for the main laboratory tests used for the assessment of secondary hemostasis (coagulation) and interpret their changes in the context of the main coagulopathies*
3. *Ask for the laboratory investigations used in the assessment of fibrinolysis and interpret their changes*
4. *Ask for the investigations used to monitor anticoagulant therapy and interpret their changes*

I. HEMOSTASIS - BRIEF PHYSIOLOGY OVERVIEW

Hemostasis represents **the spontaneous cessation of bleeding** that occurs in **2 stages: primary and secondary,** with the participation of 3 groups of factors**: vascular, platelet (thrombocytes) and plasma.**

A. PRIMARY hemostasis (VASCULAR PLATELET phase)

- **Definition:** generation of the **„white" platelet plug** that requires participation of both *vessels* as well as *platelets.*
- **Stages:**
  - **Vasoconstriction (vascular phase)** - initiated by the *endothelial lesion* and causes *blood flow to decrease through the damaged vessel*. The latter process is also favored by increased tissue pressure (extravascular) which is the mechanism that explains the haemostasis resulting from the compression of the injured area or the placement of the tourniquet.
  - **„White" platelet plug formation (*platelet* phase)** comprises: i) **adhesion** of platelets to subendothelial structures (basal membrane and collagen fibers) exposed by the lesion, process done with the help of the *von Willebrand factor (f.vW) from the level of the endothelium and the glycoprotein (GP) Ib receptor found at platelet level*; ii) platelet **activation** with the release of dense (ADP, serotonin) and alpha secretory (platelet factor 4, fibrinogen, fvW, thrombospondin, etc) granule contents (platelet degranulation), process leading to conformational changes of the GP IIb/IIIa platelet receptor that allow it to bind to fibrinogen; and iii) platelet **aggregation** (stimulated by ADP and thromboxane TxA2) occurs via the bridges created by the fibrinogen bound to GP IIb/IIIa receptors of adjacent platelets. This process leads to **„white" platelet plug formation** and **primary hemostasis** finalization.

Remember!

- **Primary** haemostasis causes bleeding to stop in **small vessels**.
- Dysfunction of primary hemostasis causes VASCULAR and PLATELET hemorrhagic syndromes characterized by:
  - spontaneous haemorrhage or bleeding with *immediate* onset after the injury
  - *skin haemorrhage* in the form of *purpura* (vascular or thrombocytic purpura), petechiae, bruising (early) and *mucous membranes haemorrhage (nose, mouth etc.)*.

B. SECONDARY hemostasis (COAGULATION)

- **Definition:** generation of the **„red" fibrin clot** that requires the activation of the *plasma clotting factors* (Fig. 3.1). The presentation of the coagulation process divided into 2 pathways is used for teaching purposes to describe the coagulation tests without reproducing the complexity of the coagulation process.
- **Stages:**
  - **The intrinsic pathway** - initiated by the contact of ***intravascular*** factors with negatively charged surfaces (*in vitro*, glass, *in vivo*, subendothelial heparan sulphate, activated platelets' surface). This pathway includes factors such as F. XII Hageman

(which initiates *in vitro* the coagulation via the intrinsic pathway), XI, IX, VIII, prekalicrein and macromolecular kininogen.

– **The extrinsic pathway** - initiated by contact between blood and factors present at the ***extravascular*** level - tissue factor (F. III, tissue thromboplastin) expressed by sub-endothelial cells (initiating *in vivo* extrinsic coagulation, *the most important* for coagulation *in situ* , both under physiological as well as pathological conditions.

– Both activation pathways converge to the ***common pathway*** that starts with **factor X activation**. **Factor Xa together with F.Va, Ca^{2+} ions and platelet phospholipid (PP3)** form the complex called prothrombinase responsible for the enzymatic conversion of *prothrombin* (F.II) into **thrombin** (F.IIa). Thrombin is the *central protease of the coagulation cascade* responsible for cleavage of **fibrinogen** (F.I.) into **monomer fibrin** which subsequently becomes **soluble polymer fibrin**, and ultimately with the generation of a **stable, insoluble fibrin net** under the action of factor XIIIa.

Remember!

– The term ***thrombus*** is used to define the *intravascular* fibrin clot in a living person.

– The term ***clot*** is used to define the fibrin clot formed: i) at *extravascular* level (at the site of a lesion) in a living person, ii) *in vitro*, and iii) in deceased persons.

– **Secondary** hemostasis causes the bleeding to stop in the **medium and large vessels**.

– Dysfunction of secondary haemostasis (coagulation) causes PLASMATIC haemorrhagic syndromes or COAGULOPATHIES characterized by:
  - hemorrhages with *late* onset after the injury
  - hemorrhages in the form of *hematomas* and *haemarthrosis.*

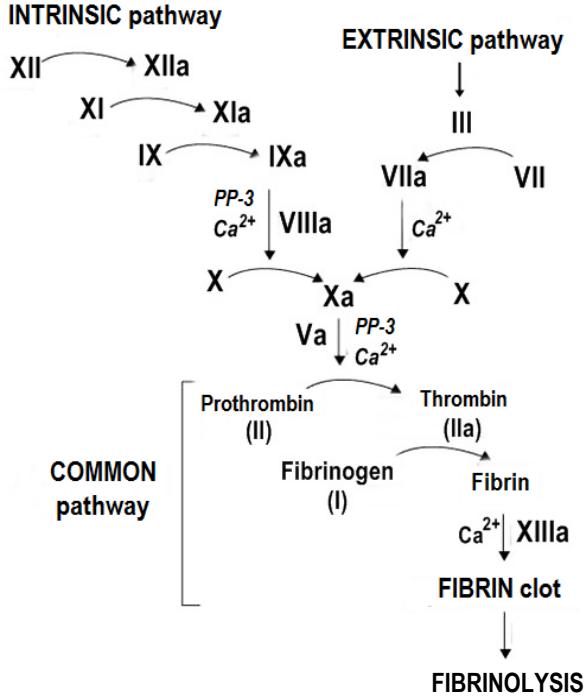

Figure 3.1. COAGULATION cascade.

II. LABORATORY INVESTIGATION OF PRIMARY HEMOSTASIS

A. PLATELET Count

Platelet count, a component of the complete blood count, is a ***screening*** assay in the assessment of the **platelet phase** of primary hemostasis.

- **Indications:**
  – To identify a low platelet count (***thrombocytopenia***) that can be associated with ***bleeding***:
    - **cutaneous:** petechiae, purpura
    - **mucous:** epistaxis (nosebleeds), hematuria, meno-/metrorrhagia, digestive bleeding
    - **gum:** from tooth extraction sites
  – To identify an elevated platelet count (***thrombocytemia, thrombocytosis***) that can be associated with the risk of ***thrombosis.***
- ***Normal values*: 150 000 – 400 000/mm^3**
- ***Pathological changes*:**
  – ***Thrombocytopenia*** (< 150000/mm^3) can be caused by:
    - *Decreased production*:
      - aplastic anemias
      - neoplastic bone marrow infiltration
      - deficiency of vitamin B_{12}
      - radio / chemotherapy
    - *Increased destruction / excessive consumption*:

- disseminated intravascular coagulation (DIC)
- splenomegaly and hyper-splenism
- immune thrombocytopenia
- drug-induced thrombocytopenia (heparin)

Table 3.1. Clinical effects induced by different levels of thrombocytopenia.

Platelet count ($x10^3/mm^3$)	Clinical effect
500 - 100	No clinical effect
100 - 50	Moderate haemorrhage after injury
50 - 20	Purpura may occur Haemorrhage after injury
<20	Purpura common Spontaneous haemorrhage from mucous membranes Intracranial haemorrhage (rare)

– ***Thrombocytosis*** (> 400 000/mm^3) can be:
  - *primary:* in myeloproliferative disorders, especially essential thrombocythaemia
  - *secondary (reactive):* in infections/ inflammations, cancer, post-splenectomy

Observation!
In the presence of a decreased number of platelets in a **venous blood sample**, it is recommended to repeat the measurement from a **capillary blood sample** in order to exclude a *pseudothrombocytopenia* (determined by platelet clumping in automatic analyzers, particularly when blood was collected on EDTA).
If thrombocytopenia persists, it is further recommended to perform a **capillary peripheral blood smear** that will confirm its presence or not.

B. PLATELET indices

1. Mean Platelet Volume (MPV)

- ***Normal values*: 8 - 13 μm^3** (fl)
- ***Pathological changes***
  – *Increased MPV* is present in:
    - immune thrombocytopenia and thrombocytopenia of: vitamin B_{12} or folic acid deficiency
    - primary thrombocytosis in myeloproliferative disorders (**not** in reactive thrombocytosis)
    - platelet disorders (impairment of platelet *function*, but with normal platelet count: eg Bernard-Soulier syndrome - adhesion defect)
    - hyperthyroidism
    - diabetes mellitus (especially in the presence of microvascular complications)
    - atherosclerosis (especially in smokers)
    - vitamin D deficiency
  – *Decreased MPV* is present in:
    - aplastic anemia
    - after chemotherapy

2. Platelet Distribution Width (PDW) - is a platelet index that quantifies the variation in platelet size (volume). It is calculated as a coefficient of variation by using the MPV and is expressed as a percentage.

- ***Normal values*: 8 - 16%**

C. PERIPHERAL BLOOD smear

- **Indications:**
  a) Confirmation of platelet abnormalities identified by the platelet count:
  – in *thrombocytopenia*, platelets are <u>rare and isolated</u>
  – in *thrombocytosis*, platelets tend to form <u>piles</u>
  – in case of *alteration of the platelet function*, <u>shape</u> and size abnormalities appear (anisocytosis, giant platelets in Bernard - Soulier syndrome)
  b) **The study of erythrocyte and leukocyte morphology** - can highlight the:
  – presence of macrocytes and hypersegmented neutrophils in association with thrombocytopenia in megaloblastic anemia
  – presence of leukocyte blasts in association with thrombocytopenia in leukemias

D. SPECIAL platelet tests

Are performed for the diagnosis of platelet adhesion and aggregation defects and consist of:

1. Assessment of Von Willebrand factor (vWF):

- **vWF is a multimeric circulating glycoprotein** with major roles both in:
  – *primary* haemostasis (platelet adhesion and aggregation)

  - *secondary* hemostasis – has the role to transport coagulation factor F.VIII (prevents its degradation and favors its procoagulant activity).
- vWF circulates in the blood under 2 forms: *free* (90%, synthesized by endothelial cells) and *inside platelets* (10%, synthesized by megakaryocytes and stored in the alpha granules)
- In clinical practice we assess:
  - **vW antigen** (FvW:Ag) – *quatitative* test
  - **vWF plasmatic activity** (that of ristocetin cofactor, FvW:RCo) - *qualitative* test, measuring vWF *function*
- ***Pathological changes:***
  - Its plasma activity *decreases* in:
    - *congenital* deficiency (qualitative or quantitative) called **von Willebrand <u>disease</u>** (the *most common hereditary coagulopathy* that associates the platelet adhesion defect with the decrease of F.VIII activity)
    - *acquired* deficiency called **von Willebrand <u>syndrome</u>** from: monoclonal gammopathies, lymphoproliferative disorders, autoimmune diseases
  - Its plasma activity *increases* in: pregnancy, estrogen therapy (including oral contraceptives), inflammation, acute myocardial infarction (vWF is considered an acute phase reactant), some thrombocytopenias (idiopathic thrombocytopenic purpura, hemolytic uremic sdr.).

2. Aggregometry

It is used only in special units in the diagnosis of congenital or acquired thrombocytopathies (adherence, aggregation and platelet-release reaction defects), not being routinely performed.

E. BLEEDING time (BT)

- **Definition:** the time required to spontaneously stop a bleeding caused by a standardized incision. It is a ***screening*** test for the ***overall assessment of primary hemostasis*** (vascular-platelet phase).
- **Ivy technique** (inflate the sphygmomanometer cuff to a constant pressure of about 40 mmHg that is maintained throughout the test). A standard incision is made using a device that is applied to the forearm and the timer is triggered at the same time. The drop of blood that spontaneously appears is absorbed on filter paper every 30 seconds. When the incision no longer bleeds, stop the stopwatch and note the elapsed time. It is currently rarely used.
- ***Normal values*: 2 - 8 min**
- ***Pathological changes*:**
  - **Bleeding time prolongation** occurs in:
    - <u>VASCULAR</u> hemorrhagic syndromes:
      - *Hereditary*
      - *Acquired* (senile purpura, C avitaminosis, Cushing disease)
    - <u>PLATELET</u> hemorrhagic syndromes:
      - ThrombocytoPENIA
      - ThrombocytoPATHIES:
        - ***Adhesion*** defect (Bernard-Soulier syndrome, von Willebrand disease)
        - ***Aggregation*** defect (Glanzmann thrombasthenia, aspirin/NSAID induced)

Remember!

- BT is **contraindicated in treatment with *aspirin/ non-steroidal anti-inflammatory (NSAIDs)*** drugs. In these conditions, the test will be performed 7 days after drug discontinuation.
- BT is **normal in coagulation disorders**.

F. BONE MARROW smear

- **Indication:** to assess platelet production in the bone marrow from *megakaryocytes* in order to establish the mechanism of thrombocytopenia**:**
  - an **increased number of megakaryocytes** suggests that thrombocytopenia is the result of **increased platelet peripheral destruction or consumption**
  - a **decreased** number of megakaryocytes is strongly suggestive for **failure of bone marrow production**.

III. LABORATORY INVESTIGATION OF SECONDARY HEMOSTASIS (COAGULATION)

- **Indications:**
  - Diagnosis of the congenital defects of clotting factors
  - Assessment of the bleeding risk prior to surgical, obstetrical, dental maneuvers or invasive diagnostic procedures
  - Identification of the causes of abnormal bleeding: internal hemorrhages, hematomas, hemarthrosis
  - Anticoagulant treatment monitoring!

A. Activated Partial Thromboplastin Time, aPTT

- **Definition:** coagulation time of the platelet-poor plasma collected on anticoagulant in the presence of *"partial thromboplastin",* calcium and a contact activator. The term „partial thromboplastin" denotes the fact that in order to trigger coagulation (prothrombin to thrombin activation) the phospholipid fraction of a tissue extract (tissue factor being absent *in vitro*) is used. The most widely used phospholipid is *cephalin* (phosphatidyl-ethanolamine) and its role is to mimic the effect of PP3 (the plasma being platelet poor). The most commonly used contact activator is *kaolin* for activation of F.XII Hageman (i.e., intrinsic pathway of coagulation).
- **Clinical significance - indications:**
  - Screening test for the evaluation of plasma factors from the:
    - **<u>intrinsic pathway</u>:** factors **XII, XI, IX, VIII**
    - **<u>common pathway</u>**: factors **X, V, II, I.**

    Prolongation of aPTT signifies the **deficit or inhibition** of these coagulation factors.
  - Test used to identify the **plasmatic presence of inhibitors of the *intrinsic and common pathways*.**
  - Test used to monitor the **anticoagulant therapy with <u>standard</u> (unfractionated) HEPARIN** where the target value is an increase of **1.5-2.5 times of the aPTT.**
- ***Normal values*: 26 - 37 sec.**
- ***Pathological changes*:**
  - **aPTT is prolonged in:**
    - **deficit (congenital or acquired) of clotting factors in the intrinsic or common pathways,** the most common being:
      - hemophilia **A = f. VIII** deficit
      - hemofilia **B = f. IX** deficit
    - treatment with <u>standard</u> heparin
    - disseminated intravascular coagulation (DIC)
  - **aPTT is decreased (shortened)** in the hypercoagulability states.

Remember!

- The test is not modified by **fractionated heparin** therapy, term used to define **low molecular weight heparins**, e.g. fraxiparin, enoxaparin, dalteparin, etc.
- The test is ***normal in deficiency of factor VII*** from the extrinsic pathway.

B. Prothrombin Time (PT) or Quick Time

- **Definition:** coagulation time of the platelet-poor plasma collected on anticoagulant in the presence of tissue thromboplastin (tissue factor & phospholipids) and calcium.
- **Clinical significance - indications:**
  - Screening test for the evaluation of plasma factors from the:
    - **<u>extrinsic pathway</u>:** factor **VII** and
    - **<u>common pathway</u>**: factors **X, V, II, I**
  - Screening test for the evaluation of **vitamin K-dependent factors**: factors **II, VII, IX, X**. Prolongation of PT signifies the ***deficiency or inhibition*** of these coagulation factors.
  - Treatment monitoring test of **<u>indirect</u> ORAL ANTICOAGULANT therapy:**
    - ***warfarin*** and
    - ***acenocoumarol***

    also known as **antivitamin K drugs (AVK)**.
- ***Normal values*: 11,5 – 13,5 sec.**

(each laboratory establishes its own NV range)

- ***Pathological changes*:**
  - **PT is prolonged** if the plasma levels of F. **II, VII, IX** and **X** are **< 40%** of normal in:
    - Chronic liver disease
    - Vitamin K deficiency
    - DIC
  - **PT is decreased (shortened)** in the hypercoagulability states.

The Koller test was classically performed to differentiate between the PT prolongation due to *vitamin K deficiency* and *liver disease*. PT is assessed before and after vitamin K (10 mg/day for 3 days, intramuscularly) administration:

Interpretation:

- ✓ with PT normalization, ***vitamin K deficiency*** is diagnosed
- ✓ the lack of PT normalization points to a coagulation abnormality due to liver ***disease***.

Observations!

- There is no warfarin in Romania, only acenocumarol preparations (Trombostop, Sintrom) are authorized.
- In case of anticoagulation with either heparin or AVK, both aPTT and PT are prolonged because both drugs inhibit factors in the common final pathway (IIa, X). The decision to use aPTT for monitoring heparin therapy and PT for AVK, respectively, was the result of empirical observations on the accuracy of these tests in current practice.
- In monitoring AVK therapy, preferentially **INR** (not PT) is used.

International Normalized Ratio (INR)

- **Definition:** standardized PT expression according to a formula that takes into account the *sensitivity* of different commercially-used thromboplastin preparations used in laboratories.
- **Clinical significance**: allows to ***determine the optimal oral anticoagulant dose,*** independently of the reagent used (commercial thromboplastins have different sensitivities and influence the PT value).
- **Normal values: INR = 0,8 - 1,2**
  (in absence of AVK treatment)
  Optimal values: **INR = 2,0 - 4,0** in patients treated with AVK anticoagulants

Remember!

- For patients treated with oral AVK-type anticoagulants, control of treatment compliance should be done by assessing the **INR at least once a month** (ideally once every 3 weeks).
- If the **INR drops below 1.5**, there is a risk of **thrombosis** and the anticoagulant dose should be *increased* or vitamin K sources (eg cabbage) should be eliminated from the diet.
- If **INR increases over 5**, there is a risk of bleeding and the dose of anticoagulant should be *reduced*.

C. Thrombin Time, TT

- **Definition:** coagulation time of plasma collected on anticoagulant in the presence of a known amount of **thrombin** and calcium. The test by-passes the intrinsic and extrinsic pathways of coagulation, ***directly measuring the ability of thrombin to convert fibrinogen to fibrin***.
- **Clinical significance - indications:**
  - Evaluation of the **common pathway** plasma factors: **X, II, V, I.**
  - Plasma detection of **fibrin formation and polymerization inhibitors:**
    - fibrin degradation products (FDP)
    - paraproteins
- **Normal values: 12 - 14 sec.**
- **Pathological changes:**
  - **TT is increased (prolonged) in**:
    - *primary* fibrinogen deficiency (primary fibrinolysis, hypo- and afibrinogenemias - rare)
    - *secondary* fibrinogen deficiency: DIC
    - the presence of FDP in plasma
    - the presence of **thrombin inhibitors** in the plasma:
      - **standard heparin** (indirect effect via antithrombin III)
      - **injectable direct thrombin inhibitors:** eg, argatroban

Remember!

- TT is **NOT** recommended for monitoring therapy with **new direct oral anticoagulants** that are **direct inhibitors** of Factors **IIa (dabigatran)** and **Xa (rivaroxaban, apixaban).**
- The test *does NOT distinguish* primary fibrinolysis from DIC (secondary fibrinolysis).

Observation!

Because prolongation of aPTT, PT or TT may result from the *deficiency of coagulation factors* described above or the presence of *coagulation inhibitors in plasma*, **correction tests** may be performed, consisting in the addition of normal plasma over the patient's plasma. Lack of aPTT, PT or TT correction means the presence of *coagulation inhibitors in plasma*.

D. Fibrinogen assessement

- **Definition:** Fibrinogen is a glycoprotein of hepatic and platelet origin, representing at the same time clotting factor I present in the

highest amount in plasma which participates both in:
  - ***primary hemostasis*** by triggering platelet aggregation
  - **secundary hemostasis** where it represents the substrate for thrombin action (which transforms fibrinogen into fibrin)
- **Clinical significance - indications:**
  - Diagnosis of DIC (consumption coagulopathy where there is a *decrease in fibrinogen concentration*)
  - Monitoring of hemostasis in conditions characterized by:
    - *low concentrations of fibrinogen* that predispose to haemorrhage: in chronic liver disease
    - *increased concentrations of fibrinogen* that predispose the patient to *excessive thrombosis* in: AMI, neoplasms, chronic inflammation, pregnancy, oral contraceptives use
  - Evaluation of primary or congenital hypofibrinogenemias (rare)
- **Normal values: 200–400 mg/dL**
- **Pathological changes:**
  - **Low values favor HEMORRHAGE** and occur in: severe chronic hepatopathy and DIC (commonly), primary hypofibrinogenemia
  - **Elevated values favor THROMBOSIS** and occur in: chronic inflammation, neoplasms, oral contraceptive use, acute myocardial infarction (fibrinogen is also an important *acute phase reactant*)

E. Assessement of the individual activity of coagulation factors

- **Indications:**
  - for diagnostic purposes in case of a prolonged aPTT or PT
  - monitoring the therapy of patients diagnosed with certain factors' deficiency.

IV. LABORATORY INVESTIGATION OF FIBRINOLYSIS

Fibrinolysis is the process by which degradation/ remodelling of the fibrin thrombus under the action of **plasmin** takes place.
Plasmin cleaves **fibrin** and **fibrinogen** with the release into the plasma of:
- fibrin degradation products
- D-dimers

1. Fibrin Degradation Products (FDP)

- **Definition:** *high molecular weight* degradation products of **fibrinogen and <u>soluble</u> (non-crosslinked) fibrin**
- **Normal values: < 10 mg/L**
- **Pathological changes:**
  Increased values (> 40 mg/L) are found in:
  - primary fibrinolysis
  - secondary fibrinolysis (DIC)
  - thrombolytic therapy
  - thrombosis
  - pulmonary embolism
  - myocardial infarction

2. D-dimers (DD)

- **Definition:** *low molecular weight* degradation products of **<u>insoluble</u> (covalently cross-linked in a stable net) fibrin**, currently considered to have a higher diagnostic specificity than FDP.
- **Clinical significance - indications:**
  - Screening test for **pulmonary thromboembolism and deep vein thrombosis of inferior limbs** where it has a ***negative predictive value*** - ie, obtaining a normal result in patients suspected of these conditions excludes their presence in > 95% of cases
  - Screening test for **DIC (secondary fibrinolysis)** where D-dimers' increase is the marker of *fibrinolysis activation*
  - Test useful in **monitoring thrombus lysis during thrombolytic therapy** (eg in coronary artery disease)
  - Test useful for the diagnosis of **subclinical thrombophilia** in patients with primary or secondary infertility.
- **Normal values: < 500 µg/L**

- **Pathological changes:**
  Increased values are found in:
  - pregnancy, estrogen treatment
  - after surgery
  - cardiovascular disorders: chronic atrial fibrillation, heart failure, aortic dissection
  - cancers (e.g., breast cancer)
  - infections, inflammation
  - burns
  - elderly people

Observation!
Thrombophilias are a group of conditions characterized by ***increased risk of recurrent thrombosis*** due to the existence of hereditary or acquired risk factors. Their diagnosis involves performing special tests, as follows:

- Dosage of soluble anticoagulant factors: S and C proteins and antithrombin III
- Genetic tests to highlight F.V (Factor V Leiden) and II gene mutations
- Highlighting the presence of antiphospholipid and anticardiolipin antibodies

Remember!
- The D-dimer test does **not** detect fibrinogen degradation products (absence of covalent bonds) and is negative when only they are present in the circulation, e.g. in primary fibrinolysis.

V. SCREENING TEST CHANGES IN HEMOSTASIS DISORDERS

The screening tests currently used in the laboratory diagnosis of haemostasis disorders are shown in Tab. 3.2, followed by their most frequent changes.

Table 3.2. Screening HEMOSTASIS tests

Test	Explors
• Platelet count	• Platelet phase
• BT	• Vascular and platelet phase
• aPTT	• Intrinsic pathway
• PT/INR	• Extrinsic pathway
• TT	• Common pathway

After performing the screening tests, the following situations may arise:

- **aPTT ↑ and PT normal**
  Deficit: factors **VIII**, **IX**, XI, XII
  Causes: hemophilia A or B, von Willebrand disease (+ BT ↑), non-specific inhibitors of these factors (e.g., antiphospholipid antibodies in systemic lupus erythematosus), standard heparin therapy.
- **PT ↑ and aPTT normal**
  Deficit: factors **VII**, X, II
  Causes: chronic liver disease, vitamin K deficiency, AVK therapy (warfarin, acenocoumarol)
- **PT normal and aPTT normal in the presence of bleeding**
  Deficit: factor XIII
  Causes: primary hemostasis dysfunction, factor XIII isolated deficiency, treatment with low molecular weight heparins.
- **DIC**: platelet count ↓, BT ↑, fibrinogen ↓, aPTT ↑, PT ↑, TT ↑, FDP ↑, D-dimers ↑.
- **Primary fibrinolysis**: *N platelet count*, BT ↑ (FDP prevent platelet aggregation), fibrinogen ↓, aPTT ↑, PT ↑, TT ↑, FDP ↑, *D-dimers N* (absence of fibrin thrombi)

Observation!
In the presence of high doses of AVK, aPTT will also be prolonged (FIX is vitamin-K dependent), but because F.VII has the shortest lifespan, it will decrease first and will modify the PT.

VI. ANTICOAGULANT THERAPY MONITORING

A. Standard heparin (Unfractionated)

- **Indications:**
  - Prophylaxis and treatment of venous thrombo-embolism:
    - Prophylaxis of post-operative deep vein thrombosis in patients undergoing major abdomino-thoracic surgery or who, for other reasons, are at risk of developing thromboembolic disease
    - Prophylaxis and treatment of pulmonary embolism
  - Atrial fibrillation with embolization risk.

- **Mechanism of action**: Increases the **inhibitory effect of antithrombin (ATIII)** on thrombin (IIa), F IXa, F Xa, F XIa and F XIIa, with the greatest effect on thrombin.
- **Monitoring:**
  - **aPTT**
    - Target: aPTT prolongation between **1.5 to 2.5** times the normal value
  - **Platelet count**
    - Daily, in order to detect heparin-induced thrombocytopenia (HIT) that typically occurs 2 to 5 days after heparin exposure
    - If platelet count drops by 30-50%, consider HIT, withdraw heparin (the antidote is protamine sulphate) and replace it with one of the injectable or oral direct thrombin inhibitors.
  - **Avoid concurrent use of aspirin and other NSAIDs.** Aspirin irreversibly inhibits platelet function. Current evidence indicates that aspirin can increase the risk of bleeding in patients anticoagulated with heparin.

B. Low Molecular Weight Heparin (LMWH, Fractionated Heparin):

enoxaparin (Clexane), nadroparin (Fraxiparin), dalteparin, reviparin, tinzaparin and a structurally related compound: fondaparinux (Arixtra).

- **Indications:** Prophylaxis and treatment of venous thrombo-embolism (subcutaneous administration)
- **Mechanism of action:** LMWH determine the increase of the **antithrombin III inhibitor effect on F. Xa - inhibition of factor Xa**
- **Monitoring:** assessement of the activity of F.Xa (**aPTT does not prolong** except for overdose cases)
  - is not necessary in patients without complications
  - is necessary in children, obese/ malnourished patients, in the case of nephropathy, long-term treatment, pregnancy, bleeding or thrombosis.

C. Vitamin K antagonists (antivitamin K, AVK):

warfarin, acenocoumarol

- **Indications:**
  - Treatment of arterial and venous thrombosis to prevent embolization
  - Prophilaxis of thromboembolic disease in atrial fibrillation, mechanical heart valves, thrombophilias and high thrombo-embolic risk surgery.
- **Mechanism of action:**
  - Inhibition of vitamin K-dependent factors activation (**II, VII, IX,** and **X**)
  - Requires 2-7 days to reach therapeutic levels (for immediate anticoagulation, one should begin with heparin).
- **Monitoring: target INR values:**
  - **2,0–3,0** for patients with: atrial fibrillation, pulmonary embolism, deep venous thrombosis
  - **2.5–3.5** for patients with: prosthetic heart valve or recurrent systemic embolism.

D. Direct injectable Thrombin Inhibitors:

argatroban and bivalirudin

- **Indications:** Substitutes for heparin when HIT is suspected or confirmed
- **Monitoring:** aPTT target: prolongation of 1,5 - 3 times.

E. Novel Oral Anticoagulants (NOAC) or Direct Oral Anticoagulants (DOAC)

are:

- **direct <u>ORAL</u> inhibitors of thrombin (F.IIa)** - dabigatran (*Pradaxa*) and
- **direct <u>ORAL</u> inhibitors of F.Xa ('xabans')** - rivaroxaban (*Xarelto*), apixaban (*Eliquis*), edoxaban (*Savaysa*).

- **Indications:** NOAC are currently considered superior (and used in priority) to indirect oral anticoagulants (AVK type) in:
  - prevention and treatment of vein thromboembolism after major orthopedic surgery (hip and knee replacement)
  - prevention and treatment of stroke and systemic embolism in patients with atrial fibrillation.
- **Monitoring:** not yet routinely available (disadvantage!).

Observation!

Recently the FDA approved antidote medication (previously unavailable for NOAC, which was another major disadvantage of therapy due to the risk of spontaneous bleeding) for dabigatran - Idarucizumab (Praxbind) and for 'xabans' - Andexanet alfa (Andexxa).

CHECKPOINT!

***1. Which is the standard assay used to monitor therapy with antivitamin K (AVK) drugs?**
A. INR
B. aPTT
C. BT
D. TT
E. Platelet count

***2. Which is the test used to monitor standard heparin therapy?**
A. BT
B. PT
C. TT
D. aPTT
E. Platelet count

***3. Which is the screening test used for the overall assessment of primary hemostasis?**
A. Bleeding time
B. Prothrombin time
C. aPTT
D. Thrombin time
E. Dosage of fibrinogen

***4. Which test explores the intrinsic pathway of coagulation?**
A. BT
B. TT
C. INR
D. aPTT
E. All of the above

***5. The D-dimer test is used in the exclusion diagnosis of:**
A. Deep vein thrombosis
B. Atrial fibrillation
C. Estrogen therapy
D. The presence of lupus anticoagulants
E. Deficiency of vitamin K

***6. The platelet count in patients treated with standard heparin can identify:**
A. Pulmonary thromboembolism
B. Drug induced thrombocytopenia
C. Deep vein thrombosis of lower limbs
D. DIC
E. None of the above

7. Which of the following may be the cause of a prolonged aPTT and a normal prothrombin time?
A. Standard heparin treatment
B. Low molecular weight heparin treatment
C. K avitaminosis
D. Oral anticoagulant treatment
E. Haemophilia

8. Which of the following concerning the INR are true?
A. If it falls below 1.5 there is a risk of bleeding
B. If it increases over 5 there is a risk of thrombosis
C. It is the standardized expression of the prothrombin time
D. Allows the optimal dose establishment for standard heparin
E. For correct treatment monitoring it must be determined at least once a month

9. Which of the following tests explore primary haemostasis?
A. Platelet count
B. Bleeding time
C. D-dimer test
D. aPTT
E. Thrombin time

10. Which of the following define von Willebrand's disease?
A. Prolonged prothrombin time
B. Increased D-dimers
C. Decreased platelet count
D. Prolonged bleeding time
E. Prolonged aPTT

CASE STUDIES

1. A 3-year-old boy is brought to the hospital for a painful, swollen left ankle after a minimal trauma. In the past 48 h he has refused to walk and play due to the pain.

Hemostasis tests:

Thrombocyte count: 384,000 /mm^3
BT: 5 min
PT: 12,7 sec
aPTT: 80 sec.

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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2. A 70-year-old patient is transferred from the emergency unit to the ICU because of an altered general status, fever (40°C), epistaxis and hematuria. The pacient was hospitalized for a posttraumatic hip fracture and has had a bladder catheter introduced one week ago. Examination shows purpura and petechiae on the thorax and superior limbs. Prolonged bleeding occurs at the site of venopunction.

Hemostasis tests:

Platelet count: 84,000 /mm^3
BT: 13 min
PT: 17 sec
aPTT: 50 sec

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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NOTES

4. INVESTIGATION OF THE ESOPHAGUS, STOMACH, INTESTINE AND EXOCRINE PANCREAS DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Request and interpret the investigations used in the positive diagnosis of the main disorders of the esophagus, stomach and intestine

2. Request and interpret the investigations used for the diagnosis and treatment monitoring of the Helicobacter pylori infection

3. Request and interpret the investigations used to diagnose malabsorption syndromes

4. Request the investigations used for the early detection of colorectal cancer

5. Request and interpret the laboratory investigations used in the diagnosis of exocrine pancreatic insufficiency

I. INVESTIGATION OF THE ESOPHAGUS DISORDERS

Indications:

Patients who manifest:

- **Pyrosis** (heartburn) or retrosternal/chest pain (acid reflux)
- **Dysphagia** (difficult swallowing)
- **Odynophagia** (painful swallowing)

The investigations used for the diagnosis of esophagus disorders are:

1. **Esophagoscopy**
2. **Barium meal**
3. Special tests: **esophageal pH determination, esophageal manometry**

A. Esophagoscopy

• Clinical value:

- allows **direct examination** of the esophageal lesions in:
  - *reflux esophagitis*: brittle mucosa, with erosions and ulcerations (4 stages of severity A-D according to the Los Angeles classification)
  - benign (scarring) or malignant (carcinoma) *esophageal stenosis*
  - *esophageal varices*
  - *achalasia*
- allows **biopsy** sampling in order to diagnose **Barrett's esophagus** and **esophageal cancer**

• Indications:

The **alarm signs** for **gastroesophageal reflux** that require **endoscopic examination** (to exclude an esophageal cancer !) are:

- Dysphagia
- Odynophagia
- Weight loss
- Anemia
- Upper digestive haemorrhage
- Family history of upper gastrointestinal cancer

Observation!

In young patients without alarm signs, we do not start with the endoscopic investigation, but we initially apply the *therapeutic test using proton pump inhibitors* (PPIs).

B. Barium meal

• Clinical value:

- outlines the contour of the upper parts of the digestive system (esophagus, stomach, duodenum) on X-ray (barium sulphate is a radiopaque compound)
- allows the evaluation of the *speed and quality of the esophageal transit* in disorders like :
  - *achalasia*
  - *esophageal spasm*
  - *esophageal stenosis*
- useful in the diagnosis of **hiatal hernia** if the examination is performed in the *Trendelenburg position* (patient is placed head down on a table inclined at an angle of about 45 degrees from the floor)

Observation!

The barium meal evaluating all segments of the digestive tract has been nowadays replaced by **endoscopy** (eso-gastro-duodenoscopy) or other imaging techniques (CT, MRI).

C. SPECIFIC examinations

Specific investigations are relatively rarely used in specialized services, only for selected cases.

1) ESOPHAGEAL pH measurement

- **Principle**: esophageal pH monitoring (5 cm above the lower esophageal sfincter) for 24 hours, using a portable monitoring device
- **Clinical value:**
  - it used to be considered *the gold standard* for the diagnosis of the **gastroesophageal reflux disease** (GERD):
    - a pH value **< 4** indicates **acid reflux**
- **Indications:**

Although pH measurement is **the most sensitive** test for ***gastroesophageal reflux disease (GERD)***, currently it is indicated in specific cases:

- Patients with **typical GERD symptoms** who do not respond to the classic therapy with proton pump inhibitors (PPI)
- Patients with **atypical GERD symptoms** (chronic cough, bronchial asthma)
- Patients who **underwent anti-reflux surgery**

b) ESOPHAGEAL manometry

- **Principle:** direct measurement of the esophageal pressure at rest and during swallowing with the aid of a manometer
- **Normal values:**
  - *pressure at rest*: between +2 and –2 cmH_2O
  - *pressure during swallowing*: between +40 and +80 cmH_2O

• Clinical value:

- diagnosis of *esophageal motility disorders* responsible for some of the dyspeptic syndrome symptoms
- it used to be considered the *gold standard* for the diagnosis of **achalasia**, a primary esophageal motility disorder characterized by the absence of esophageal peristalsis in the inferior esophagus, incomplete relaxation of the lower esophageal sphincter (LES) in response to swallowing and high resting LES pressure

• Indications:

- patients with suspected esophageal motility disorder (achalasia, diffuse spasm)
- pacients with indication for anti-reflux surgery

Remember!

In patients with **GERD**, manometry is **not routinely indicated**, except for the cases that do not respond to the PPI therapy and have normal eso-gastro-duodenoscopy aspect.

II. INVESTIGATION OF STOMACH AND DUODENUM DISORDERS

Indications:

Patients with:

- organic or functional dyspepsia **(epigastric pain/heartburn, nausea, vomiting, fullness sensation after eating, abdominal distension or disconfort)**
- **upper gastrointestinal bleeding**
- **weight loss**

The main investigations for the diagnosis of the stomach and duodenum disorders are:

- **Gastro-duodenoscopy**
- **Barium meal**
- **Identification of Helicobacter pylori infection**
- **Identification of occult bleeding** - in cases with possible gastrointestinal bleeding
- **Measurement of serum gastrin** - essential for the diagnosis of Zollinger-Ellison syndrome (gastrin-secreting pancreatic tumor)

A. Gastro-duodenoscopy

• Clinical value:

- it is the ***main*** **investigation** currently used in the **diagnosis and treatment follow-up of stomach and duodenal diseases**
- allows taking **gastric biopsies** if needed during the examination

• Indications:

- persistent/recurrent GERD
- persistent/recurrent vomiting
- epigastric pain of unknown etiology
- acute and chronic gastritis
- gastro-duodenal ulcers
- upper gastrointestinal bleeding (+ therapeutic purpose)
- anemia with unknown origin
- Addison-Biermer anemia (every 6-12 months)

• Taking a gastric mucosa biopsy is **mandatory** for:

- the **exclusion of the malignant nature** of **lesions in gastric ulcer** where the **anatomo-pathological diagnosis is mandatory** unlike

duodenal ulcer where the biopsy and histopathological diagnosis are not routinely required!
- detection of **dysplastic lesions**
- identifying the **H. pylori infection**

• **The characteristics of gastric ulcers in endoscopy** are:
- mucosal defect of **> 5 mm** diameter, covered with fibrin
- lesions **< 5 mm** in diameter are defined as **erosions but can be classified as ulcerations in the context of confirmation of the affectation of the muscular mucosal layer in the histopathological examination**
- the ulcer is usually solitary

Observation!
Ulcers identified at the **distal duodenum** level raise suspicion of:
- **their ischemic origin**
- **Crohn's disease**
- **Zollinger-Ellison sdr.**

B. Barium meal

• Indications:
Although rarely used nowadays, barium meal examination can be used as a viable diagnostic alternative when endoscopy is not available, in patients suspected of:
- *giant hypertrophic gastritis* (Menetrier disease)
- *gastro-duodenal ulcers*: direct signs (ulcerative niche) or indirect signs (notches, convergence of the folds or accelerated evacuation from the duodenal bulb, etc.)
- *gastric cancer*: malignant infiltratitive rigid niche, malignant vegetations
- *pyloric stenosis*: hypotonic dilation with abundant gastric fluid and delayed gastric emptying

C. Diagnosis of HELICOBACTER PYLORI infection

Helicobacter pylori (HP) infection is the main cause of gastro-duodenal ulcers, therefore the diagnosis of such an infection through direct or indirect methods is **mandatory**.

1. DIRECT methods (invasive) – an **endoscopy** together with **gastric biopsies** are necessary, in order to identify H. pylori infection through:
- ***histology assays*** (special stains)
- ***rapid urease test*** from the biopsy sample (a pH indicator modifies its colour in the presence of H. pylori that produces a high amount of urease)
- ***culture assays*** (special media)

2. INDIRECT methods (non-invasive):

a) Serum anti-H. pylori antibody measurement
- NO accuracy for the diagnosis because:
  - a positive result indicates either a current or a previous infection
  - patients with acute HP infection in an early stage may present with undetectable levels of anti-HP IgG antibodies
  - there is NO relationship between the serum levels of IgG antibodies and the severity of HP infection
- the test CANNOT be used to assess the *therapeutic response* since the IgG anti-HP antibodies may require up to 1 year to decrease to 50% after the infection has been eradicated.

b) UREA breath test
- can be used as a ***screening test*** for the **identification of HP infection**
- a radioactive isotope (C^{13}) is used to mark an urea sample, which will be swallowed by the patient
- HP secrets *urease* that splits urea and generates $^{13}CO_2$ that will be absorbed and measured in the patient's breath

c) Fecal HP antigen measurement
- high accuracy (over 95%) for both **diagnosis** as well as **assessment of therapeutic efficiency** - it confirms the eradication of HP or its persistance after treatment
- nowadays has superior diagnostic value to the urea breath test, by the use of immunological assays with monoclonal antibodies.

III. INVESTIGATION OF SMALL BOWEL DISORDERS

Indications:
Patients with:
- **coprologic syndrome**: acute or chronic, fermentation or putrefaction diarrhea
- **malabsorption syndromes**: dyspepsia (abdominal distension, belching, flatulence), abdominal cramps, chronic diarrhea and/or steatorrhea, appetite modifications, weight loss
- **various defficiencies**: iron, B12 and folic acid, liposoluble vitamins (A,D,K), water, minerals (calcium, magnesium, potassium), weight loss (moderate to cashexia)

• Etiology of malabsorption:
- **DIGESTIVE disorders:**
  - Impairment of *intraluminal digestion*:
    - pancreatic failure
    - lack of bile salts in the intestine
  - Impairment of *intestinal absorbtion*:
    - *primary* disorders of the intestinal mucosa (lactose intolerance, celiac disease)
    - *secondary* intestinal mucosal damage in chronic inflammatory bowel diseases (eg Crohn's disease or regional ileitis)
- **METABOLIC and ENDOCRINE disorders:**
  - diabetes mellitus
  - hyperthyroidism
  - adrenal failure

The investigation of small bowel disorders and malabsorption syndromes comprises:

- **Static investigations:**
  - are useful in the **etiological diagnosis** of malabsorption syndromes:
    - **Stool examination**
    - Increased levels of inflammatory markers (ESR, leucocytosis, CRP) - in *inflamatory bowel diseases*
    - *Serum autoantibodies against gliadin and transglutaminase* – in celiac disease
  - are useful for the assessment of **metabolic and nutritional consequences** of chronic malabsorption by performing:
    - **Blood tests**
    - Bone X-ray and osteodensitometry (osteoporosis, osteomalacia)
- **Dynamic investigations** - are specific tests designed to diagnose the *type of malabsorption*, most commonly for *carbohydrates* (lactose tolerance test, D-xylose assay) and lipids (radioactively labeled triolein or oleic acid breath test) and are performed only in specialized clinics
- **Barium follow through** – in order to explore small bowel motility
- **Tests that explore intestinal bacterial proliferation**
- **Abdominal ultrasound:** useful in case of suspicion of intestinal occlusion, where the intestinal loops appear dilated and full of fluid, with thickening of the intestinal wall.
- **Biopsy of the intestinal mucosa:**
  - is obtained through GI tract endoscopy (duodenoscopy, balloon enteroscopy)
  - is **mandatory** for the ***positive etiologic diagnosis*** of the malabsorption syndromes
- **Capsule endoscopy** (expensive, rarely performed in practice):
  - useful for the diagnosis of gastrointestinal bleeding (in which gastroscopy and colonoscopy did not reveal the etiology), of small bowel tumors and occult inflammatory bowel diseases!
  - is contraindicated in the case of suspected intestinal strictures.

A. STATIC Investigations

1. STOOL examination

a) MACROSCOPIC examination – shows:
- changes of appearence, colour and smell
- the presence of pathological elements such as: mucus, puss, blood, undigested food (also called lientery), intestinal parasites

b) MICROSCOPIC examination – shows:
- **Muscle fibres (creatorrhea)**:
  - a large quantity of ***undigested*** muscle fibres is called creatorrhea and demonstrates a pancreatic failure or accelerated peristaltic movement of the bowels
- **Lipids (steatorrhea):**
  - are normally present in a low quantity or absent in the stool (2-6 g per day for 100 grams of lipid intake)
  - can be present in the stool as *neutral lipids* (orange-red in the Sudan III stain) or as *fatty acids* (dark blue in the Nile blue stain)

- an **increase in lipid excretion in the stool > 7 g/day** is called **steatorrhea** and can be a consequence of:
  - *hepatic disorders* (low synthesis of bile salts)
  - *obstructive jaundice* (due to the absence of bile salts in the intestine)
  - *pancreatic disorders* (decreased secretion of pancreatic lipase)
  - *intestinal disorders* (decreased lipid absorption due to increased bacterial proliferation, lesions of the intestinal mucosa, etc.)

- **Starch:**

- is highlighted using the Lugol solution (undigested starch appeares blue, whilst partially digested starch gains a dark purple colour)
- is present in small amounts in normal stool
- a large amount of undigested starch usually indicates pancreatic failure

- **Cellulose:**

- is found in the stool in the *digestible* (completely digested in the colon) and *indigestible* (which escapes the action of microbial flora) form
- the presence of digestible cellulose in high amounts in the stool indicates increased peristaltic movements or a mastication defect

- **Cells:**

- *erythrocytes*: digested in upper gastro-intestinal bleeding, undigested in hematochezia (the passage of fresh blood)
- *leukocytes*: suggest an inflamatory condition and represent an indication to perform stool culture
- *parasite eggs*: their presence represents an indication for the coproparasitological examination - at least 3 samples should be analysed at a 2-3 days interval!

c) CHEMICAL examination – consists of:

- **pH measurement:**

- is done by using litmus paper; normal stool has a *neutral* or *slightly alkaline* pH
- an acidic pH results from increased *fermentation* processes
- an alkaline pH results from increased *putrefaction* processes

- **OCCULT BLEEDING test:**

  - **The Guaiac Fecal Occult Blood Test** (gFOBT) or the **Hemoccult test**

- is used to detect the presence of fecal occult blood (blood not macroscopically visible in the feces) recommended to be performed **annually in people aged over 45-50 years** as a **screening test** for identification of subjects with **indication for colonoscopy**
- the test involves placing a faecal sample on guaiac paper and applying hydrogen peroxide which, in the presence of blood, yields a blue reaction product within seconds
- the patient requires a 2-3 day preparation in advance:
  - „white diet" - no intake of red meat (contains myoglobin) or certain vegetables (contain chlorophyll), iron supplements or NSAIDs (false positive results)
  - no vitamin C or citrus fruits (false negative results)
  - the test will NOT be performed during menstruation or in patients with active bleeding due to hemorrhoids

  - **The Immunochemical Fecal Occult Blood Test** (iFOBT) or the **Fecal Immunochemical Test** (FIT):

- FIT only detects human blood by using anti-hemoglobin antibodies
- is more accurate that gFOBT since drugs and food do not interfere with the test and no prior preparation of the patient is needed

2. BLOOD tests

- **Complete blood count** - can show the presence of ***microcytic or macrocytic anemia***
  - Serum ferritin, serum iron, total iron-binding capacity and transferrin saturation are performed if the MCV is low (iron deficiency anemia).
  - Serum levels of B12 and folate are performed if the MCV is high (megaloblastic anemia)
- **Serum electrolytes** - a low level of electrolytes can be due to chronic diarrhea:
  - *hypocalcemia* (plus an increase of alkaline phosphatase suggests the presence of osteomalacia due to vitamin D deficiency)
  - *hypomagnesemia*
  - *hypokalemia*
- **Serum protein levels** - are indicators for the nutritional status:

- *hypoproteinemia* and *hypoalbuminemia* are present with advanced malabsorption syndrome

- **Coagulation tests** - *prolongation of the protrombin time* (PT, Quick time) is suggestive for vitamin K deficiency due to lipid malabsorbtion
- **Lipid profile** - can show *hypolipidemia* and *hypocholesterolemia* in lipid malabsorption

B. DYNAMIC Investigations

1. Lactose tolerance test

• Principle:

- fasting plasma glucose is determined and 50g lactose mixed with 400 ml water and a barium sulfate packet are then administered orally
- blood glucose is determined at 60 and 120 minutes
- an abdominal X-ray is performed at 60 minutes

• The test is positive for lactose intolerance if 3 types of manifestations occur:

- **clinical:** diarrhea (hyperacidic stools), abdominal colic, flatulence, borborygmi (a few tens of minutes after administration)
- **biological:** lack of increase in blood glucose by more than 25% of the fasting plasma value (flat blood glucose curve)
- **radiological:** dilution of the barium mass (by hypersecretion), aeroenteria with intestinal distension and very accelerated intestinal transit (barium reaches the colon 60 minutes after administration)

C. BARIUM FOLLOW THROUGH

• Clinical value:

- indirect evaluation of small bowel motility by tracking the contrast substance through the intestine
- allows the detection of a possible **lesion** of the **wall** of the jejunum or ileum, or of an **obstruction** by an *inflammatory or proliferative process*

D. TESTS THAT EXPLORE INTESTINAL BACTERIAL PROLIFERATION

1. HYDROGEN breath test (HBT)

• Principle:

- oral administration of 50 g of ***lactulose*** that will be degraded by bacteria with the release of hydrogen
- **early increase of hydrogen levels in the exhaled air** indicates:
  - a rapid degradation of lactulose by bacteria in the small intestine (SIBO - Small Intestinal Bacterial Overgrowth);
  - an accelerated intestinal transit in the colon

2. C^{14} GLYCOCHOLIC acid breath test

• Principle:

- radiolabelled **bile salts** are administered orally
- bacteria act on bile salts and release radioactive glycine
- radioactive glycine will be metabolized, generating $^{14}CO_2$ which will be eliminated by breathing out
- early increase in radioactivity of the exhaled air indicates:
  - bacterial proliferation in the proximal intestine
  - accelerated peristaltic movements in the colon

Observation!

In the last decade, special attention has been paid to the **study of the human microbiota** (the microroganisms that populate the digestive system: saprophytes and pathogens, fungi, viruses) that are 10 times more abundant than the cells of the human body. The current paradigm postulates a central role for the gut microbiota in modulating metabolic, nutritional, inflammatory and immune processes and, more recently, psychic processes. Colonization of the gastrointestinal tract of the newborn (sterile at birth) depends on: the type of birth (natural or caesarean), the type of food (breast or milk preparations), gestational age, environmental and genetic factors. The intestinal microbiota is considered to be life-stable and specific for an individual (similar to fingerprints) and also self-regenerating (regeneration of the same microbial population) after episodes of aggression (eg, antibiotic therapy).

The *in vitro* evaluation of the quantitative and qualitative changes of the microbiota, currently available through expensive molecular gene studies (nowadays used only for research purposes) has led to the identification of associations between these changes and numerous chronic pathologies. Thus, in chronic inflammatory bowel diseases, i.e. Crohn disease and ulcerative colitis, a reduction in the number and variety of commensal intestinal bacteria has been reported. Moreover, the excessive activation of Th2 lymphocytes, responsible for the increased prevalence of atopic terrain and childhood allergies is favored by intestinal dysbiosis. In recent years, intestinal dysmicrobism has been

studied in connection with the predisposition for the diseases of the century: obesity, Alzheimer disease and depression.

Fecal microbiota transplantation or fecal bacteriotherapy is the therapeutic intervention by which fecal bacteria are transplanted from a healthy individual to another individual to restore colonic microbiota (the introduction of healthy 'saprophytic bacteria' either by enema instillation of feces or by oral administration of freeze dried encapsulated fecal microbiota). Fecal transplantation is currently being used with successful results in the treatment of recurrent Clostridium difficile infection. In recent years, bacteriotherapy has also been tested in other digestive (enterocolitis, constipation, irritable bowel syndrome) but also extradigestive disorders (Parkinson's disease and multiple sclerosis).

IV. INVESTIGATION OF LARGE BOWEL DISORDERS

Indications:
Patients with:
- **abdominal pain**
- **constipation, diarrhea**
- **occult hemorrages**
- **hematochezia** (fresh blood in the stool)

Investigation of the large bowel consists of:
1. **Abdominal X-rays**
2. **Colonoscopy**
3. **Barium enema**
4. **Fecal calprotectin test**

1. The abdominal X-ray

• Indicates:
- an excessive quantity of air in the colon
- air-fluid levels in bowel obstruction
- radioopaque foreign bodies: calculi or swollen objects (frequently in pediatrics)
- Chilaiditi syndrome (the radiological finding of a colonic interposition between the liver and the diaphragm)

2. Colonoscopy

There are 2 technical variants:
- ***complete*:** visualization of the entire colon up to the ileo-cecal valve, using the colonoscope
- ***sigmoidoscopy:*** *flexible* (can visualize the distal colon up to the splenic flexure) or *rigid* (can visualize the last 20-25 cm of the colon) is addressed especially to patients with diarrhea or lower abdomen symptoms (eg rectorrhagia).

• Clinical value:
- it is both a ***diagnostic*** and ***therapeutic*** procedure since it allows biopsies, polypectomies, hemostasis for lesions with active bleeding, dilations of stenotic segments with a stent insertion in critical bowel obstructions
- it is the ***gold standard*** in **early detection of colorectal cancer** in patients with **occult bleeding detected with the Hemoccult tests.**

Observation!
Virtual colonoscopy involves the insufflation of CO_2 into the previously cleansed colon and represents an alternative to classical colonoscopy to visualize colon tumor masses.

3. Barium enema

• Principle:
- Comprises the opacification of the colon with barium paste introduced with caution through the rectum. The progression of the radiopaque substance is followed by means of a radiography screen and various images are obtained in various incidences

• Clinical value:
- diagnosis of tumoral lesions (benign polyps or malignant tumors), ulcers, stenoses
- it is less sensitive than the colonoscopy especially for the detection of smaller lesions (approximately a quarter of tumor formations with a diameter of less than 1 cm are not visualized by means of the barium enema)
- *air contrast barium enema* (air is introduced after the elimination of barium) - is used to examine the lining of the colon mucosa

Remember!
Both methods, colonoscopy and barium enema, require an **adequate preparation of the patient** (strict diet, laxatives, evacuation enemas) in order for the procedures to succeed.

4. FECAL CALPROTECTIN test

- **Definition:** protein abundant in the PMN neutrophil cytoplasm, with antimicrobial (by calcium and zinc binding) and antiproliferative properties, released from cells during their

activation and/or degradation in chronic intestinal inflammation

- **Normal values (adults):** < 100 µg/g feces
- **Clinical value: non-invasive marker of chronic intestinal inflammation**
  - screening test used to differentiate *irritable bowel syndrome* (functional disorder) from **inflammatory bowel disease, infections, polyps and colorectal cancer** where calprotectin levels are elevated (over 200 µg/g feces)
- **Indications:**
  - identification of patients with abdominal symptoms that require invasive investigations
  - establishing the degree of disease activity, the stage of mucosal healing and the risk of relapse in patients with inflammatory bowel disease
  - monitoring the response to treatment in children with inflammatory bowel disease (invasive procedures are difficult to perform and require general anesthesia)

V. INVESTIGATION OF THE EXOCRINE PANCREAS DISORDERS

Indications:
In patients with acute and chronic pancreatic disorders:

A. Serum and urine assessment of pancreatic enzymes:
- useful for the diagnosis of **acute pancreatitis**
- inconclusive in **chronic pancreatitis**, except for acute flare-ups
- a correlation with *imaging techniques* (ultrasound, computed tomography, MRI) must be performed in order to rapidly and non-invasively detect the degree of organ destruction

B. Secretory tests – evaluate the pancreatic functional reserve in patients with exocrine pancreatic failure

C. Stool examination - useful to identify the pancreatic exocrine failure in patients with chronic pancreatitis

A. Assessment of PANCREATIC ENZYMES

1. Serum Amylase

• **Normal values:** 25 – 125 U/L

• **Increased values** - can be found in:

- **Pancreatic disorders**: acute pancreatitis or exacerbation episodes of chronic pancreatitis:
  - increases are *important* (5-12 times the normal values), *early* (in the first 24h), but *transient* - reaching a normal level after 3-5 days
  - serum levels DO NOT correlate with the severity of the pancreatitis, but persistently increased values show an active process
- **Extrapancreatic disorders:**
  - salivary glands disorders (parotiditis, salivary calculi)
  - digestive tract disorders (perforated ulcer with peritonitis, bowel obstruction, mesenteric infarction, any kind of gall bladder disorder)

• **Decreased values**

- pancreatic failure
- severe hepatic disorders

2. Urine Amylase

• **Normal values** (24 h) = 6,5 – 48 U/h

• **Increased values:**
  - in acute pancreatitis increases occur rather *late* (after normalization of serum amylase), but are *persistent* (7-10 days), due to a transient renal impairment that inhibits tubular reabsorbtion of amylase

3. Serum Lipase

• **Normal values:** 10 - 160 U/L (differs depending on the method of assessement)

• **Increased values:**
  - appear *slower* as compared to the serum amylase, but their *persistence* is greater (for 3-8 days)
  - has *increased specificity* for the pancreas as compared to serum amylase
  - ***concomitantly*** increased serum levels of lipase and amylase increase the diagnostic accuracy in **acute pancreatitis** and exclude salivary glands disorders

Observation!
Investigation of the pancreas also includes:

- biochemical tests: complete blood count, plasma ionogram, blood glucose, CRP, serum creatinine, acid-base balance parameters, hepatic tests
- imaging techniques: CT - "gold standard" for the detection of pancreatic diseases, abdominal ultrasound and MRI.

B. SECRETORY tests

1. Fecal elastase 1 (FE-1) test

- **Principle:**
  - elastase-1 (E1) is a stable pancreatic enzyme that passes through the small intestine without being degraded and concentrates 5-6 times in the feces
- **Normal value** > 200 µg/g of feces
- **Pathological values:** elastase secretion is extremely low in pancreatic failure, and therefore its concentration decreases in the stool:
  - < 100 µg/g feces in severe forms
  - 100-200 µg/g feces in mild-moderate forms
- **Clinical value:**
  - ***early*** diagnosis of **exocrine pancreatic failure**, especially in children with **cystic fibrosis** (confirmation of the diagnosis)

C. STOOL examination

- **Macroscopic examination** - shows:
  - abundant, soft, light-yellow, shiny, greasy-like, rancid-smelling stools (due to steatorrhea)
  - the presence of undigested food
- **Microscopic examination** - shows:
  - neutral fatty drops (Sudan III stain)
  - undigested muscle fibers (creatorrhea)
  - alkaline reaction of the stool
- **Lipid digestion test:**
  - during a 3 day period, the patient receives a regimen with 100-130 g of lipids/day
  - lipid stool levels are determined (normal values: < 2-6 g/day)
  - elimination of **> 7 g/day** = steatorrhea
  - the test does NOT establish the severity of pancreatic lesions, because chronic pancreatitis can exist without steatorrhea
- **Stool nitrogen evaluation**
  - protein malabsorbtion induces an increase in stool nitrogen elimination
  - as with steatorrhea and creatorrhea, nitrogen appears in the stool only in advanced stages of pancreatic failure (over 70% damage of the pancreas)

CHECKPOINT

1. Which of the following are true about the gastric biopsy?
A. Is mandatory in duodenal ulcer
B. Allows the detection of dysplastic lesions
C. Is required to confirm the benign character of a gastric ulcer
D. Allows the diagnosis of H. Pylori infection
E. Is indicated only when the esophageal manometry is normal

***2. Which of the following tests is used to monitor treatment efficiency in Helicobacter pylori (HP) infection?**
A. H. Pylori identification in gastric biopsies
B. Serum anti-H. Pylori antibodies
C. Stool H. Pylori antigen
D. Urea breath test
E. Urease test

***3. Which of the following is mandatory for the positive etiologic diagnosis of a malabsorption syndrome?**
A. Stool examination
B. Hydrogen breath test
C. Capsule endoscopy
D. Intestinal mucosa biopsies
E. Fecal occult bleeding tests

4. Which of the following are correct regarding the Hemoccult test?
A. It becomes positive in upper gastrointestinal bleeding
B. Is a screening test that indicates which patient needs a colonoscopy
C. Does not require the patient preparation
D. It ishould be performed once a year in patients over 45-50 years old
E. It represents the gold standard for early detection of colorectal cancer

5. Which of the following are true about serum amylase in acute pancreatitis?
A. Major increased levels occur early, but are transient
B. Major increased levels occur late, but are persistent
C. Serum levels are correlated with the severity of pancreatitis
D. Persistently high levels can indicate an active process
E. Is due to the transient renal impairment that stimulates amylase tubular reabsorption

6. Which of the following are tests that contribute to the etiologic diagnosis of the malabsorption syndrome?
A. Biological inflammatory syndrome
B. Proteinemia
C. Complete blood count
D. Coprologic examination
E. Assessement of anti-gliadin and anti-transglutaminase antibodies

7. Which of the following statements are true about the increase of serum pancreatic enzymes in acute pancreatitis?
A. High specificity in the case of amylase
B. Concomitant increase of amylase and lipase increases diagnostic accuracy
C. It is early, important but transient in the case of amylase
D. It is slower but persistent in the case of lipase
E. It is parallel to the severity of pancreatitis in the case of amylase

8. Which of the following regarding the barium meal are true?
A. It is useful in the diagnosis of hiatal hernia if performed in the Trendelenburg position
B. It is the gold standard in the diagnosis of gastro-oesophageal reflux
C. Allows direct visualisation of oesophageal lesions in reflux esophagitis
D. Allows the assessement of the speed of the esophageal transit
E. It is the gold standard in the diagnosis of achalasia

9. Which of the following statements about the measurement of anti-Helicobacter pylori (anti-HP) antibodies in the serum are true?
A. It is the gold standard for identifying the infection
B. A positive result indicates either an acute or a chronic infection
C. It has an accuracy of 95% for the diagnosis of infection
D. It is the test that confirms the eradication of the infection after treatment
E. There is no correlation between the antibody titer and the severity of the infection

CASE STUDIES

1. A 55-year-old male patient with chronic alchoholism is brought to the emergency room with severe epigastric pain, nausea, vomiting, severely alterated general state.

Laboratory investigations:

Erythrocytes = 4.500.000/mm^3
Hb = 13 g/dL
Ht = 43%
Leukocytes = 16.000/mm^3
Blood glucose level = 200 mg/dL
Serum calcium = 8 mg/dL
Serum amylase = 520 U/L
Serum urea = 41 mg/dL
Serum creatinine = 1.2 mg/dL

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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2. A 55-year-old patient presents to a physician for epigastric pain that started 4 weeks ago and has been progressively aggravated. At the beginning the pain had a burning character and improved after food intake; now it is a dull epigastric pain associated with persistent fatigue. The patient has been a smoker from the age of 25.

Laboratory investigations:

Erythrocytes = 3.200.000/mm3
Hb = 10.7 g/dL
Ht = 31%

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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NOTES

5. INVESTIGATION OF LIVER AND GALLBLADDER DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Request the tests that define the 4 biohumoral syndromes characteristic of the liver diseases

2. Know and interpret the tests that define the syndrome of hepatocytolysis and the one of cholestasis in the context of the acute and chronic liver diseases

3. Know and interpret the tests that highlight the effects of chronic alcohol consumption on liver function

4. Know the investigations that evaluate fibrosis, steatosis and inflammation in chronic liver disease

5. Identify the types of jaundice with the help of laboratory tests and interpret their changes in the etiopathogenic context

I. LIVER FUNCTIONAL INVESTIGATION

- Liver functional investigation has the following **indications:**
  - diagnosis and monitoring of the evolution of chronic liver diseases
  - the presence of jaundice
  - the presence of coagulation disorders
  - use of medication with hepatocytotoxic effect
  - annual checkup in diabetic patients
  - suspicion of a chronic condition in order to assess liver damage
- Liver tests (LTs) are biochemical tests that can be further grouped into markers that assess the:
  1. Hepatocellular injury
  2. Cholestasis
  3. Liver function
  4. Fibrosis and inflammation
- In addition to these tests, supplementary investigations can be carried out which include:
  - **Complete blood count**
  - **Immunological tests**
  - **Imaging techniques**
  - **Non-invasive tests for liver fibrosis assessement**
  - **Percutaneous liver biopsy**

A. BIOHUMORAL SYNDROMES IN HEPATIC DISORDERS

1. MARKERS OF HEPATOCELLULAR INJURY

• Explore the damage/destruction of hepatocytes (*hepatocytolysis*), which is followed by the release into the circulation of cellular constituents, mainly ***hepatocytolysis enzymes*** and ***storage substances*** (oligoelements, vitamins).

a) Hepatic cytolysis enzymes

They increase in **all** acute and chronic hepatobiliary disorders. Their serum values **do NOT** always correlate with the severity of hepatocytolysis, therefore their monitoring in ***dynamics*** and ***evaluation in comparison with cholestasis enzymes*** is more useful. The main enzymes of hepatocytolysis are aminotransferases and LDH.

i) Aminotransferases (AST and ALT)

They are enzymes present both in the liver as well as in other tissues, whose serum level increase has the general significance of *cell damage*. For the evaluation of the liver injury the following are determined: AST (GOT), ALT (GPT) and the AST/ALT ratio (de Ritis coefficient).

- **Aspartate aminotransferase (AST) or serum glutamic oxaloacetic transaminase (GOT)**
  - **Normal values: < 40 U/L**
- it is located mainly at *mitochondrial* level *(80%, only 20% in the cytoplasm)*
- is present in significant amounts in the *liver, myocardium* and in smaller amounts in skeletal muscle, kidneys, erythrocytes, brain.
- when compared to ALT it is a <u>*less specific indicator of hepatic cytolysis*</u>.

- **Alanin aminotransferase (ALT) or serum glutamic pyruvic transaminase (GPT)**
  - **Normal values: < 40 U/L**
- is located exclusively in the *cytosol*
- is present in large quantities in the *liver*, its increase being ***specific for hepatocytolysis***
- in liver lesions the serum level *increases faster and more* as compared to AST

- **The AST/ALT ratio** (*de Ritis ratio*) has a mean value around **1**.

Clinical value:

- **In hepatic disorders:** AST and ALT increase as follows:
  - **> 10 times** in *acute hepatocytolysis*: acute viral hepatitis, acute toxic hepatitis, acute obstruction of the extrahepatic biliary tree
  - **5-10 times** in *active chronic hepatitis* and in *decompensated liver cirrhosis*
  - **2-5 times** in *persistent chronic hepatitis*
- **In viral HEPATITIS: ALT increase** is ***greater*** than the one of AST (**de Ritis ratio <1**)
- **In chronic ALCOHOLIC hepatitis**: **AST increase** is ***greater*** as compared to ALT because alcohol induces mitochondrial injury which leads to AST release (de Ritis ratio > 1, possibly associated WITH jaundice). Values > 2 have been associated with the risk of **evolution of chronic ethanolic liver disease towards fibrosis and cirrhosis.**
- In **severe hepatic injuries** - e.g., acute fulminant toxic hepatitis: a *severe drop* in ALT and AST signifies the *extreme severity of hepatocytolysis* (severe reduction of functional hepatic parenchyma).
- In **extrahepatic injuries** - e.g., acute myocardial infarction, shock, acute pancreatitis, rhabdomyolysis, intravascular hemolysis: **AST increase** is greater than the one of ALT (de Ritis ratio > 1 but WITHOUT jaundice association).

ii) Lactate dehydrogenase (LDH)

- is an intracellular enzyme widely distributed in the body, being found mainly in the *myocardium, erythrocytes, kidneys, lungs, liver and skeletal muscle*. LDH has 5 isoenzymes, of which the *LDH_5 isoenzyme is specific for the liver.*
  - **Normal values: 240-480 U/L**

Clinical value

- *moderate* increases in LDH are found in acute viral hepatitis, alcoholic hepatitis, liver cirrhosis, biliary tract disorders.

b) Hepatic storage products

They are mainly increased in ***acute*** hepatic disorders, proportional to the severity of liver injury.

- ***Increased Serum Iron***: is an indicator of severe hepatocellular injury that occurs in *acute viral or toxic hepatitis*.
  - **Normal values:**
    - **60 – 160 µg/dL in men**
    - **50 – 150 µg/dL in women**
- ***Increased Serum Vitamin B_{12}***: the liver represents the main storage site of vitamin B_{12}. The increased level of plasma vitamin B_{12} occurs in acute hepatitis, but also in decompensated cirrhosis and drug-induced cholestasis.
  - **Normal values = 160 – 925 ng/L**

2. MARKERS OF CHOLESTASIS (Cholestatic enzymes)

These are enzymes secreted into the bile by *hepatocytes* (gamma-glutamyl transferase - GGT, alkaline phosphatase - ALP, 5'-nucleotidase) and the *epithelial cells of the bile ducts* (GGT), respectively. Increased serum levels reveal **biliary stasis (cholestasis)**, which can be **intrahepatic** and/or **extrahepatic**. Their diagnostic significance is further increased if the cholestatic enzymes are ***simultaneously measured*** with the enzymes of hepatocellular injury.

a) Gamma glutamyl transpeptidase/ transferase (GGT) – is the **specific** indicator of cholestasis; it is secreted by the epithelial cells of the bile ducts and the liver. GGT secretion significantly increases in ***chronic alcohol consumption*** and is correlated with the duration and amount of alcohol consumed when GGT increase is isolated or preponderant versus that of ALP. GGT may be used to monitor alcohol withdrawal.

- is the **indicator of cholestasis** when the increase is associated with that of ALP (having the same route of excretion).
  - **Normal values:**
    - **11–58 U/L in men**
    - **7–32 U/L in women**

b) Alkaline phosphatase (ALP) – is the **non-specific** indicator of cholestasis; it has several isoforms that originate from the bones, intestine, liver and placenta. In the absence of bone disorders or pregnancy, elevated ALP serum levels usually reflect *cholestasis*. To confirm the hepatic origin of ALP it is recommended to measure both the **bone isoform** of ALP as well as **5'-nucleotidase**:

- ALP increase is of ***bone origin*** if the bone ALP isoform is also increased
- ALP increase is of ***hepatic origin*** if 5'-nucleotidase is increased
  - **Normal values:** < 120 UI/L

Clinical value:

- **Parallel increase of GGT and ALP** – can be:
  - **Mild** (2 x normal value) in:
    - acute viral or toxic hepatitis
  - **Moderate** (2-10 x normal value) in:
    - chronic active hepatitis (viral or autoimmune)
    - hepatic cirrhosis
  - **Marked** (>10 x normal values) in:
    - mechanical obstruction of the extrahepatic biliary tree (eg., pancreatic head cancer, common bile duct stones, etc.)
    - primary biliary cholangitis - increases of GGT and ALP ***precede jaundice*** !
    - primary liver tumors and liver metastases
- **Markedly elevated GGT compared to ALP** occurs in:
  - chronic alcoholic hepatitis
  - alcoholic cirrhosis
  - drug-induced cholestasis (eg., oral contraceptives, anti-thyroid drugs, anabolic hormones)
- **Isolated increase of GGT without ALP serum level increase** occurs in:
  - alcoholic liver steatosis

3. MARKERS OF LIVER FUNCTION

Explore the decreased liver function, which is characteristic for chronic liver disease.

a) Assessement of bile pigments in the blood and urine

- **Total Bilirubin (TB)**
  - **Normal values = 0,8-1 mg/dL**

Clinical value:

- TB between **1,5 and 2,5 mg/dL** = subicterus
- TB **> 2,5 mg/dL** = **jaundice**

- **Unconjugated (UB) / Indirect (IB) Bilirubin**
  - **Normal values = 0,2 -0,7 mg/dl**

Increases in:

- hemolysis = **hemolytic jaundice**
- hepatic cytolysis = **hepatocellular jaundice**

- **Conjugated (CB)/Direct (DB) Bilirubin**
  - **Normal values**: **0,1- 0,3 mg/dL (or < 20% of TB)**

Increases in:

- extrahepatic biliary obstructions = **obstructive jaundice**
- intrahepatic biliary obstruction associated with hepatic cytolysis = **hepatocellular jaundice**

- **Identification of Bile Pigments in Urine**

- This test is performed by *semi-quantitative* methods during urine examination:
  - ***Urobilinogen (Ubg):***
- increases (urobilinogenuria) in *hemolytic jaundice* and *hepatocellular jaundice*
- is absent in *obstructive jaundice*
  - ***Bilirubin***:
- is absent in *hemolytic jaundice*
- increases (bilirubinuria) in *hepatocellular jaundice* and *obstructive jaundice*

b) Impairment of the Liver Synthetic Function

Depending on the disease severity, one or all three intermediary metabolisms can be affected.

○ *Impairment of Protein Metabolism*

1. Serum cholinesterase (pseudo-cholinesterase)

- It is an enzyme synthesized by the liver, pancreas, heart and cerebral white matter. Also known as pseudocholinesterase, it should not be mistaken for acetylcholinesterase ("true" cholinesterase). Although the biological role of cholinesterase is unknown, its values ***drop early***, in chronic liver disease (e.g., cirrhosis, chronic hepatitis, liver metastases, liver congestion in heart failure) - **prior to the decrease in plasma albumin**.
  - **Normal values: 4,9 - 11,9 U/mL**

2. Serum Proteins

- All plasma proteins are synthesized by the liver (except for immunoglobulins = γ-globulins). Since albumin is the major protein fraction, the degree of ***hypoalbuminemia is an indicator of the severity*** of the impairment in liver synthetic function. In chronic liver disease, decreased serum albumin levels are associated with an **increase in globulins** due

to the increase of **γ-globulins**, with a **decrease in the albumin/globulins ratio < 1**.
- **Normal values:**
  - **Total proteins = 6,7 – 8,4 g/dl**
  - **Albumins = 3,5 – 5,5 g/dl**
  - **Albumins/globulins ratio = 1,2 – 1,5**

3. Serum proteins electrophoresis (ELFO)
- ELFO abnormalities characterize the *dysproteinemia from chronic liver disease* and are related to the presence of:
  - **Altered liver synthetic function**: decreased albumins
  - **Cholestasis**
    - increase of α2-globulins in chronic alcoholic hepatitis and cirrhosis
    - increase of β-globulins in primary biliary cholangitis
  - **Presence of the inflammatory-immune syndrome**: policlonal γ-globulins increase in chronic hepatitis and liver cirrhosis, in response to the altered capacity to eliminate intestinal toxins (with endotoxemia risk)
    - **Normal values:**
      - **Albumins = 50-60%**
      - **α1-globulins = 3-6%**
      - **α2-globulins = 7-10%**
      - **β-globulins = 11-14%**
      - **γ-globulins = 15-23%**

4. Prothrombin time (PT)
- Evaluates the liver's capacity to synthesize the vitamin K-dependent clotting factors - **II, VII, IX and X**. In this respect, the prothrombin index (PI) is preferentially used, expressed as a percentage of the normal prothrombin activity:
  - in acute and chronic hepatitis, the PI decreases to 70-80%
  - in liver cirrhosis, the PI is below 60% !
  - **Normal values:**
    PT = 11 – 14 sec
    PI = 80-100%

5. Fibrinogen
- **Decreases in advanced stages of hepatic cirrhosis (liver failure)**.
  - **Normal values: 200 – 400 mg/dl**

• *Impairment of Lipid Metabolism*

The liver plays a central role in lipid metabolism in terms of their synthesis, biliary/plasma excretion and lipid degradation. In this regard, the serum level of lipids (lipemia), triglycerides (TG) and total cholesterol (TC) is determined:
- Total serum lipids, cholesterol and triglycerides increase in *primary biliary cholangitis and obstructive jaundice*
- Total serum lipids, cholesterol and triglycerides decrease in *advanced stages of liver cirrhosis (hepatic failure)*
  - **Normal values:**
    - **Total Cholesterol 140-200 mg/dl**
    - **Triglycerides = 50-150 mg/dl**
    - **Total lipids = 400-800 mg/dl**

• *Impairment of Carbohydrate Metabolism*

The liver is the *main organ responsible for blood glucose control* and also the major site of glycogen storage (glycogenogenesis) and de novo synthesis of glucose (gluconeogenesis).
- **Normal values**:
  - **Blood glucose: 70 – 110 mg/dL**

• Pathological changes:
- Blood glucose: is normal or slightly elevated in chronic liver diseases and liver cirrhosis (due to the decreased glycogenogenesis). In the case of acute fulminant hepatitis with extensive hepatocyte necrosis and in advanced hepatic failure, severe hypoglycemia occurs (by depletion of glycogen stores and decreased neoglucogenesis).

• *Impairment of the Conjugation Function*

It is manifested by decreased serum levels of *esterified cholesterol* (< 70% of total cholesterol).
- **Normal values:**
  - **Esterified Chol. / Total Chol. = 0.7-0.8**

• *Impairment of the Detoxification Function*
- ***Increased serum NH_3*** occurs in:
  - acute fulminant hepatitis
  - hepatic cirrhosis complicated with gastrointestinal bleeding
  - presence of porto-caval shunts
  - **Normal values**: **15-45 µmol/L**
- ***Decreased serum urea*** – appears in the advanced stages of cirrhosis (liver failure).
  - **Normal values**: **15-45 mg/dL**

Remember!
High *ammonia* levels is the main factor responsible for the production of hepatic encephalopathy in patients with decompensated cirrhosis!

4. MARKERS OF INFLAMMATION

Definition: sdr. characterized by inflammatory and immunological responses underlying the pathogenic mechanisms of: acute/chronic postviral hepatitis, chronic active autoimmune hepatitis, liver cirrhosis

- ***Erythrocyte sedimentation rate (ESR)***
- Increased values (more than 30 mm/h) occur during *acute viral hepatitis, chronic active hepatitis and decompensated cirrhosis*.
  - **Normal values: 0 – 20 mm/h**

- ***Serum protein electrophoresis***
- The decrease in albumin synthesis as a marker of hepatocellular failure is usually associated with an increase in the production of **γ–globulins** (marker of hepatic reticuloendothelial tissue hyperactivity !)
- The increase of γ–globulins is generally significant:
  - up to **25-30%** in chronic hepatitis
  - **more than 30%** in liver cirrhosis

- ***Immunoelectrophoresis***
- **Increases of IgM** occur in:
  - acute viral hepatitis
  - primary biliary cholangitis
- **Increases of IgG** occur in:
  - acute viral hepatitis
  - autoimmune chronic hepatitis
- **Increases in IgA** occur in:
  - alcoholic hepatitis
  - alcoholic cirrhosis
- **Normal values:**
  IgG = 800 – 1500 mg/dL
  IgM = 50 – 250 mg/dL
  IgA = 100 – 500 mg/dL

B. SUPPLEMENTARY TESTS THAT EVALUATE HEPATIC DISORDERS

1. COMPLETE BLOOD COUNT (CBC)

- **Anemia** can be:
  - *Microcytic hypochromic* - chronic blood loss
  - *Macrocytic* with spherocytes - in patients with chronic alcoholism
  - *Megaloblastic* - vitamin B12 deficiency
  - *Normocytic normochromic* (with schizocytes) - in hemolysis due to hypersplenism
- **Leukopenia** (WBCs < 4,000/mm^3) - in patients with splenomegaly and hypersplenism
- **Thrombocytopenia** (PLT < 150,000/mm^3) - in patients with cirrhosis (through splenomegaly and hypersplenism) or chronic alcohol consumption (by alcohol-induced bone marrow suppression)

2. IMMUNE TESTS

a) Immune markers of viral infections
- *Antigens*: HBsAg, HBeAg and HBV-DNA in hepatitis B, HCV-RNA in hepatitis C
- *Antibodies*:
  - anti-HAV IgM and IgG in hepatitis A
  - anti-HBs IgM, anti-HBe IgG and anti-HBc IgG in hepatitis B
  - anti-HCV in hepatitis C

b) Autoantibodies:
- Anti-nuclear (ANA) in *autoimmune hepatitis*
- Antimitochondrial (AMA) in *primary biliary cholangitis*, etc.
- Anti-neutrophil cytoplasmic antibodies (ANCA) in *primary sclerosing cholangitis*

3. BIOCHEMICAL TESTS

a) α_1-Antitripsin
- is a glycoprotein synthesized by the liver, also representing an acute phase protein which constitutes 90% of serum α1-globulins. Its major role is to inhibit proteolytic enzymes (mainly *elastase*).

b) Alpha-fetoprotein (AFP)
Is a glycoprotein synthesized during the fetal period in the gastrointestinal tract, liver and yolk sac. After birth, AFP levels gradually decrease in the first year of life, reaching values similar to those of adults. In adults, AFP novel increases (transient or persistent) occur in:
- ***benign*** liver disease - eg., chronic hepatitis, cirrhosis
- liver ***malignancies*** - eg., hepatic carcinoma
  - **Normal values**: < **10 ng/mL**

Clinical value:
- AFP is the ***tumor marker used for monitoring patients with liver cirrhosis and risk for liver carcinoma***. For this category of patients it is recommended to perform an *abdominal ultrasound* (considered to be the

screening test for the early detection of liver carcinoma) and *AFP measurements* every 6 months:
  - increase of **AFP > 200 ng/ml** + changes seen in ultrasound are suggestive for ***hepatocarcinoma***
  - ***liver biopsy*** is required for the definitive diagnosis!

4. IMAGING INVESTIGATIONS

- ***Endoscopic examination*** (esophagoscopy) – **diagnosis and treatment of esophageal varices**
- ***Abdominal ultrasound*** - the most widely used non-invasive diagnostic method that allows:
  - description of liver size and structure
  - identification of changes in the biliary tree and the presence of gallstones
  - identification of cirrhosis and portal hypertension signs
- ***Color Doppler ultrasound*** – highlights the vascularization of a liver lesion.
- ***Contrast-enhanced abdominal CT*** - allows the evaluation of the structure and size of intra-abdominal organs, the characterization of various liver lesions and of their vascularization.
- ***MRI*** - the ***most sensitive investigation for focal liver lesions***, being capable of also evaluating liver fibrosis.

5. LIVER BIOPSY

Performed under ultrasound, laparoscopic or computed tomography control, it is the **gold standard** for the diagnosis of liver disease with the following main indications:

- the diagnosis of chronic diffuse liver diseases (chronic hepatitis and cirrhosis)
- the assessment of disease activity for a correct therapeutic approach
- diagnosis of unexplained hepatomegaly and/or identification of the etiology of inconclusive ultrasounds images in the setting of persistently altered liver tests.

Liver biopsy is contraindicated in the case of un-cooperative patients, with prolonged INR, no. platelets < 60,000/mm^3, ascites, extrahepatic cholestasis.

6. NON-INVASIVE TESTS FOR THE EVALUATION OF LIVER FIBROSIS

The tests used for the non-invasive evaluation of liver fibrosis range from simple (APRI - Aspartate aminotransferase to Platelet Ratio Index) to complex (FibroTest, ELF – Enhanced Liver Fibrosis test).

- **Liver elastography (FibroScan)** is a modern technique that allows quantification of ***liver elasticity*** (inversely proportional to the degree of hepatic fibrosis), based on the propagation of shock waves within the liver tissue. The obtained values allow **staging of the severity of fibrosis (F0-F4)** - *stage F4 is characteristic for cirrhosis*.
- **FibroTest -** is a non-invasive test that allows the quantification of ***liver fibrosis and inflammation***. It combines five standard biomarkers, which are introduced into an algorithm: *GGT, Total bilirubin, Alpha-2-macroglobulin, Apolipoprotein A, Haptoglobin*.
- **FibroMax -** it is a non-invasive test currently accepted as the ***universal marker of fibrosis in the evaluation of chronic liver disease***. It comprises 5 sets of tests and was designed to ***replace, in selected cases, the percutaneous liver biopsy***; currently it is part of the *inclusion criteria in the antiviral treatment plan of patients with chronic viral hepatitis B or C*.

II. INVESTIGATION OF JAUNDICE

- **Definition**: Jaundice (icterus) is a yellow discoloration of the sclerae, mucous membranes and skin, produced by bilirubin accumulation in tissues and detectable when the serum total bilirubin is greater than **2,5 mg/dL**.
- **Classification:**
  - *Jaundice with predominant **un-conjugated/ indirect bilirubin (UCB/IB)***
    Typical Ex.: ***hemolytic jaundice*** (pathological intravascular or extravascular hemolysis)
  - *Jaundice with predominant **conjugated/direct bilirubin (CB/DB)***
    Typical Ex.:

- **hepatocellular jaundice** in: acute viral/toxic hepatitis, chronic active hepatitis, cirrhosis
- **obstructive jaundice** in: biliary lithiasis, pancreatic head carcinoma, cholangio-carcinoma (rare)

A. Characteristics of hemolytic jaundice (PREHEPATIC)

- ***predominantly unconjugated (indirect) hyperbilirubinemia*** with lemon-yellow, **flavin** jaundice
- ***acholuric jaundice*** (without bilirubinuria)
- ***hyperchromic feces*** (via increased production of stercobilin)
- ***urobilinogenuria*** (with increased production of urobilin)
- **aminotransferases and cholestasis enzymes are normal**, but there is an isolated **AST** increase, from the lysed red blood cells.

• Biological markers

- **Serum:**
  - DB < 20% of TB
- **Urine:**
  - Ubg (++)
  - Bilirubinuria: absent

B.Characteristics of HEPATOCELLULAR JAUNDICE (PARENCHYMAL)

- ***mixed hyperbilirubinemia, predominantly conjugated***, with ***rubin jaundice***
- ***choluric jaundice*** (presence of bilirubinuria, urine has a yellow-brown color)
- ***normally colored*** (reduced cholestasis) or ***discolored*** (intense cholestasis) feces
- ***urobilinogenuria***
- *aminotransferases and cholestasis enzymes are increased*, but ***hepatic cytolysis is more important than cholestasis***

• Biological markers:

- **Serum:**
  - DB = 20-50% of TB
- **Urine:**
  - Ubg (++)
  - Bilirubinuria (++)

C. Characteristics of obstructive jaundice (POSTHEPATIC obstruction)

- ***predominantly conjugated (direct) hyperbilirubinemia*** with ***verdin*** jaundice and pruritus
- ***choluric jaundice*** (presence of bilirubinuria, urine has a dark brown color)
- ***hypochromia of the feces***
- ***absence of urobilinogenuria***
- *aminotransferases and cholestasis enzymes are increased*, but ***cholestasis is more important than hepatic cytolysis***

• Biological markers:

- **Serum:**
  - DB > 50% of TB
- **Urine:**
  - Ubg: absent
  - Bilirubinuria (++)

CHECKPOINT

1. Which of the following changes occur in acute viral hepatitis?
A. Sudden decrease of ALT and AST
B. Increase of ALT greater than AST increase
C. De Ritis ratio > 1
D. De Ritis ratio < 1
E. Sudden decrease of LDH_5

2. Which of the following changes suggest a liver disease caused by chronic alcohol consumption?
A. Increased ALT and AST over 10 x normal values
B. AST increase is greater than ALT increase
C. De Ritis ratio < 1
D. GGT isolated increase
E. Increased IgA

***3. Which of the following characteristics defines hemolytic jaundice?**
A. Absence of urobilinogenuria
B. Indirect hyperbilirubinemia
C. Predominantly conjugated hyperbilirubinemia
D. Isolated increase of ALT
E. Bilirubinuria

4. Which of the following characteristics define hepatocellular jaundice?
A. Predominant increase of GGT and ALP over aminotransferases
B. Predominantly indirect hyperbilirubinemia
C. Urobilinogenuria
D. Bilirubinuria
E. Acholuric jaundice

***5. Which of the following decreases early on in chronic liver disease?**
A. Serum albumins
B. Factors II, VII, IX and X
C. Esterified cholesterol
D. Serum cholinesterase
E. Blood sugar

6. Which of the following parameters are decreased in hepatic failure?
A. GGT
B. Fibrinogen
C. Serum urea
D. Serum ammonia
E. Blood sugar

7. Which of the following define the acute hepatocytolysis syndrome?
A. Increased serum iron
B. Unconjugated hyperbilirubinemia
C. 10-fold increase of aminotransferases
D. Marked increase of GGT and ALP
E. Acholuric jaundice

***8. Which of the following statements regarding the cholestasis enzymes are true?**
A. ALP increase is of bone origin if 5'nucleotidase is also increased
B. Parallel increases in GGT and ALP occur in acute viral hepatitis
C. ALT and AST are specific indicators of cholestasis
D. The isolated increase of GGT without ALP increase occurs in chronic ethanolic hepatitis
E. Isolated GGT without ALP increase precedes jaundice in primary biliary cholangitis

9. Which of the following changes can be caused by obstructive jaundice?
A. Predominant increase of indirect bilirubin
B. Presence of bilirubin in urine
C. Presence of urobilinogen in urine
D. Predominant increase of hepatocytolysis enzymes
E. Increased cholesterol

10. Which of the following are changes that may occur in hepatocellular jaundice in acute viral hepatitis?
A. Predominant increase of hepatocytolysis enzymes
B. Hyperchromia of feces
C. Predominant increase of cholestasis enzymes
D. Presence of bilirubin in urine
E. Presence of urobilinogen in urine

CASE STUDIES

1. A 45-year-old patient presents herself to the emergency room for altered general state, fever (38.8°C), nausea, abdominal pain. The general clinical examination reveals moderate jaundice, painful abdomen on palpation in the right upper quadrant, hepatomegaly, BP = 90/60mmHg, HR = 120b/min.

Laboratory investigations:

Blood:

AST = 205 U/L
ALT = 310 U/L
GGT = 876 UI/L
ALP = 880 UI/L
TB = 6,54 mg/dL
DB = 4,82 mg/dL

Urine:

Ubg (-)
Bilirubin (+++)

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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2. A 50-year-old patient with chronic alcoholism is rushed to the emergency room for hematemesis. The clinical examination reveals jaundice, splenomegaly, liver with a sharp lower edge and nodular surface.

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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NOTES

6. INVESTIGATION OF ACID-BASE DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Enumerate the main parameters that define the acid-base balance

2. Identify the primary and secondary (compensatory) changes that define an acid-base disorder

3. Establish the degree of compensation in a primary acid-base disorder (non-compensated, partially compensated or fully compensated)

4. Recognize a mixed acid – base disorder

5. List the main causes for each metabolic and respiratory acid-base disorder

I. ACID-BASE PARAMETERS - BRIEF PHYSIOLOGY OVERVIEW

Acid–base homeostasis, i.e. the ability of maintaining the extracellullar fluid pH between **7,35 – 7,45**, is fundamental for maintaining life.

The pH is a measure of the hydrogen ion concentration in a solution and is calculated as the negative logarithm of the protons concentration, (is inversely correlated with the acidity).

$$pH = -\log[H^+] = \log\frac{1}{[H^+]}$$

Maintaing a normal pH is possible due to the existence of the following elements:

- *buffer systems (as the first line of defense)*
- *the lungs (the second line of defense)*
- *the kidney (the third line of defense)*

A buffer system is an aqueous solution containing a *weak acid* and its *conjugate base* (or a *weak base* and its *conjugate acid*). The main role of such an association is the rapid intervention in order to **prevent large variations of blood pH**. The extracellular buffer systems (e.g., plasma bicarbonate) act instantaneously, the interstitial ones within minutes and the intracellular ones within hours.

The main extracellular buffer system is the ***bicarbonate/carbonic acid*** – **HCO_3^-/H_2CO_3** with the following characteristics:

- Results from the dissociation of H_2CO_3 in cells containing the enzyme *carbonic anhydrase* (e.g. erythrocytes, tubular renal cells) and who have the *ability to bind* (e.g. erythrocytes on intracellular buffer systems) or to *eliminate* (tubular renal cells via the H^+ or K^+/H^+ ATP-ases) the protons (H^+) that result from H_2CO_3 dissociation ($H_2CO_3 \rightarrow HCO_3^- + H^+$)
- **High** concentration in the extracellular fluids (an average value of 24 mmol/L in plasma)
- A dissociation constant with a value close to that of the extracellular pH (pKa = 6,1)
- Its components are easily adjustable:
  - HCO_3^- by the ***kidneys*** → HCO_3^- is the **metabolic component** of the buffer system
  - H_2CO_3 by the ***lungs*** → H_2CO_3 is the **respiratory component** of the buffer system
- **Rapid** intervention in the presence of a large amount of acid in order to buffer it:
  - $HCO_3^- + H^+ \rightarrow H_2CO_3$
  - $H_2CO_3 \leftrightarrow CO_2 + H_2O$

The Henderson – Hasselbalch equation

– describes the relationship between the parameters of the buffer system that characterize the status of the acid-base balance:

1. pH = the pH of the solution in which the HCO_3^-/H_2CO_3 buffer system is found.

Normal value: pH = ~ 7,4

2. pKa (the dissociation constant) = the pH of the solution that allows the dissociation of 50% of the H_2CO_3 into $HCO_3^- + H^+$

Normal value: pKa = 6,1

3. The bicarbonate concentration [HCO_3^-]:

Normal value: [HCO_3^-] = ~ 24 mmol/L

4. The carbonic acid concentration ([H_2CO_3]) depends upon $PaCO_2$ according to the following relation: $[H_2CO_3] = 0,03 \times PaCO_2$ where:

- 0,03 = the solubility constant for CO_2 in H_2O
- $PaCO_2$ = the partial pressure of CO_2 in the arterial blood

Normal value: $PaCO_2$ = ~ 40 mmHg

$[H_2CO_3]$ = ~ 1,2 mmol/L

5. The ratio $[HCO_3^-]/[H_2CO_3]$ or $[HCO_3^-]/[0,03 \times PaCO_2]$ = the ratio that dictates the pH value
Normal value is ~ 20

According to the Henderson-Hasselbalch equation:

$$pH = pKa + \log\frac{[HCO_3-]}{[H_2CO_3]}$$

$$pH = pKa + \log\frac{[HCO_3-]}{[0,03x\ PaCO_2]}$$

$$pH = 6,1 + \log\frac{24}{1,2} = 6,1 + \log 20 = 6,1 + 1,30 = 7,4$$

ACID-BASE DISORDERS (ABD) COMPENSATION

The acid-base compensatory mechanisms can restore the ratio **$[HCO_3^-]/[H_2CO_3]$ to its normal value (≈ 20)**, regardless the ABSOLUTE values of the 2 components:

A. METABOLIC acid-base disorders

- are characterized by a **PRIMARY modification of bicarbonate (HCO_3^-)**, which leads to a **SECONDARY, compensatory change of carbonic acid (H_2CO_3)**. Metabolic ABD have a **respiratory compensation** via the changes in lung ventilation. The compensation process is *prompt (minutes)*, via the carotid and aortic arch chemoreceptors, which are highly sensitive to pH disturbances :
  - an **acidic pH** ($[HCO_3^-]$ ↓ with a $[HCO_3]/[H_2CO_3]$ < 20) will induce *hyperventilation*, which leads to:
    - a compensatory decrease of $PaCO_2$ and $[H_2CO_3]$
    - normalization of $[HCO_3^-]/[H_2CO_3]$
  - an **alkaline pH** ($[HCO_3^-]$ ↑ with a $[HCO_3]/[H_2CO_3]$ > 22) will induce *hypoventilation* that leads to:
    - a compensatory increase of $PaCO_2$ and $[H_2CO_3]$
    - normalization of $[HCO_3^-]/[H_2CO_3]$

B. RESPIRATORY acid-base disorders

- are characterised by a **PRIMARY modification of $PaCO_2$** and thus, the modification of carbonic acid (H_2CO_3) which will then induce a **SECONDARY modification of bicarbonate levels**. Respiratory ABD are compensated via the **metabolic processes** controlled by the kidneys. This compensation takes place *slowly (12-24 hours)* and reaches its peak after *3-5 days*. The renal compensatory mechanisms are based upon the kidneys' ability to *modify the reabsorption/generation of HCO_3^- and H^+ excretion processes*, in the form of titratable acidity (NaH_2PO_4) and ammonium chloride (NH_4Cl), respectively:
  - an **acidic pH** ($PaCO_2$ ↑ and $[H_2CO_3]$ ↑ with $[HCO_3]/[H_2CO_3]$ < 20), will lead to:
    - a compensatory increase in HCO_3^- reabsorption/generation and H^+ excretion and passing of *acidic* urine, respectively
    - normalisation of the $[HCO_3^-]/[H_2CO_3]$
  - an **alkaline pH** ($PaCO_2$ ↓ and $[H_2CO_3]$ ↓ and $[HCO_3]/[H_2CO_3]$ > 22), will lead to:
    - a compensatory decrease in HCO_3^- reabsorption/generation and H^+ excretion and passing of *alkaline* urine, respectively
    - normalisation of the $[HCO_3^-]/[H_2CO_3]$

II. ASSAYS THAT DEFINE THE ACID-BASE BALANCE

A. ARTERIAL BLOOD GASES analysis

- **Principle:** assessement of the **pH, $PaCO_2$ and PaO_2** in the **arterial blood** with the help of electrodes, followed by the automatic calculation of acid-base parameters (Table 6.1).

Table 6.1. The main parameters resulting from the arterial gases analysis.

Parameter	DEFINITION	NORMAL VALUE
pH	H^+ concentration in the arterial blood	7.35-7.45
$PaCO_2$	CO_2 amount in the plasma	35-45 mmHg

Total CO_2	The total CO_2 concentration in the plasma, found as $[HCO_3^-]$ and $[H_2CO_3]$	23-28 mmol/L
$[HCO_3^-]$	The plasma HCO_3^- concentration: Total CO_2 – (0,03 x $PaCO_2$)	22-26 mmol/L
Base excess (BE)	Base excess/deficit in the blood, depending on pH, $PaCO_2$ and Ht levels	0±3 mmol/L
PaO_2	Plasma O_2 levels	> 80 mmHg
$SatO_2$ (%)	O_2-saturated Hb found in the plasma	> 95%

Remember!
BE defines the **metabolic ABD** as follows:
- positive values define *base excess and the deficit of non-volatile acids*
- negative values define the *base deficit and the excess of non-volatile acids* and are used to assess the HCO_3^- correction in **metabolic acidosis**:

HCO_3^- deficit (mmol/L) = 0,3 x Weight (kg) x BE

B. THE ANIONIC GAP

The anionic gap (AG) refers to the plasma concentration of anions that participate in maintaining plasma electric neutrality, but ARE NOT determined during usual lab tests. The AG includes:
- **organic** anions: ketoacids, lactate
- **inorganic** anions: phosphate, sulphate

AG (mmol/L) = $[Na^+]$ – $[(Cl^-) + (HCO_3^-)]$

- **Normal values**: 12 ± 4 mmol/L
- **Clinical value**: metabolic acidosis (MA) classification:
  - *METABOLIC ACIDOSIS with increased ANIONIC GAP* in which the decreased serum $[HCO_3^-]$ levels are the consequence of bicarbonate consumption for the buffering of protons (H^+) that result from acid dissociation when this is present in high amounts:

    Increased AG = $[Na^+]$ – $[Cl^- + HCO_3^- \downarrow]$

  - *METABOLIC ACIDOSIS with normal ANIONIC GAP* in which the decreased serum $[HCO_3^-]$ levels result from digestive or renal loss of bicarbonate; plasma neutrality is maintained because of a compensatory increase in renal reabsorption of chloride ions.

    Normal AG = $[Na^+]$ – $[Cl^- \uparrow + HCO_3^- \downarrow]$

III. PRINCIPLES FOR ABD DIAGNOSIS

1. The pH change suggests the type of ABD:
   - **Acidosis if the pH is < 7,35**
   - **Alkalosis if the pH is > 7,45**

2. If the $[HCO_3^-]$ changes occur in the **SAME** direction as the pH change:
- **The primary ABD is a METABOLIC one and can be:**
  - ***Metabolic acidosis*** – primary decrease of $[HCO_3^-]$ < 22 mmol/L
  - ***Metabolic alkalosis*** – primary increase of $[HCO_3^-]$ > 26 mmol/L
- **The secondary/compensatory change is a RESPIRATORY one**. According to the degree of compensation, the primary metabolic disorder can be:
  - ***Decompensated/Uncompensated*** – $PaCO_2$ is normal, and the pH is abnormal
  - ***Partially compensated*** – $PaCO_2$ change occurs in the same direction as the one of HCO_3^-, but the pH still remains abnormal
  - ***Fully compensated*** – $PaCO_2$ change occurs in the same direction as the one of HCO_3^-, and the pH is back to normal

3. If the $[HCO_3^-]$ changes occur in the **OPPOSITE** direction as the pH change:
- **The primary ABD is a RESPIRATORY one** and can be:

- ***Respiratory acidosis*** – primary increase in $PaCO_2$ levels > 45 mmHg
- ***Respiratory alkalosis*** – primary decrease in $PaCO_2$ levels < 35 mmHg

– **The secondary/compensatory change is a METABOLIC one**. According to the degree of compensation, the primary respiratory disorder can be:
- ***Decompensated/Uncompensated*** – $[HCO_3^-]$ is normal, and the pH is abnormal
- ***Partially compensated*** – $[HCO_3^-]$ change occurs in the same direction as the one of $PaCO_2$, but the pH still remains abnormal
- ***Totally compensated*** – $[HCO_3^-]$ change occurs in the same direction as the one of $PaCO_2$, and the pH is back to normal

4. **If $PaCO_2$ and $[HCO_3^-]$ changes occur in opposite directions and the pH is highly abnormal - there is a MIXED ABD due to the association of 2 primary ABD that modify the pH in the *same* direction**:
– *Metabolic acidosis* ($[HCO_3^-]\downarrow$) plus *Respiratory acidosis* ($PaCO_2\uparrow$) can lead to a **highly acidic pH** $\Rightarrow$ MIXED acidosis
– *Metabolic alkalosis* ($[HCO_3^-]\uparrow$) plus *Respiratory alkalosis* (PaCO2$\downarrow$) can lead to a **highly alkaline pH** $\Rightarrow$ MIXED alkalosis

5. **If $PaCO_2$ and $[HCO_3^-]$ levels are modified in the same direction, but the pH levels are normal** - the ABD can be:
– A primary fully compensated ABD AFTER THERAPY

or

– A mixed ABD that results from the association of *2 primary ABD that modify the pH in opposite directions*:
- *Metabolic acidosis* ($[HCO_3^-]\downarrow$) plus *Respiratory alkalosis* ($PaCO_2\downarrow$) OR
- *Metabolic alkalosis* ($[HCO_3^-]\uparrow$) plus *Respiratory acidosis* ($PaCO_2\uparrow$)

Remember!
A fully compensated ABD (normal pH) prior to any therapeutic measure is always a mixed ABD. In other words, a **total** compensation of a primary ABD IS NEVER the proof of pulmonary or renal compensation efficiency, but the **consequence of an APPROPPRIATE THERAPEUTIC INTERVENTION**.

STEP BY STEP APPROACH IN ACID-BASE BALANCE WORK-UP

1. Identify the type of ABD according to the pH value: ACIDOSIS/ALKALOSIS.
2. Identify the primary change in the case of simple ABDs: METABOLIC/ RESPIRATORY.
3. Identify the secondary (compensatory) change in the case of simple ABDs (respiratory/metabolic).
4. Establish the degree of compensation (absent, partial, total).
5. Identify a mixed ABD (with normal or abnormal pH).
6. Calculate the anionic gap, in case of metabolic acidosis.
7. Recognize the chloride-responsive or resistant type, in case of metabolic alkalosis.

1. METABOLIC ACIDOSIS

- **Definition:**
  – pH < 7,35
  – primary $\downarrow$ of $[HCO_3^-]$ < 22 mmol/L
- **The degree of compensation** – is established according to $PaCO_2$ changes (Table 6.2).

Table 6.2. The degree of compensation in a metabolic acidosis (examples).

pH	$[HCO_3^-]$ (mmol/L)	$PaCO_2$ (mmHg)	Interpretation
7,23	**15**	**37**	*Decompensated* metabolic acidosis
7,32	**15**	**30**	*Partially compensated* metabolic acidosis
7,41	**15**	**24**	*Fully compensated metabolic acidosis*

- **Classification and main causes**

Metabolic acidosis is classified according to the anionic gap (AG) in**:**

a) Metabolic acidosis with <u>increased</u> AG:
– *Ketoacidosis*: in diabetes mellitus, alcoholism, starvation
– *Lactic acidosis*: in shock, alcoholism, liver failure

- *Toxic*: intoxications with methanol, ethylenglycol (antifreeze), salicylates *in advanced stages*
- *Chronic kidney disease mainly in its end-stage of renal failure* (decreased renal excretion of phosphate, sulphate, urate)

b) Metabolic acidosis with <u>normal</u> AG:

- *Digestive loss of HCO_3^-:* chronic diarrhea
- *Renal loss of HCO_3^-:* hypoaldosteronism

2. METABOLIC ALKALOSIS

- **Definition:**
  - pH > 7,45
  - primary ↑ in $[HCO_3^-]$ > 26 mmol/L
- **The degree of compensation** – is established according to $PaCO_2$ changes (Table 6.3)

Table 6.3. The degree of compensation in a metabolic alkalosis (examples).

pH	$[HCO_3^-]$ (mmol/L)	$PaCO_2$ (mmHg)	Interpretation
7,57	**34**	**38**	*Decompensated* metabolic alkalosis
7,49	**34**	**47**	*Partially compensated* metabolic alkalosis
7,43	**34**	**52**	*Fully compensated* metabolic alkalosis

- **Classification and main causes**

Metabolic alkalosis is classified according to the response to the **administration of 0,9% sodium chloride into:**

a) CHLORIDE-RESPONSIVE metabolic alkalosis (the administration of 0,9% NaCl corrects the metabolic alkalosis). In the presence of water, Na^+ and Cl^- depletion, the kidneys are able to compensate by reabsorbing HCO_3^- in order to maintain the electroneutrality of the plasma, whilst the administration of 0,9% NaCl will cause a renal elimination of the bicarbonate excess (correction of the fluid depletion will correct the ABD too). This type of alkalosis is due to:

- *Digestive loss of water, Na^+ and Cl^-* due to severe vomiting
- *Renal loss of water, Na^+ and Cl^-* due to excessive administration of thiazide/loop diuretics

b) CHLORIDE-RESISTANT metabolic alkalosis (0,9% NaCl administration **does not** correct the metabolic alkalosis) is due to:

- *Excessive HCO_3^- intake* (antiacid medication)
- *Excessive administration of metabolic precursors of HCO_3^-* (Ringer lactate solution in resuscitation, high amount of citrated blood in transfusions)
- *Increased renal reabsorption of HCO_3^-:* hyperaldosteronism.

3. RESPIRATORY ACIDOSIS

- **Definition:**
  - pH < 7,35
  - primary ↑ of $PaCO_2$ levels > 45 mmHg
- **The degree of compensation –** is established according to bicarbonate changes (Table 6.4).

Table 6.4. The degree of compensation in a CHRONIC respiratory acidosis (examples).

pH	$PaCO_2$ (mmHg)	$[HCO_3^-]$ (mmol/L)	Interpretation
7,22	**60**	**24**	*Decompensated* respiratory acidosis
7,34	**60**	**32**	*Partially compensated* respiratory acidosis
7,42	**60**	**38**	*Fully compensated* respiratory acidosis

- **Classification and main causes**

a) Acute respiratory acidosis – is caused by *acute hypoventilation* that occurs in minutes to hours time and is decompensated in most of cases (not enough time for renal compensation to occur). The most frequent causes are:

- *CNS centers depression (decrease activity of the respiratory center)* in drug intoxications (e.g. barbiturates)
- *Thoracic lesions* due to severe thoracic trauma

- *Respiratory muscles paralysis* in myasthenia gravis
- *Acute respiratory airway obstructions* due to **severe** asthma crisis or aspiration of foreign bodies
- *Acute pulmonary edema*

b) Chronic respiratory acidosis – is caused by *chronic hypoventilation* and is always followed by maximal renal compensation. The most frequent causes are:

- *COPD*
- *Extreme obesity*

4. RESPIRATORY ALKALOSIS

- **Definition:**
  - pH > 7,45
  - primary ↓ in $PaCO_2$ levels < 35 mmHg
- **The degree of compensation –** is established according to [HCO_3^-] changes (Table 6.5).

Table 6.5. The degree of compensation in a respiratory alkalosis (examples).

pH	$PaCO_2$ (mmHg)	[HCO_3^-] (mmol/L)	Interpretation
7,62	**25**	**24**	*Decompensated* respiratory alkalosis
7,56	**25**	**21**	*Partially compensated* respiratory alkalosis
7,42	**25**	**16**	*Fully compensated* respiratory alkalosis

- **Classification and main causes**

a) Acute respiratory alkalosis – is caused by *acute hyperventilation*, with no posibility of renal compensation. The most frequent causes are:

- *Hyperventilation that results from increased activity of respiratory centres* in:
  - panic attacks
  - hyperpyrexia
  - salicylate intoxication in the **initial stage**
  - severe pain
- *Hyperventilation that results from hypoxia* in*:*
  - pulmonary embolism (initial phase)
  - **moderate** asthma crisis

b) Chronic respiratory alkalosis - is the result of *chronic hyperventilation*, compensated by increased renal bicarbonate elimination. The most common cause is pregnancy (3rd trimester).

CHECKPOINT

***1. Which of the following statements regarding ABD is true?**
A. They always determine pH variations above normal limits
B. Can be fully compensated without therapeutical interventions
C. Are fully compensated if the ratio between HCO_3^-/H_2CO_3 equals 20
D. Recruitment of pulmonary compensatory mechanisms occurs in primary respiratory disturbances
E. Recruitment of metabolic compensatory mechanisms occurs in primary metabolic disturbances

***2. Which type of ABD ocurs in the presence of the following arterial blood gas analysis: pH = 7.55, $PaCO_2$ = 19 mmHg, HCO_3^- = 24 mmol/L?**
A. Decompensated metabolic alkalosis
B. Partially compensated metabolic alkalosis
C. Partially compensated respiratory alkalosis
D. Decompensated respiratory alkalosis
E. Mixed alkalosis

***3. What ABD occurs in the presence of the following arterial blood gases values: pH = 7.55, $PaCO_2$ = 44 mmHg, HCO_3- = 56 mmol/L?**
A. Partially compensated metabolic alkalosis
B. Decompensated metabolic alkalosis
C. Partially compensated respiratory alkalosis
D. Decompensated respiratory alkalosis
E. Mixed alkalosis

***4. Which ABD most likely corresponds to the following blood parameters in a 15-year-old patient with a pH = 7.51, $PaCO_2$ = 49mmHg, HCO_3^- = 38 mmol/L?**
A. Partially compensated metabolic alkalosis
B. Partially compensated respiratory alkalosis
C. Decompensated respiratory alkalosis
D. Mixed alkalosis
E. Mixed acidosis

***5. What ABD occurs in the presence of the following arterial blood gas analysis in a patient with COPD and vomiting: pH = 7.40, $PaCO_2$ = 50 mmHg, HCO_3^- = 30 mmol/L?**
A. Fully compensated metabolic acidosis
B. Fully compensated respiratory alkalosis
C. Partially compensated respiratory acidosis
D. Partially compensated metabolic acidosis
E. Mixed ABD

6. Which of the following are true regarding the acid-base disturbances?
A. Ketoacidosis leads to metabolic acidosis with normal anionic gap
B. Renal failure leads to metabolic alkalosis
C. COPD leads to chronic respiratory acidosis
D. Chronic diarrhea leads to metabolic acidosis with increased anionic gap
E. Severe pain leads to acute respiratory alkalosis

7. A pH level of 7.4:
A. Corresponds to a HCO_3^-/ H_2CO_3 ratio of 20
B. Corresponds to a HCO_3^-/ H_2CO_3 ratio < 18
C. Corresponds to a HCO_3^-/ H_2CO_3 ratio > 22
D. Indicates a fully compensated metabolic acidosis, if $PaCO_2$ and $[HCO_3^-]$ levels are decreased
E. Indicates a fully compensated respiratory acidosis, if $PaCO_2$ and $[HCO_3^-]$ levels are increased

8. The following changes can be found in a chronic decompensated respiratory acidosis:
A. pH = 7.4
B. pH = 7.28
C. $PaCO_2$ = 65 mmHg
D. $[HCO_3^-]$ = 30 mEq/L
E. $[HCO_3^-]/[H_2CO_3]$ ratio > 22

***9. If pH = 7.10, $PaCO_2$ = 52 mmHg, HCO_3^- = 13 mmol/L, the most likely ABD is:**
A. Decompensated primary metabolic acidosis
B. Decompensated primary respiratory acidosis
C. Mixed acidosis
D. Partially compensated primary metabolic acidosis
E. Partially compensated primary respiratory acidosis

***10. Which ABD most likely corresponds to the following parameters resulting from blood gas analysis in a 45-year-old patient with a severe asthma crisis: pH = 7.29, $PaCO_2$ = 62mmHg, HCO_3^- = 24 mmol/L**
A. Partially compensated respiratory acidosis
B. Partially compensated respiratory alkalosis
C. Decompensated respiratory acidosis
D. Mixed alkalosis
E. Mixed acidosis

CASE STUDIES

1. A 50-year-old patient with a history of gastric ulcer is brought to the hospital for severe vomiting that appeared in the setting of weight loss.
Arterial blood gas analysis shows:
pH = 7,53
$PaCO_2$ = 47 mmHg
$[HCO_3^-]$ = 35 mmol/L

Analyse the acid-base status and diagnose the ABD.

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2. A young man with a history of type 1 DM is rushed to the emergency room for an altered general state and fever (40°C) caused by a respiratory infection. His blood tests show: Na^+ = 132 mmol/L, Cl^- = 80 mmol/L, Blood glucose = 240 mg/dL.
Arterial blood gas analysis shows:
pH = 7,42
$PaCO_2$ = 23 mmHg
HCO_3^- = 12 mmol/L

Analyse the acid-base status and diagnose the ABD.

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NOTES

7. INVESTIGATION OF FLUID AND ELECTROLYTE DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Request and interpret the laboratory tests that evaluate the fluid and electrolyte imbalances

2. Recognize fluid imbalances based on suggestive clinical signs and laboratory data for hemodilution and hemoconcentration assessment

3. Recognize and interpret changes in plasma osmolarity

4. Recognize and interpret sodium metabolism disorders

5. Recognize and interpret potassium metabolism disorders

I. INVESTIGATION OF BODY FLUID COMPARTMENTS

Total body water (TBW) – represents aproximately 60% of a person's body weight, depending on the age and gender, and is distributed into 2 major compartments:

- intracellular fluid compartment (ICF)
- extracellular fluid compartment (ECF)

1. The intracellular fluid compartment (ICF) is the largest and represents aproximately **40%** of the body's weight (two thirds of the TBW)

2. The extracellular fluid compartment (ECF) represents **20%** of the body weight (one third of the TBW) and comprises:

a) The plasma volume (PV) – represents **25% of the ECF** and **5%** the body's weight.

b) The interstitial fluid – represents **75% of the ECF** and **15%** of the body's weight. It also comprises the *lymph* (2-3% of the body's weight)

! Important rule: 20 + 40 = 60
ECF (20%) + ICF (40%) = TBW (60%)

c) The transcellular fluid – represents **1,5-2%** of the body weight (aproximately 1 litre). This compartment comprises: digestive fluids (1/2 of the transcellular fluid), bile, sweat, spinal fluid, pleural, pericardial, peritoneal, synovial and intraocullary fluids. Abnormal increase of this compartment is denominated ***the third space***, since it does not participate in normal fluid exchanges.

Example:

An adult who weighs 70 kg, has a total of 42 litres of body water (0,6 x 70), divided into the:

• intracellular fluid compartment (ICF) = 28 L

• extracellular fluid compartment (ECF) = 14 L:

- plasma volume = 3.5 L
- interstitial fluid = 10.5 L

In healthy individuals, the composition and volume of the two compartments remain constant, due to the preservation of the balance between fluid intake and fluid loss.

II. MEASUREMENT OF FLUID COMPARTMENTS

The dilution principle

- The volume (V) of a hydric compartment in which a certain substance is injected, can be determined by knowing the initial concentration of that substance (Q) and its concentration after it has been equally distributed in the studied compartment (C). The volume of that hydric compartment can be determined using the formula:

V=Q/C.

- The main substances that can be used for fluid compartment measurement are presented in Table 7.1.
- The substance may leave the studied compartment through one of the following mechanisms:
  - urine excretion
  - transfer into another compartment in case of different concentrations
  - metabolisation of the substance
  - perspiration or breathing

- Regardless the studied compartment, the substances used must meet the following conditions:
  - to be measurable
  - to remain long enough in the assessed compartment
  - to be non-toxic
  - to not modify the balance of the fluids existing in the studied compartment

Table 7.1. Main substances used to assess the fluid compartments.

Compartment	Indicators
TBW	$^{3}H_2O$, $^{2}H_2O$, antipyrine
ECF	^{22}Na, thiosulphate, inuline
ICF	Calculated as (TBW-ECF)
Plasma vol.	^{125}I-albumine, Evans blue (T-1824)
Interstitial fluid	Calculated as (ECF-plasma volume)

1. Assessment of TOTAL BODY WATER (TBW)
A certain amount of a substance, usually *radioactive water* (tritium), *heavy water* (deuterium) or *antipyrine*, which can distribute uniformely in all the fluids of the body, will be administered to the patient, either orally or intravenously. A few hours later, after the substance was allowed to reach the tissues, a blood sample is taken in order to measure the plasma concentration of the substance that was administered. Total body water can be then measured using the dilution principle.

2. Assessment of the EXTRACELLULAR FLUID COMPARTMENT (ECF)
The extracellular volume can be estimated using substances that diffuse into the plasma and interstitial fluid, but DO NOT pass through the cellular membrane. Such substances are: *sodium thiocyanate, sodium thiosulphonate, inuline, radioactive sodium*. **Sodium thiocyanate** is used more frequently than other substances, the estimated value of the ECF being 26% (higher than the normal value of 20%) due to partial distribution in the gastric mucosa and some other parenchymal organs.

a) Plasma volume can be measured using:
- substances that remain in the vascular system and do not enter the red cells, such as:
  - Evans blue (T-1284)
  - radioactive albumin (^{125}I – albumin)

b) Interstitial fluid volume can not be measured directly, because no substance can diffuse exclusively into this compartment. Therefore, it will be calculated as the difference between the extracellular fluid and plasma volume.

3. Assessment of the INTRACELLULAR FLUID COMPARTMENT (ICF)
ICF can not be measured directly using the dilution technique, due to the fact that no substance can remain exclusively into this compartment (after intravenous administration). Therefore, the ICF volume is obtained from the difference between TBW and ECF.

III. DISTURBANCES OF BODY FLUID COMPARTMENTS

- **General concepts**

- **Volume** - volume disturbances are hypovolemia/dehydration and hypervolemia/ hyperhydration, respectively, both being associated with abnormalities of extracellular fluid volume.
- **Tonicity** of a solution is linked to its effect upon the volume of a cell, for instance the erythrocyte:
  - isotonic solutions do not modify the cell volume
  - hypotonic solutions determine a swelling of the cell
  - hypertonic solutions can cause cellular dehydration

1. Hypovolemia

- **Definition:** represents a **decrease in extracellular fluid volume** caused by **both water and sodium deficit** (unlike *dehydration that reffers only to a water deficit*).
- **Causes:** the most frequent causes of extracellular fluid loss include: vomiting, diarrhea, severe burns, high doses of diuretics and chronic kidney disease.
- **Clinical signs:**
  - diminished skin turgor
  - dry skin and mucosae
  - tachychardia
  - orthostatic hypotension.

2. Hypervolemia

- **Definition:** represents an **increase in extracellular fluid volume** due, in most cases, to high sodium concentration (unlike *hyperhydration which reffers only to an increase of the volume of water*).
- **Causes:** the most frequent causes which are responsible of hypervolemia include: cardiac failure, nephrotic syndrome, liver cirrhosis.
- **Clinical signs:**
    - edema
    - increased body weight
    - orthopnea.

In both types of fluid disorders, the diagnosis is firstly a clinical one, the major signs and symptoms being detailed in Table 7.2.

Table 7.2. The main signs/symptoms in fluid disorders.

Characteristics	Dehydration	Hyperhydration
History	Vomiting, diarrhea, reduced liquid intake	Excess in sodium intake, renal impairment, cardiac failure, excess of corticosteroids, excess of i.v. fluid administration
Pulse	Weak, rapid	Strong
Blood presure	↓	N/↑
Skin	Reduced skin turgor	Edema
Eye balls	Hypotonic, sunken into the eye socket	N
Mucosae	Dry	N
Thirst	↑	-
Weight	Acute weight loss	Acute weight increase
Urine output	Olyguria, concentrated urine	Variable
Consciousness	↓	↓
Venous circulation	↓	Venous distention, pulmonary edema
Capilary nail refill time	↑	N
Ht, urea	Hemo-concentration	Hemodilution

Body weight

Body weight must be measured daily, in the same conditions as the day before. A decrease/increase in weight of 2% is considered mild, between 5% and 8%, moderate and above 8%, severe.

Skin turgor

Water allows the skin and tissues to maintain a certain elasticity, which is referred to as the skin turgor. It can be evaluated by making a skin crease using the first and index fingers. The skin should normally get back to its original state after releasing the crease. A loss of water between 3-5% (especially in children) determines a loss of skin elasticity, the teguments remaining deformed for a few seconds. This clinical sign loses its clinical value in older pacients, where the skin loses elasticity due to ageing.

Sunken anterior fontanelle in infants

A depressed anterior fontanelle represents a sign of dehydration and it is caused, most frequently, by fluid loss or by a decrease in the pressure of cerebrospinal fluid (CSF). The anterior fontanelle is a diamond-shaped membrane located at the intersection of the sagittal, coronal and frontal cranium sutures that usually closes between 9 months and 2 years. In normal conditions, if palpated, the anterior fontanelle should be firm, flat, and delineated from the near cranium bones. A bulging, tensioned fontanelle, with visible pulsation is a sign of high intracranial pressure or fluid retention.

Capillary nail refill test

Pressure that is applied on the nail for 5 seconds will determine a discoloration of the nail, which indicates that the blood was forced to leave the tissue. The time required for the blood to return in order for the nail to regain its pink colour should be < 2 seconds. Increased pallor of the nail, over 2 seconds can indicate: dehydration, shock, hypothermia.

IV. INVESTIGATION OF PLASMA ELECTROLYTES

- **Definition:** electrolytes are negative and positive ions present in the body fluids:
  - **sodium** is the main **extracellular cation**
  - **potasium** is the main **intracellular cation**
  - **proteins and phosphate** represent **intracellular anions**
  - **chloride and bicarbonate** are the main **extracellular anions**

The normal values for plasma electrolytes are detailed in Table 7.3.

Table 7.3. Plasma electrolytes.

Cations	**Plasma** (mmol/L)
Sodium (Na^{+})	135 - 145
Potasium (K^{+})	3.5 - 5
Calcium (Ca^{2+})	2.20 - 2,67
Magnesium (Mg^{2+})	0.7 -1.1
Anions	
Chloride (Cl^{-})	98 - 106
Bicarbonate (HCO_3^{-})	22 - 26
Phosphate (HPO_4^{2-})	1.3 - 2.1
Sulphate (SO_4^{2-})	0.1 - 0.65

- **Osmolality/Osmolarity**

The number of particles disolved in a volume unit can be reffered to as osmolality or osmolarity

- **Osmolality** reffers to the *number of osmols per kilogram of water* (therefore, the total volume will be 1 litre of water to which a small volume of substance can be added)
- **Osmolarity** reffers to the *number of osmols per litre of solution* (therefore, the water volume is less than 1 litre). Due to the low concentration of disolved substances in the body fluids, the difference between osmolality and osmolarity can be neglected. Plasma osmolarity can be estimated using the following formula:

Serum osmolarity (mOsm/L) = 2 x plasma Na^{+} (mmol/L)

This simple formula can be used only in cases in which plasma glucose and urea are in normal range. If one or both are pathologically increased, their concentrations should be considered for the calculation of plasma osmolarity. Therefore, the formula for osmolarity becomes:

Total plasma osmolarity (mOsm/L) = 2 x [Na^{+} + K^{+}] + [urea]/6 + [glucose]/18

(Sodium and potassium are measured in mmol/L, while urea and glucose in mg/dL)

- **Normal values:** 275-295 mOsm/L

A. DISORDERS OF SODIUM METABOLISM

1. Hypernatremia

- **Definition:** increase of plasma levels of **sodium > 145 mmol/L**
- **Causes:** water deficit (usually it is proof of an absolute deficit in total body water) or increased sodium intake/retention.
- **Clinical signs:** thirst (main symptom), neurological signs due to disturbances of intracellular volume (neuromuscular signs, confusion, seizures, coma).

Table 7.4. The main causes of hypernatremia.

1. Water loss:
- **Extrarenal causes:**
  - Respiratory: tachypnea
  - Skin: increased sweating in high fever, burns
  - Gastrointestinal: vomiting, diarrhea (when water loss > sodium loss)
- **Renal causes:**
  - Pituitary diabetes insipidus
  - Renal diabetes insipidus
  - Osmotic diuresis (glucose, urea, manitol)
  - Excessive diuretic administration

2. Hypertonic solution administration:
hypertonic saline, sodium bicarbonate, total parenteral nutrition

3. Increased mineralocorticoid production
- Primary hyperaldosteronism
- Cushing syndrome

4. Other causes:
- Lack of acess to water (bedridden patients)

- **Diagnosis:** Plasma sodium, osmolarity, Ht, urea (Table 7.6).

2. Hyponatremia

- **Definition:** decreased plasma levels of **sodium < 135 mmol/L**
- **Causes:** water excess (dilution hyponatremia) or a decrease of sodium intake/increased sodium loss. The major causes of hyponatremia are detailed in Table 7.5.
- **Clinical signs:** neurological manifestations especially in acute hyponatremia (headache, confusion, stupor, convulsions and coma).

Table 7.5. The main causes of hyponatremia

1. Dilution hyponatremia:

a. Water and sodium retention from:

- decompensated liver cirrhosis
- congestive heart failure
- acute kidney injury (oliguric phase)
- renal failure

b. Pathological ADH production and release:

- opioid drugs
- postoperative pain, emotional stress
- inadequate ADH secretion occuring within paraneoplastic syndromes (ex. pulmonary tumors)

2. Decreased sodium intake:

- low sodium diet (associated with increased loss in patients treated with diuretics)

3. Excessive sodium loss:

a. Extrarenal causes:

- gastrointestinal: vomiting, diarrhea (when sodium loss > water loss)
- third space formation: acute pancreatitis, peritonitis, intestinal occlusion

b. Renal causes:

- mineralocorticoid deficit (hypoALDO)
- glucocorticoid deficit (Addison disease)
- diuretics
- osmotic diuresis (glucose, urea, manitol)
- sodium loss nephropathies

- **Diagnosis:** Plasma sodium, osmolarity, Ht, urea (Table 7.6).

a) Calculation of plasma osmolarity:

  - normal – false hyponatremia
  - increased – check for hyperglycemia
  - decreased – check the hydration status

- *In case of hypovolemia*, check urinary sodium:
  - Urinary Na^+ < 20 mEq/L: gastrointestinal losses, diuretics (late)
  - Urinary Na^+ > 20 mEq/L: renal losses, diuretics (early)
- *In case of hypervolemia*, check urinary sodium:
  - Urinary Na^+ < 20 mEq/L: liver disease, heart failure
  - Urinary Na^+ > 20 mEq/L: renal disease

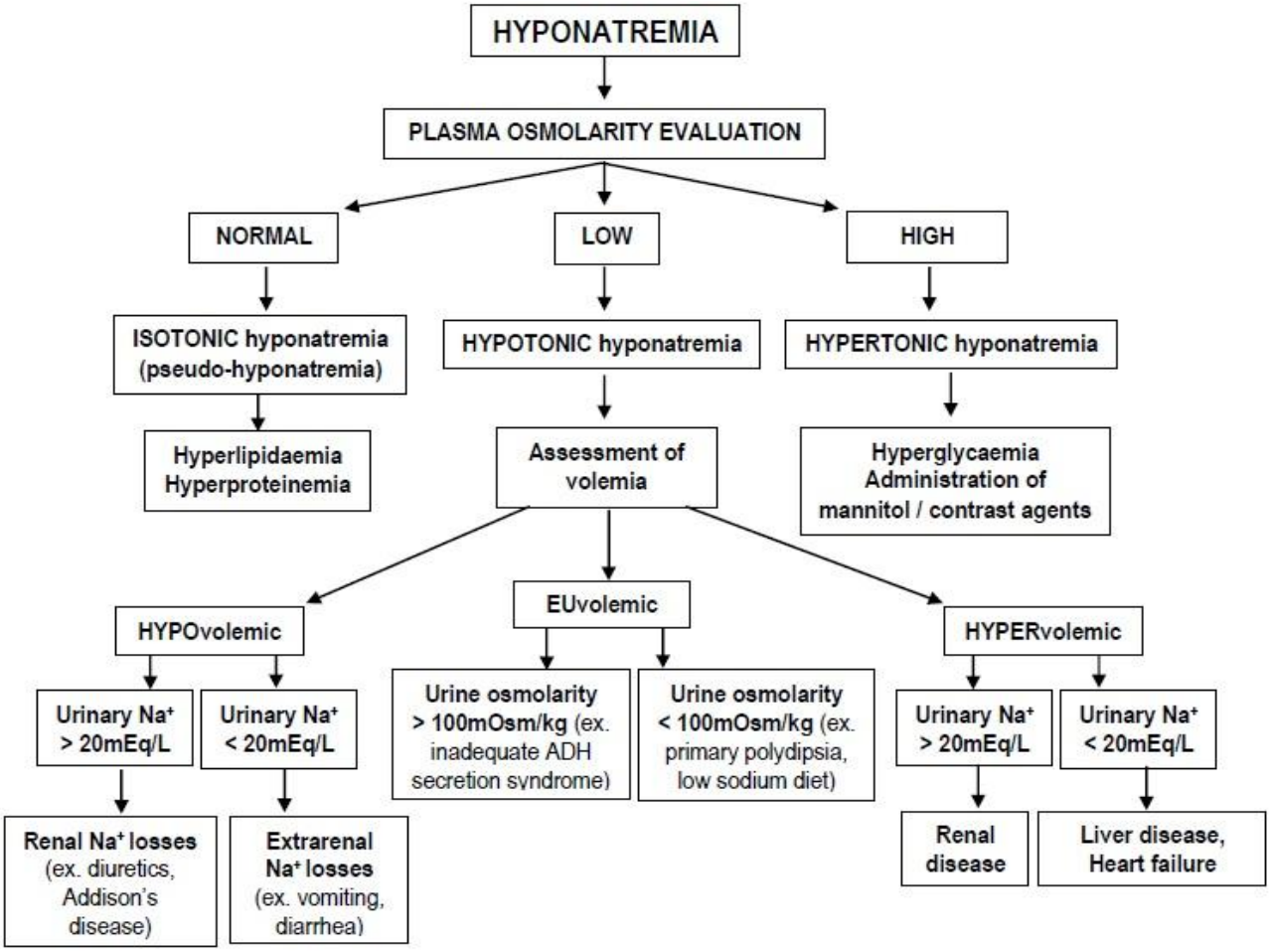

Figure 7.1. Hyponatremia diagnosis algorithm

b) Calculation of sodium deficit for therapeutic correction:

- Na^+ deficit in women:

  (140 – current Na^+) x Weight x 0,5

- Na^+ deficit in men:

  (140 – current Na^+) x Weight x 0,6

Observations!

Deficit correction is performed with:

- NaCl 0,9% => 500 ml sol NaCl 0,9% contain aprox. 75 mmol of sodium
- NaCl 5,85% => 1 ml sol NaCl 5,85% contains 1 mmol of sodium.

Hyponatremia can also be the consequence of laboratory determination errors (pseudo-hyponatremia) in case of:

- hyperlipidemia (lipemic serums)
- hyperproteinemia (hyperglobulinemia from multiple myeloma, macroglobulinemia)

situations where plasma osmolarity is normal.

Table 7.6. Manifestations of hypo- and hypernatremia.

	Hyponatremia	**Hypernatremia**
LAB tests: Plasma sodium	<135 mmol/L	>145 mmol/L
Plasma osmolarity	Reduced	Increased
	Hemodilution:	Hemo-concentration
	↓ Ht	↑ Ht
	↓ Urea	↑ Urea
CLINICAL signs	***Water enters the cells*** **Muscles:** cramps and weakness, reduced deep tendon reflexes **Nervous system:** headache, disorientation, seizures and coma (brain edema due to 'water intoxication') **Digestive system:** appetite loss, nausea, vomiting, cramps, diarrhea	***Water exits the cells*** **Nervous system:** headache, disorientation, anxiety, decreased nervous reflexes, seizures and coma. ***Compensatory mechanisms:*** - thirst - increased ADH release with oliguria

B. DISORDERS OF POTASSIUM METABOLISM

Potassium is the most abundent intracellular cation and a major factor responsible for the intracellular osmolarity.

1. Hypokalemia

- **Definition:** decrease in plasma levels of potassium **< 3.5 mmol/L**.
- **Clinical features:** muscle weakness, polyuria; cardiac hyperexcitability with severe hypokalemia (Table 7.7). The diagnosis must be established by measuring the plasma level of potassium.
- **Causes:** potassium deficit or abnormal potassium migration into the cells. The most frequent causes are renal or gastrointestinal losses:

– **Increased renal losses:**
  - mineralocorticoid excess
  - diuresis: diuretics, osmotic diuresis
  - metabolic alkalosis
  - iatrogenic

– **Gastrointestinal losses:**
  - vomiting
  - diarrhea

– **Shift between the intra- and extracellular compartments:**
  - acute alkalosis
  - insulin therapy
  - glucose administration

- **Calculation of the potassium deficit**

– The K^+ deficit is calculated using the formula:

(4 – current K^+) x Weight x 0.4

Observation!

Deficit correction is performed with KCl 7.45% sol. => 1 ml sol. 7.45% KCl contains 1 mmol of potassium (max. 150 mmol K^+ per day may be administered).

2. Hyperkalemia

- **Definition:** increase in plasma levels of potasium **> 5 mEq/L**
- **Clinical features (Tab. 7.7):** muscle weakness, cardiac toxicity, which in severe casese can lead to ventricular fibrillation or asistoly.
- **Causes:** the main cause is chronic kidney disease/renal failure, but it can also be present in metabolic acidosis or poorly controlled diabetes mellitus.

- **Excessive intake:**
  - potassium supplements
  - blood transfusions
- **Decreased renal output:**
  - decreased glomerular filtration rate: chronic kidney disease and renal failure
  - decreased tubular secretion: hypoaldosteronism and potassium-sparing diuretics
- **Shift from the intracellular compartment to the extracellular compartment**
  - acidosis
  - cell lysis: hemolysis, trauma, burns, tumor necrosis
  - digoxin overdose
  - lack of insulin

Table 7.7. Clinical features of hypo- and hyperkalemia

	Hypokaliemia	**Hyperkaliemia**
Lab test Serum K^+ Acid-base balance	< 3.5 mmol/L Metabolic alkalosis	> 5 mmol/L Metabolic acidosis
Gastro-intestinal	Appetite loss, Nausea, Vomiting, Abdominal distension,	Nausea, Vomiting, Intestinal cramps, Diarrhea
Neuromuscular	Weakness, Fatigue, Muscle cramps, Paresthesia, Palsy	Weakness, Dizziness, Paresthesia, Palsy
Central nervous syst.	Confusion, Depression	
Cardiovascular	Orthostatic hypotension, Digoxin intoxication risk, ECG changes, Cardiac arrythmias	ECG changes, Cardiac arrest in severe hyperkalemia

CHECKPOINT

*1. A 56-year-old patient presents with nicturia, fatigue and muscle hypotonia. The laboratory tests show the following: BP = 160/100 mmHg, Na^+ = 150mmol/L, K^+ = 2.8mmol/L, urea = 26mg/dL, creatinine = 0.8mg/dL, serum glucose = 100mg/dL.
Which is the most likely diagnosis?
A. Diabetes mellitus
B. Acute kidney injury
C. Primary hyperaldosteronism
D. Hypoaldosteronism
E. Addison disease

*2. A patient presents the following electrolyte values: Na^+ = 105mmol/L and K^+ = 6mmol/L.
Which is the most likely cause for these changes?
A. Insuline deficit
B. ADH deficit
C. Parathyroid hormone excess
D. Renine excess
E. Aldosterone deficit

*3. A 58-year-old female patient, diagnosed with pulmonary cancer, has been manifesting nausea, vomiting and headaches for over a week. Plasma levels of sodium: 108mEq/L.
Which of the following can represent a cause for the patient's hyponatremia?
A. Inadequate ADH secretion syndrome
B. Thiazide and loop diuretic administration
C. Hypoaldosteronism
D. Hyperaldosteronism
E. Increased adrenal cortex activity

4. Which of the following parametres are used to calculate plasma osmolarity?
A. Urea
B. Creatinine
C. Plasma glucose levels
D. Fibrinogen
E. Serum proteins

*5. A female diagnosed with type I diabetes presents after insulin administration increased sweating, weakness and muscle hypotonia.
Which of the following can be the cause for the patients clinical manifestations?
A. Isotonic dehydration
B. Hypokalemia due to insulin administration
C. Hyperkalemia due to insulin administration
D. Isotonic hyperhydration
E. Metabolic acidosis

6. A 42-year-old female patient presents with weakness and muscle cramps, vomiting and diarrhea. BP = 75/50mmHg, HR = 120 b/min. Laboratory analyzes show: Na^+ = 123 mmol/L, K^+ = 6.8 mmol/L, Ht = 54%, serum urea = 62 mg/dL, serum creatinine = 0.7 mg/dL, blood glucose = 40 mg/dL .
Which of the following statements are correct?
A. Clinical signs show extracellular dehydration
B. Clinical signs may be caused by hyponatremia
C. Clinical signs may be caused by hypokalaemia
D. The values of Ht and serum urea are suggestive of hemodilution
E. A probable cause of the hydroelectrolyte imbalance is Addison's disease

7. What manifestations do you think a patient who presents diarrhea for three days could have?
A. Hypertension
B. Hypotension
C. Dry mucous membranes
D. Sunken eyeballs
E. Low heart rate

8. A 55-year-old man was caught for 8 hours in a road accident, presenting with crushed arms and multiple fractures. At admission, the patient was conscious, breathing spontaneously, HR = 50 b/min, and BP = 80/40 mmHg.
What parameters of the serum ionogram can turn out modified?
A. Calcium
B. Magnesium
C. Potassium
D. Sodium
E. Phosphate

CASE STUDIES

1. A 64-year-old female patient is admitted with severe dyspnea and lower limb edema. Her history shows hypertension. The following lab results were obtained shortly after hospital admission:
Na^+ = 123 mmol/L
K^+ = 5.9 mmol/L
Cl^- = 106 mmol/L
Urea = 68 mg/dL
Creatinine = 2.9 mg/dL

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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2. A 47-year-old female patient, diagnosed with a brain tumor is admitted into hospital with severe headache, thirst, weakness and polyuria. The lab tests show the following values:
Na^+ = 150 mmol/L
K^+ = 4.2 mmol/L
Diuresis = 3.600 ml/24h

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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NOTES

8. LABORATORY ASSESSMENT OF KIDNEY DISEASES (I)
Blood tests. Assessment of glomerular and tubular function.

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. *Ask for blood tests that indicate nitrogen retention and interpret them in acute kidney injury (AKI) vs chronic kidney disease (CKD)*
2. *Ask for blood tests that evaluate regulatory kidney function and interpret them in AKI vs. CKD*
3. *Ask for and interpret the blood tests that evaluate the endocrine function in CKD*
4. *Ask for and interpret the main immunological investigations used in the diagnosis of glomerulonephritis*
5. *Use an on-line platform for GFR calculation and interpret the results according to CKD stadialization*
6. *Ask for and interpret the tests indicating acute and chronic tubular dysfunction*

The evaluation of renal function has **two major indications**:

a) Diagnosis of renal disorders – in patients with: *reno-urinary pain, micturition disorders, quantitative changes and/or* the *presence of pathological compounds in urine*

b) Monitoring of patients at high risk for developing renal disorders (patients with diabetes mellitus, hypertension, atherosclerosis, personal/familial history of kidney disease, chronic treatment with nephrotoxic drugs). The main goal of this monitoring refers to the:

- ✓ *Early* detection of *glomerular/tubular dysfunction*
- ✓ Adjustment of drug dosage according to the renal excretion capacity

The tests that explore renal disorders can be classified into:

I. The biochemical blood assessment
II. Tests that explore glomerular dysfunction
III. Tests that explore tubular dysfunction
IV. Imaging techniques
V. Urine analysis

I. BIOCHEMICAL BLOOD ASSESSMENT

Blood tests explore the impairment of the renal function, offering information regarding the subjacent etiological mechanisms.

A. TESTS THAT EXPLORE RENAL FUNCTION IMPAIRMENT

The renal functions that can be explored through biochemical blood tests are:
- the uremic toxins excretory function
- the blood homeostasis regulatory function
- the endocrine function

a) Tests that explore the EXCRETORY function. Indicators of nitrogen retention (azotemia)

Nitrogen retention or **azotemia** represents ***the increased concentration of serum catabolytes*** (urea, creatinine, uric acid) which are **indicators of *decreased glomerular filtration rate* (GFR).** The excretory function is altered both in *acute kidney injury* (formerly known as acute renal failure), as well as in *chronic kidney disease* (formerly known as chronic renal failure):

- **Acute kidney injury (AKI)** – represents the *sudden* (but potentially reversible) decrease of GFR, that usually occurs in **healthy kidneys**
- **Chronic kidney disease (CKD) –** represents the *slow, progressive* and *irreversible* decrease of **GFR < 60 ml/min/1,73 m²**, for at least 3 months in **kidneys with preexisting damage.**

1. Serum urea

- **Source**: it represents the final product of nitrogen catabolism, synthesized by the liver from ammonia during the process of *ureogenesis*. The serum concentration of urea depends on:
  - tissue protein catabolism
  - protein exogenous intake

- *renal function* (urea is filtered in the glomerulus, is reabsorbed and is secreted in the tubules)
- ammonia liver detoxification function
- extrarenal excretion (skin and GI tract during markedly increased serum levels)
- volemia

- **Normal values: 15-45 mg/dL**
- **Pathological changes:**
  - **Increased values (uremia) are due to:**
    - ***Renal causes:***
      - **in AKI**: urea *rapidly* increases (by 10-20 mg/dl in 48 hours) and it can get to values of *200 - 400 mg/dl* in less than 1 week
      - **in CKD:** plasma urea increases *slowly*, reaching values *> 300 mg/dl in the end stage of the disease (chronic uremia)*

Remember!
Serum urea is a ***good indicator of azotemia, but it cannot be correlated with the severity of GFR decrease.***

    - ***Extrarenal causes***:
      - increased protein catabolism (e.g., digestive hemorrhages, massive tissue destructions, neoplasms)
      - massive protein ingestion
      - hypovolemia
  - **Decreased values are due to:**
    - end-stage cirrhosis (hepatic failure)
    - protein malnutrition
    - hypervolemia

2. Blood urea nitrogen (BUN)

For establishing the etiology of azotemia in AKI, the **BUN : creatinine ratio** can be calculated. Blood urea nitrogen can be determined by dividing the value of urea by 2,2.

- **Normal values: 7-20 mg/dL**

3. Serum creatinine

- **Source**: It is a breakdown product of creatinphosphate in the muscle. Values are dependent on:
  - muscular mass (gender and age)
  - *renal function* (90% of the creatinine is filtered and 10% is secreted in the proximal tubule)
- **Normal values: 0,6 - 1 mg/dL (women)**
  0,8 - 1,3 mg/dL (men).
- **Pathological changes:**
  - **Increased values:**
    - ***Renal causes:***
      - **in AKI** creatinine increases by ≥ 0,3 mg/dl in 48 hours and can reach values *≥ 4 mg/dl in less than 1 week*
      - **in CKD** there is a 0,5-1 mg/dl increase every 1-2 years, reaching values *> 10 mg/dl in the end stage of the disease*
    - ***Extrarenal causes*:**
      - tissue necrosis: rhabdomyolysis, IIIrd degree burns
      - hypovolemia
  - **Decreased values**:
    - severe hepatic dysfunction
    - decreased muscle mass (muscle dystrophy, myasthenia gravis)
    - protein malnutrition
    - hypervolemia

Remember!:
Serum creatinine is **not a good indicator for the early stages of GFR decrease** because its serum level increases only when the number of functional nephrons decreases to 50-75%. Doubling of serum creatinine level signifies the reduction by ***50% of the functional nephron mass.***

4. BUN:Creatinine Ratio

- **Normal values: 10 – 20**
- **Clinical value:**

BUN:creatinine ratio is used for the **differential diagnosis of azotemia in acute kidney injury**, condition which can be induced by one of the following 3 causes:

- *prerenal* (functional)
- *renal* (intrinsic)
- *postrenal* (obstructive)

- **In PRERENAL azotemia –** GFR decreases due to **renal hypoperfusion** induced by ***hypovolemia*** (severe hemorrhage, gastro-intestinal fluid loss, burns, congestive heart failure, severe acute pancreatitis):
  - *at glomerular level*: the GFR decrease induces the decrease of filtered urea and creatinine
  - *at tubular level*: an important part of urea is reabsorbed in the proximal convoluted tubule (PCT), while the whole quantity of filtered creatinine is eliminated through urine
  - *BUN : creatinine* ratio **> 20**

- **In RENAL azotemia** – the most frequent cause of AKI is **the acute tubular necrosis *(ischemic or nephrotoxic)*** which induces the retrodiffusion of primary urine in the PCT and the decrease of GFR due to renal blood flow disorders:
  - *at glomerular level*: the GFR decrease induces the decrease of filtered urea and creatinine
  - *at tubular level*: filtered urea and creatinine are reabsorbed in plasma, but the urea excess in the plasma can be eliminated via extrarenal pathways (skin, gastrointestinal tract), while the excess of creatinine accumulates in plasma
  - *BUN : creatinine ratio* < **10**
- **In POSTRENAL azotemia** – GFR decreases due to **urinary tract obstruction,** induced by obstructive uropathy which occurs bilaterally or on a solitary kidney (anatomical/functional):
  - *at glomerular level*: the decrease of GFR induces the parallel filtration decrease of both urea and creatinine
  - *at tubular level*: filtered urea and creatinine cannot be properly eliminated due to renal obstruction, thus they both accumulate in the blood
  - *BUN : creatinine ratio* is **normal = 10-20**

5. Uric acid

- **Source:** final catabolism product of purine nucleotides (originating from nucleic acids structure) within the liver. Serum concentration depends on:
  - nucleoproteins' catabolism
  - *renal function* (uric acid is glomerularly filtered and is reabsorbed/secreted in the proximal convoluted tubule)
- **Normal values: 2 – 7 mg/dl (Men)**
  2 – 5,7 mg/dl (Women)
- **Increased values (hyperuricemia)**:
  - ***Renal cause*** – uric acid is a **good indicator of the end stage of CKD** (i.e. renal failure) when its plasma level can reach values > 10 mg/dl
  - ***Extrarenal causes*:**
    - gout
    - increased nucleoproteins' catabolism in leukemias, chemotherapy, radiotherapy
- **Decreased values***:* hepatic failure

b) Tests exploring the kidney's ability to maintain BLOOD HOMEOSTASIS

1. Serum electrolytes - plasma ionogram

Evaluates the function of *plasma electrolytes preservation or elimination*. The ionogram is useful in appreciating the hydro-electrolyte imbalances induced by:

- **AKI:** the oligoanuric phase and then the diuretic phase (polyuric)
- **CKD:** the initial phase (compensatory phase) and then the terminal phase (renal failure)
- aldosterone secretion impairment
- diuretic therapy

Table 8.1. Changes of plasma ionogram in AKI and CKD

	AKI initial phase CKD end stage (oligo-anuria)	**AKI diuretic phase CKD initial phase (polyuria)**
Na^+	↑/↓	↓
K^+	↑	↓
Ca^{2+}	↓	↓
Mg^{2+}	↑	↓
Cl^-	↑	↓
HCO_3^-	↓	↓
HPO_4^-	↑	N
SO_4^{2-}	↑	N

2. Acid-base balance parameters

Reveal the **metabolic acidosis** induced by the inability to maintain the acid-base balance in AKI and the final stage of CKD (reabsorption & generation of HCO_3^- simultaneously with H^+ excretion).

Table 8.2. Normal values and pathological variations of acid-base parameters in renal failure.

Parameters	**NV**	**AKI/CKD**
pH	7,35-7,45	↓
HCO_3^- (mEq/l)	22-26	↓
$PaCO_2$ (mmHg)	35-45	↓

3. Proteinemia and protein electrophoresis

Reveal the *hypoproteinemia and dysproteinemia* induced by protein loss at glomerular membrane level in **nephrotic syndrome** (Table 8.3).

Table 8.3. Normal values and pathological variations of proteinemia and electrophoresis in nephrotic syndrome.

Parameters	NV	Nephrotic syndrome
Proteinemia (g/dl)	6,7 – 8,4	↓
ELFO (%)		
Albumins	50-60	↓
α_1 – globulins	3-6	N
α_2 – globulins	7-10	↑
β– globulins	11-14	↑
γ –globulins	15-23	↓

4. Serum lipidogram

- **Normal value:**
  - **Lipids = 400-800mg/dl**
  - **Total Cholesterol = 140-200mg/dl**
- Serum lipids and total cholesterol increase in **nephrotic syndrome.**

c) Tests that explore the ENDOCRINE function

Are recommended for **patients with CKD** and include:

1. Serum level of erythropoietin

In patients with CKD, the secretion of erythropoietin is impaired, so it's serum concentration will be ***decreased***, leading to severe *normocytic normochromic anemia*.

Exogenous substitution therapy with human recombinant erythropoietin or erythropoiesis – stimulating agents is effective in treating this secondary anemia.

- **Normal values: 4,3 – 29 U/L**

2. Serum level of parathyroid hormone (PTH)

PTH serum level ***increases*** due to *secondary hyperPTH induced by hypocalcaemia and hyperphosphatemia ⇒ CKD – associated mineral bone disease.*

- **Normal value: 10-65 ng/L**

3. Serum level of 1,25-dihidroxi-cholecalciferol (1,25 –$(OH)_2$ – D_3):

The serum level of 1,25 –$(OH)_2$ – D_3 ***decreases*** *due to renal synthesis deficit of the active form of vitamin D_3* (decrease of the activity of 1 α-hydroxylase, responsible for the second hydroxylation) ⇒ *hypocalcaemia* and *osteomalacia*

- **Normal values: 20-75 ng/ml**

B. IMMUNOLOGICAL INVESTIGATIONS

1. Serum autoantibodies:

- **Antinuclear antibodies (ANA profile)**
  - represent a group of antibodies directed against the nucleus of self cells.
  - their presence is specific for different autoimmune diseases (anti double stranded DNA antibody – systemic lupus)
- **Anti glomerular basement membrane antibodies**
  - antibodies against type IV collagen in the basement glomerular and alveolar membrane
  - they are present in rapidly progressive glomerulonephritis (ex. Goodpasture syndrome, associated to pulmonary manifestations).
- **ANCA (anti neutrophil cytoplasmic antibodies)**
  - antibodies against antigens from the cytoplasm of neutrophils, seen typically in patients with immune vasculitis, that can associate different forms of glomerulonephritis.

2. Serum Complement - C3, C4 fractions

- The C3 component takes part in the activation of the *classical* and *alternate* complement pathway while the C4 fraction refers to the activation of only the *classical* complement pathway (it's concentration will be normal if the complement was activated only through the alternate pathway).
- C3 levels will be decreased, while C4 levels will be normal during poststreptococcal glomerulo-nephritis (this diagnosis also associates the increase in the titer of ASLO antibodies - antistreptolysin O - and anti-DNAse B antibodies)
- Both fractions are decreased when they are consumed at renal level (membrane proliferative glomerulo-nephritis, lupus nephritis)

3. Serum immunoglobulins and immuno-ELFO

– Useful in determining the level of different immunoglobulines (i.e., IgA in mesangial IgA nephropathy, or IgG and light k and λ chains in multiple myeloma).

Observation!
The detection of a possible infection with hepatitis B / C virus or HIV is mandatory if the diagnosis of glomerulonephritis is suspected.

II. TESTS THAT EVALUATE GLOMERULAR DYSFUNCTION

Glomerular dysfunction is manifested by **GFR decrease < 90 ml/min/1,73m^2 WITH or WITHOUT proteinuria.**
GFR can be measured directly (the renal clearance method) or can be estimated indirectly (by using several formulas).

1. Endogenous creatinine clearance (CrCl)

- **Definition:** The clearance of a substance refers to the removal of that substance from the blood, expressed as **the volume of blood or plasma** (ml) **cleared of the substance per time unit** (minute).

$$Cl_x \text{ (ml/min)} = (U_x \times V) / P_x$$

Where:
Cl = clearance
V = urinary flow (ml/min)
U = urinary concentration of the substance in the 24h urine
P = plasmatic concentration of the substance

For GFR evaluation substances that are *eliminated through glomerular filtration* but are NOT reabsorbed and NOT secreted within the tubules must be used. These requirements are fulfilled by exogenous substances (inulin, radioactively marked EDTA – that are not used in clinical practice) and only partially fulfilled by endogenous creatinine, which is eliminated through glomerular filtration up to 90% while 10% is secreted in the PCT. Therefore, GFR is overestimated by 10-20 ml/min/1,73m^2 , but this is found to be within *reasonable limits*. However, in kidney failure the secreted fraction increases, therefore the clinical value of creatinine clearance is lost.

- **Normal values**: 90 -140 ml/min/1,73m^2 (M);
  80 -120 ml/min/1,73m^2 (F)

Remember!:
CrCl **CANNOT identify** a moderate decrease of GFR **between 40 and 70 ml/min/1,73 m^2** (the „*blind*" diagnosis zone of the creatinine clearance).

2. Assessment of the glomerular filtration rate (eGFR)

Due to the difficulty in precisely collecting diuresis, especially in the case of patients with low compliance, the use of formulas that estimate the GFR is preferred because they are based on a single determination of the **serum concentration** of **creatinine and/or,** more recently of **cystatin C.**

- **Cystatin C** is a low molecular weight protein, part of the lysosomal enzyme inhibitor family (mainly inhibits cystein-proteinases) preventing the extracellular catabolism of peptides and proteins. This substance is *produced by all nucleated cells*, its production rate being constant throughout life. ***Cystatin C production is not influenced by muscle mass (gender, age), food or drug intake.*** Due to the low molecular weight and the positive electronic charge, cystatin C is freely filtered in the glomeruli and is then completely reabsorbed in the PCT cells, where it is also metabolized/degraded. Thus, in the absence of tubular lesions, cystatin C is NOT seen in the urine (is determined only in the blood).
- GFR can be measured using different formulas:
  - ✓ **CKD-EPI (*C*hronic *K*idney *D*isease *E*pidemiology *C*ollaboration) –** the most accurate formula to estimate GFR, especially if GFR > **60**ml/min/1,73m^2 (according to KDIGO 2013 Guidelines).
  - ✓ **MDRD (*M*odification of *D*iet in *R*enal *D*isease) –** less accurate than CKD-EPI, especially if GFR > 60ml/min/1,73m^2 (according to KDIGO 2013 Guidelines).
- These formulas can be accessed *on line* (*mdrd.com*) allowing the stadialization of CKD (Table 8.4) according to the plasma level of **creatinine** *and/or* **cystatin C**.

Table 8.4. CKD stadialization according to the GFR decrease rate and the severity of renal injury.

Stage	GFR (ml/min/1,73 m^2)	Severity of renal damage
G1	≥ 90	Chronic renal injury with normal GFR
G2	60 – 89	Chronic renal injury with slightly decreased GFR
G3a	45 – 59	CKD with slight to moderate decrease of GFR
G3b	30 – 44	CKD with moderate to severe decrease of GFR
G4	15 – 29	CKD with severe decrease of GFR
G5	< 15	Renal failure

III. TESTS THAT EVALUATE TUBULAR DYSFUNCTION

Tubular dysfunction represents the decrease of the kidney's ability to *concentrate and dilute urine,* occurring *rapidly* in **acute tubular necrosis** *(ATN)* or *slowly, progressively* in **chronic kidney disease** *(CKD):*

- **in ATN:**
  - Retrodiffusion of the primary urine in the PCT and the important decrease of GFR induce *oliguria*
  - The acute decrease of the kidney's ability to **concentrate** and **dilute** urine induces the elimination of urine with the same density as the primary urine (*isosthenuria)*
  - Decreased tubular reabsorption capacity induces *the increase of FENa$^+$ (fractional excretion of sodium)*
- **in CKD:**
  - The slow-progressive decrease of GFR is compensated by glomerular hyperfiltration at the level of remnant nephrons, which induces *polyuria*
  - The slow and progressive decrease of the kidney's ability to **concentrate** and **dilute** urine induces the elimination of urine with the same density as the primary urine *(isosthenuria)*, which won't be modified regardless the volume of diuresis *(fixed isosthenuria).*

Indicators of ACUTE TUBULAR DYSFUNCTION

1. Urinary indices → explore the decrease of the kidney's ability to concentrate urine in ATN and comprise:

- **Urine Osmolarity** (UOsm, *mOsmol/l*) – measured in urine/24 hours
- **Urinary Na$^+$** (UNa$^+$, *mmol/l*) – measured in a spontaneous urine sample
- **Fractional Excretion of sodium, FENa$^+$** – calculated according to the formula:

FENa$^+$ = [(urinary Na$^+$ / serum Na$^+$) x 100] / (Urinary Creat./Serum Creat.)

- **Clinical value:** differential diagnosis of *functional* oliguria in prerenal azotemia (due to renal hypoperfusion, with *normal* tubular function) from *organic* oliguria in renal azotemia (due to ATN, with *decreased* tubular function) (Table 8.5)

Table 8.5. Differential diagnosis of oliguria

Type of azotemia	UOsmol (mOsmol/l)	UNa$^+$ (mmol/l)	FENa$^+$ (%)
Prerenal	> 500	< 10	< 1%
Renal	< 350	> 20	> 1%

2. Early markers of ATN

- **NGAL (neutrophil gelatinase associated lipocalin or lipocain 2):** it is a gelatinase binding protein, member of the lipocaine family, initially found in the granules of human neutrophils. NGAL is markedly expressed in injured tubular epithelial cells (NGAL has a role in the conservation of tubular function through inhibition of apoptosis and of the proliferative response). The increase in the serum and urine level of NGAL has the following significance:
  - ***It is a „troponin-like"* biomarker** which:
    - identifies ATN in an early stage (few hours after the onset), ***before*** the increase of serum creatinine (after 24-48 hours)
    - allows the differential diagnosis of oliguria caused by *renal azotemia* (increased NGAL level) from *prerenal azotemia* (normal NGAL level)
  - ***It is an independent risk marker for the progression of CKD***, even in patients with normal creatinine and without albuminuria. Its values are normal in stable CKD and increase in patients with progressive CKD.

IV. IMAGING TECHNIQUES

1. Renal ultrasound

- represents the imaging ***method of choice*** for renal evaluation, being non-invasive and non-irradiating.
- highlights:
  - changes in kidney size and symmetry (impaired kidney function in the presence of normal-sized kidneys suggests acute kidney injury)
  - the existence of a bladder or ureteral obstruction associated with hydronephrosis
  - abnormal kidney masses (cysts, tumors, abscesses)
  - *Doppler ultrasonography* can be used to investigate the patency of the renal vein or artery

2. Computed tomography (CT)

- is the imaging ***method of choice for renal colic and suspected kidney stones***
- highlights:
  - the presence of abnormal renal masses
  - the presence of retroperitoneal tumors or fibrosis or other causes of ureteral obstruction
  - renal vascularization by CT angiography
  - stage of a kidney or bladder tumor
- this method has the disadvantages of being radiation-based and of possessing nephrotoxic potential through the contrast agent administered.

3. Positron emission tomography (PET)

with F-18 fluorodeoxyglucose

- is useful for detecting infections (e.g. in a cyst), inflammation or tumours
- is often used along with CT (PET/CT)

4. Magnetic resonance imaging

- can be used as a non-irradiating alternative to CT for staging prostate, kidney or bladder cancer and for high quality assessement of the renal vascularization.
- the contrast agent used (gadolinium) is not recommended in patients with severe renal impairment due to its potential to induce nephrogenic systemic fibrosis.

CHECKPOINT

***1. Which of the following factors influence the serum concentration of cystatin C?**
A. Dietary protein intake
B. Liver detoxification function
C. Gender and age
D. Muscle mass
E. None of the above

2. Which of the following aspects characterize prerenal azotemia?
A. Slow and progressive decrease of GFR
B. Decreased BUN : creatinine ratio < 10
C. Increased BUN : creatinine ratio > 20
D. Is induced by acute tubular necrosis
E. Is induced by hypovolemia

3. Which of the following changes of plasma ionogram are present in the oligoanuric stage of acute tubular damage?
A. Hyponatremia
B. Hypokalemia
C. Hypercalcemia
D. Hypermagnesaemia
E. Hyperphosphatemia

4. Which of the following changes define dysproteinemia in the nephrotic syndrome?
A. Decreased albumins
B. Increased α_1 – globulins
C. Decreased α_2 - globulins
D. Increased β-globulins
E. Increased γ-globulins

5. Which of the following are characteristic for post-renal azotemia?
A. At glomerular level the decrease in GFR causes the decrease of urea as well as creatinine filtration
B. At tubular level filtered urea and creatinine cannot be properly eliminated due to renal obstruction, thus they both accumulate in the blood
C. Is caused by acute tubular necrosis
D. Is caused by hypovolemia
E. Involves the activation of anti-glomerular basement membrane antibodies (anti-GBM Ab)

***6. Which of the following changes represents a consequence of endocrine function impairment in CKD?**
A. Increased EPO synthesis
B. Normochromic normocytic anemia
C. Hyperphosphatemia
D. Hypercalcemia
E. Secondary hypoparathyroidism

***7. What is the severity of renal dysfunction if the estimated GFR through the CKD-EPI formula is 50 ml/min/1,73 m^2?**
A. CKD with mildly decreased GFR
B. CKD with mild to moderate GFR decrease
C. CKD with moderate to severe GFR decrease
D. CKD with severe GFR decrease
E. Renal failure

8. Which of the following evaluate the tubular dysfunction in acute tubular necrosis?
A. Plasma osmolarity
B. Na^+ measured in a spontaneous urine sample
C. Fractional Excretion of sodium
D. Creatinine clearance
E. Serum complement

9. In acute tubular necrosis:
A. The acute decrease of GFR causes oliguria
B. Isosthenuria appears
C. The fractional excretion of Na^+ increases
D. The slow-progressive decline of GFR is compensated by glomerular hyperfiltration
E. Nephrotic syndrome develops

10. Anti-glomerular basement membrane antibodies (anti-GBM Ab):
A. Are antibodies directed against type IV collagen in the glomerular and alveolar basal membrane
B. Are present in rapidly progressive glomerulonephritis
C. Are antibodies directed against neutrophil cytoplasmic antigens typically present in patients with immune vasculitis associated with different forms of glomerulonephritis
D. Are anti-double-stranded DNA antibodies present in systemic lupus erythematosus
E. They are present in Goodpasture syndrome associated with pulmonary manifestations

CASE STUDIES

1. A 71-year-old diabetic and hypertensive patient goes to her doctor accusing general malaise, muscle cramps, oliguria, pallor.

Blood tests:

Hb = 9,6 g/dl, MCV = 91 fl, MCH = 30 pg/E, MCHC = 33 g/dl
Ionogram: K^+ = 5,9 mmol/l, Na^+ = 131mmol/l, Ca^{2+} = 1 mmol/l, Blood glucose = 201 mg/dl
Cr = 2,1 mg/dl, Urea = 140 mg/dl, Uric acid = 9 mg/dl
GFR = 23 ml/min/1,73m^2,
Albuminuria: 100 mg/day
Urine analysis: Glucose (+)

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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2. A 60-year-old diabetic and hypertensive patient undergoes coronary angiography. Approximately 48 hours after the investigation the general condition of the patient is rapidly altered and nausea and vomiting occur. The patient says he has not urinated in the last 24 hours despite the fact that he is receiving diuretic treatment for HT.

BP = 220/115mmHg
Blood glucose = 240mg/dl
Serum urea = 130mg/dl
Serum creatinin = 2,8mg/dl

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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NOTES

9. LABORATORY ASSESSMENT OF KIDNEY DISEASES (II)
Urine examination

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. *Ask for and interpret the urine analysis parameters*
2. *Recognize the specific urinary changes in glomerular nephropathy vs. tubulointerstitial nephropathy*
3. *Recognize the specific urinary changes in upper vs. lower urinary tract infections (UTI)*
4. *Ask for and interpret the tests that evaluate the severity of chronic kidney disease (CKD)*

Urine examination has **5 components**:
• Macroscopic examination
• Physical examination
• Biochemical examination
• Microscopic examination
• Bacteriological examination

I. MACROSCOPIC URINE EXAMINATION

1. Urinary VOLUME (diuresis)

The normal urinary volume/24h, i.e. the **diuresis**, is between **800–2500 ml/day**, which varies with fluid intake and diet (usually the minimal diuresis required to maintain renal excretion function is 650 ml/day).

- **Pathological changes:**
  - **Oliguria** – decreased diuresis **< 400 ml/day**
  - **Anuria** – decreased diuresis **< 50 ml/day**
    - *Prerenal Causes:*
      - severe hypovolemia (vomiting, diarrhea, extensive burns, diuretics abuse)
      - shock states with arterial hypotension (cardiogenic, distributive shock)
      - fluid sequestration in the IIIrd space (pancreatitis, peritonitis, hypo-albuminemia)
    - *Renal Causes:*
      - Acute kidney injury (AKI) in the initial stage
      - CKD in the final stage (of renal failure)
    - *Postrenal causes:* prostate hypertrophy, urethral strictures
  - **Polyuria** – diuresis **> 2500 ml/day**
    - *Causes*
      - AKI during the diuresis recovery phase
      - CKD in the initial compensatory phase
      - diabetes insipidus
      - presence of osmotically active particles in the urine – nephrotic syndrome (proteinuria) or decompensated diabetes mellitus (glycosuria)
      - diuretics abuse
  - **Polakiuria** - high frequency of micturition with the passage of small volumes of urine, typically associated with upper or lower urinary tract infections.
  - ***Nicturia*** **-** nocturnal micturition, usually associated with a prostate pathology (adenoma, carcinoma).

2. Urine APPEARANCE

Normally, immediately after the emission the urine is *clear* and *transparent*. When left at room temperature for a certain period of time a thin cloudy formation (*nubecula)* consisting of desquamated epithelial cells and mucus can be observed, without any pathological significance.

- **Pathological changes:**

Urine becomes cloudy due to:

- increased quantity of salts (ex. urinary lithiasis)
- presence of leukocytes, bacteria or pus (pyuria, in urinary infections)
- presence of erythrocytes (ex. in acute glomerulonephritis)
- presence of lipids (nephrotic syndrome)
- presence of lymph (chyluria)

3. Urine COLOR

Normally, urine color varies from pale yellow to dark yellow due to the presence of normal metabolic compounds, ***urochromes*****.**

- **Pathological changes:** abnormal pigmentation of the urine can be:
  - **almost colorless** (diluted urine): diuretics abuse
  - **pale yellow:** diabetes mellitus/insipidus, alkaline pH
  - **yellow-orange** (concentrated urine): fever, intense sweating, acidic pH
  - **dark yellow:** presence of excessive bile pigments (bilirubin)
  - **red:** hemoglobinuria (due to intravascular haemolysis), myoglobinuria (due to traumatic muscular destruction), hematuria
  - **brown:** haematuria of glomerular origin due to the formation of methemoglobin under conditions of an acidic pH
  - **green:** *Pseudomonas* urinary infection

4. Urine ODOR

Normally, freshly passed urine has a characteristic ***aromatic odor***, due to volatile organic acids.

- **Pathological changes:**
  - ***intense ammonia odor*** (decomposition of urinary urea into NH_3) in urinary infections with aerobic flora that produces *urease* (typical ex.: *Proteus*)
  - ***acetone odor*** in ketonuria in diabetic ketoacidosis and after severe vomiting
  - ***putrid smell*** in anaerobic microbial infections, bladder tumors with overlapping infection

II. PHYSICAL URINE EXAMINATION

It is a component of the urine analysis performed by the *dipstick method*, a ***semi-quantitative screening test*** to be performed annually, even in case of apparent health.

1. Urinary pH

Normal urine is slightly acidic (**pH** of **5.5 - 6.5)** with a wide range of variation (**4.5** to **8.0**) according to food intake: vegetarian diet = alkaline pH; proteic diet = acidic pH). Interpreting the urine pH is relevant only in the absence of urinary infection, as some bacteria *acidify* (E. Coli) and some *alkalinize* (Proteus Mirabilis, Pseudomonas aeruginosa, some Klebsiella species - by producing urease) the urine.

- **Pathological changes:**
  - **acidic urine pH (<5.5):**
    - metabolic acidosis
      - with high values of the anion gap: diabetic ketoacidosis, methanol ingestion, uremia
      - with normal values of the anionic gap: diarrhea.
    - respiratory acidosis (hypoventilation)
  - **alkaline urine pH (>7):**
    - respiratory alkalosis (hyperventilation)
    - metabolic alkalosis (vomiting)
    - renal tubular acidosis
  - **neutral/slightly acidic pH (6.5-7):** impairment of urine acidification capacity in acute tubular necrosis (ATN) or CKD

2. Urine SPECIFIC GRAVITY

Normally, the specific gravity of the freshly passed morning urine is **1015-1025**.

- **Pathological changes:**
  - ***hypersthenuria* (excludes tubular dysfunction!)** – specific gravity **> *1025***.
    A high specific gravity can be due to: diabetes mellitus (high glucose concentration), nephrotic syndrome (high amounts of proteins), prerenal azotemia.
  - ***hyposthenuria*** – specific gravity < ***1015.*** Occurs after high fluid intake, ATN, CKD in the initial phase, diabetes insipidus.
  - ***isosthenuria*** – specific gravity ***1008 – 1012*** (after repeated assessement), equaling the specific gravity of the plasma, regardless the water intake.
    It signifies decreased concentration and dilution capacity of urine in ATN or CKD.

III. BIOCHEMICAL URINE EXAMINATION

1. PROTEINURIA

Normally: proteins that are glomerularly filtered are absorbed almost entirely in the proximal convoluted tubule (PCT), a small amount being eliminated in the final urine - **< 150 mg/24 hours** or **< 30mg albumins/day.**

- **Pathological changes:** excretion > **150 mg/ day** can be detected using dipsticks, *measured* in urine/24h and *analyzed* by electrophoresis of the urinary proteins

a) Comercial Test Strips (dipstick)

– used for semiquantitative evaluation of ***proteinuria*** or ***albuminuria***. On the basis of the color reactions, it can detect "traces" of proteins (5-15 mg/dl) up to more than 2000 mg/dl. The method is highly sensitive to the presence of *albumin*, but less sensitive to the presence of globulin, hemoglobin or light chains.

b) Measurement of proteinuria/24h

– is performed when the dipstick method emphasizes high levels of protein/albumin in the urine. This method has been largely replaced by more convenient tests, namely the proteinuria/urinary creatinine ratio and the urinary albumin/creatinine ratio measured in a spontaneous urine sample, respectively.

- Allows the ***quantification of proteinuria*** and can ***indicate the cause:***

✓ **In glomerular nephropathies:**
- proteinuria appears by increased filtered protein quantity, tubular reabsorption capacity being exceeded:
  – *moderate albuminuria* = 30-300 mg/day in diabetic and hypertensive nephropathy
  – *moderate proteinuria* = 1-3 g/day in nephritic syndrome (glomerulonephritis)
  – *severe proteinuria* > 3,5 g/day in nephrotic syndrome

✓ **In tubulo-interstitial nephropathies** (eg., acute pyelonephritis, ATN, Fanconi syndrome, etc.)
- proteinuria occurs by decreased tubular protein reabsorption, the filtered quantity being normal
- *proteinuria is mild* = 0.3 - 2 g/day

✓ **in extra-renal diseases** (eg., multiple myeloma, hemoglobinuria, rhabdomyolysis)
- proteinuria appears by hyperfiltration due to increased amounts of Ig light chains (Bence-Jones proteinuria), Hb, myoglobin in plasma
- proteinuria is variable between 0.2-10 g/day

c) Urinary protein electrophoresis

It reveals the ***type of proteinuria***.

- **Glomerular:**
  – *selective proteinuria* - characterized by **exclusive loss of albumins** (100%) <u>without</u> globulins, in minimal glomerular injury ⇒ *pure nephrotic syndrome*
  – *non-selective proteinuria* - characterized by loss of **albumins** (>75%) and **globulins** in severe damage of the glomerular filtration barrier ⇒ *impure nephrotic syndrome*
- **Tubular:**
  – low loss of albumins (< 30%) and increased loss of low molecular weight proteins (β2-microglobulin, α2 micro-globulin, retinol-binding protein, etc.)

Remember!

A transient proteinuria < 2 g/day associated with fever, intense exercise, prolonged orthostatism, exposure to cold is considered *physiological* or *functional*.

d) Albuminuria and urine albumin/ creatinine ratio

Albuminuria is an early marker of glomerular disease and is considered a ***screening*** and ***monitoring test*** used for patients with *diabetes mellitus* and *arterial hypertension*. It also defines the **presence of CKD** and the **severity of kidney damage** (Table 9.1.)

- **Albuminuria** is evaluated in urine/24h:
  – values > **30 mg/24h, persisting for 3 months**, define the presence of **CKD** and the level of increase defines the severity of kidney damage
- **Albumin/creatinine ratio** is determined in a spontaneous urine sample:
  – values > **30 mg/g, persisting for 3 months**, define the presence of **CKD** and are associated with a **high cardiovascular risk.**

Table 9.1. Stages of CKD according to the degree of albuminuria and the severity of renal damage (KDIGO 2013 guidelines - *KDIGO = Kidney Disease Improving Global Outcomes*)

Stage	Albuminuria (mg/24 h)	Urine albumin/creatinine ratio		Degree of renal impairment
		mg/mmol	mg/g	
A1	< 30 (normal to slightly increased)	< 3	< 30	Normal to moderate
A2	30-300 (moderately increased)	3-30	30-300	Moderate
A3	> 300 (severely increased)	> 30	> 300	Severe

2. GLYCOSURIA

Glucose is normally **absent in urine,** as it is completely reabsorbed in the proximal tubules.

- **Pathological changes:**
  - **serum glucose > 160-180mg/dl:** *renal glucose elimination threshold is exceeded*: diabetes mellitus, Cushing's syndrome, acromegaly, pancreatitis, hyperthyroidism.
  - **serum glucose is normal**, but there is (most frequently) a *decrease in the kidney threshold for glucose elimination* (eg, pregnancy, benign glycosuria) or (more rarely) an *important impairment of the PCT* (e.g., Fanconi sdr.).

3. KETONURIA

Normally the urine does not contain ketones (acetone, acetylacetic acid, beta-hydroxybutyric acid).

- **Pathological changes:** ketonuria occurs through increased production of ketone bodies when glucose can not be used as an energy substrate due to:
  - absolute/relative insulin deficit in *diabetes mellitus*
  - insufficient intake in *diarrhea, vomiting*
  - increased catabolism in *hyperpyrexia, cachexia, severe thyrotoxicosis*

Remember!

Ketonuria ***precedes*** the significant increase in serum ketone concentration (ketonemia), therefore displaying a **higher clinical value** in glycemic balance assessment in diabetic patients.

4. DETECTION OF BLOOD IN URINE

Normally, urine does not contain blood.

- **Pathological changes:** blood may be present in urine in the form of ***hematuria*** (red blood cells) or ***hemoglobinuria*** (hemoglobin resulting from erythrocyte destruction). The dipstick method *can NOT differentiate hemoglobinuria from hematuria* and may yield false positive results in the case of myoglobinuria. The **microscopic examination of urine** and the **Addis-Hamburger sediment** are therefore recommended:
  - the presence of erythrocytes in the urinary sediment excludes hemoglobinuria
  - the absence of red blood cells in the urine sediment in the settings of an intense hematuria revealed by the dipstick method raises suspicion of hemoglobinuria or myoglobinuria
- **Causes:**
  - *hematuria*: glomerulonephritis, renal lithiasis, renal tumors, renal trauma
  - *hemoglobinuria*: intravascular haemolysis
  - *myoglobinuria*: rhabdomyolysis

5. NITRITES

Normally, nitrites are not present in urine.

- **Pathological changes:** they are produced when gram negative bacteria (E. Coli, Enterococci) reduce urinary nitrates to nitrites and therefore their identification using dipsticks signifies bacteriuria. The test is either positive or negative and is not a substitute for urine culture. False-negative results may occur with certain pathogens that cannot convert nitrate to nitrite (eg, Mycobacterium tuberculosis, Pseudomonas sp) or when time is inadequate (< 4 h) for conversion of nitrate to nitrite.

6. DETECTION OF URINARY WHITE BLOOD CELLS

Normally, urine does NOT contain leukocytes detectable by using dipsticks.

- **Pathological changes:** the threshold for leukocyturia detectable by dipstick is of at least ***5-15 cells/microscopic field***. This must be confirmed by the examination of the urinary sediment. It is caused by ***urinary tract infections***.
- **Clinical value**:
  - leukocyturia should be confirmed by:
    - an increase in the number of leukocytes in the urine sediment
    - a positive *urine culture*
    - an increase in *leukocyte esterase* in the case of a negative urine culture taken on average media, which indicates sterile pyuria in the case of Mycobacterium tuberculosis or Chlamidia trachomatis infection
  - leukocyturia should be interpreted together with the presence of leukocyte casts:
    - the presence of leukocyte casts signifies an ***upper*** UTI (acute pielonephritis)
    - the absence of leukocyte casts signifies a ***lower*** urinary tract infection

7. URINARY UROBILINOGEN (Ubg)

Normally, freshly passed urine contains only small amounts of Ubg (Ubg +).

- **Pathological changes:**
  - *increased* urinary levels during hemolytic and hepatocellular jaundice
  - *absent* during obstructive jaundice.

8. BILIRUBIN

Normally, bilirubin is **absent** in urine.

- **Pathological changes:**
  - conjugated bilirubin is present in urine during mechanical or hepatocellular jaundice (choluric jaundice).

9. LIPIDURIA

Normally < 10 mg of lipids/24 hours (neutral fats, fatty acids and cholesterol) are eliminated.

- **Pathological changes: increased values > 0,5g/24 h** appear in the **nephrotic syndrome**

10. URINARY IONOGRAM

- *Evaluation of urinary sodium* in a spontaneous urine sample is useful as an indicator for pre-renal AKI differentiation, in which sodium preservation capacity is maintained (urinary Na^+ < 10 mmol/l) from intrinsic/renal AKI (ATN) in which the ability to preserve sodium is decreased and sodium is lost via urine (urinary Na^+ > 20 mmol/l)
- *Evaluation of calcium* (100-300 mg/day) and *phosphate* (400-800 mg/day) in urine is useful as an indicator of *phospho-calcic balance disorders*.

IV. MICROSCOPIC URINE EXAMINATION

A. PLAIN URINARY SEDIMENT

The microscopic examination of the normal urinary sediment (examination of at least 10 high resolution microscopic fields) is obtained from fresh morning urine, through mild centrifugation (5 minutes at 2000 rpm), in order to not destroy the cells.

1. Red blood cells

- **Normal:** 0-2 RBCs/microscopic field
- **Pathological changes:** hematuria can be:
  - microscopic: > 3 RBCs/field
  - macroscopic: field filled with RBCs
- **Erythrocyte morphology** (phase contrast microscopy) - specifies the *origin of hematuria*:
  - *erythrocyte dysmorphism present* (> 30% of RBCs are dysmorphic) $\Rightarrow$ glomerular origin
  - *normal morphology RBCs* $\Rightarrow$ non-glomerular origin
- **Causes:**
  - haemostasis disorders
  - anticoagulant overdose
  - nephropathy: glomerulonephritis
  - urinary tract infection
  - uropathy: tumors (renal cancer, transitional cell bladder carcinoma, prostate hyperplasia), lithiasis

Observation!

Depending on the moment during the act of micturition when the presence of hematuria is clinically observed, the site of origin of the bleeding may be suspected:

- haematuria obvious at the onset of urination followed by clear urine: the origin is usually urethral
- haematuria obvious throughout the micturition act: the origin is in the bladder or upstream
- haematuria obvious at the end of the micturition act: the origin is prostatic or from the base of the bladder.

2. White blood cells (WBC)

- **Normal: WBCs < 5/HPF** (high power field)
- **Pathological changes:**
  - **leukocyturia** associated with *lower UTI* (cystitis, urethritis) or *upper UTI* (pyelonephritis)
  - other causes of leukocyturia: kidney stones, interstitial cystitis, tubulo-interstitial nephritis, papillary necrosis, urinary tuberculosis

3. Epithelial cells

- **Normal**: 3-4 flat epithelial cells/microscopic field (HPF), resulting from desquamation of the epithelium that lines the urinary tract
- **Pathological changes:**
  - *transitional (urothelial) epithelial cells*: in the case of UTI, transitional cell bladder carcinoma
  - *convoluted tubule cells*: in ATN due to acute ischemia, toxic agents (ex. aristolochic acid), contrast agents, nephrotoxic drugs (eg, aminoglycosides - gentamicin, immuno-suppressants - cyclosporine, chemo-therapeutic agents - cisplatin)
  - *fatty cells*: in nephrotic syndrome

4. Microorganisms (bacteria, parasites, yeast)

- **Normal**: missing from the urinary sediment
- **Pathological changes:**
  - bacteria are usually accompanied by leukocyturia
  - Candida albicans is commonly found in the urine of diabetic, immunosuppressed patients or those undergoing antibiotic treatment
  - the most common parasite is Trichomonas vaginalis

5. Casts

- **Normal:** 0-1 hyaline casts/microscopic field
- **Pathological changes:** they represent molds of the distal or collecting tubular lumen (protein mass that contains cellular debris, blood cells, epithelial cells, bacteria), their presence indicating *renal parenchyma* injury. According to the structures they may be:
  - ***Non-cellular casts:***
    - ***Hyaline casts –*** (consisting exclusively of Tamm-Horsfall proteins) - in normal urine with high specific gravity (absence of proteinuria) or glomerular nephropathy
    - ***Granular casts*** (resulting from degeneration of cellular casts or precipitation of serum proteins in a Tamm-Horsfall mucoprotein matrix) - severe kidney disease
    - ***Waxy casts*** (resulting from degeneration of the granular casts) - in renal failure
    - ***Fatty casts*** (resulting from the binding of fatty cells, cholesterol and neutral lipids on hyaline casts) - in the nephrotic syndrome associated with lipiduria
  - ***Cellular casts:***
    - ***RBC casts*** – glomerular disease.
    - ***WBC casts*** – tubulointerstitial disease, mainly pyelonephritis. When present they may greatly help in the *differentiation of pyelonephritis from cystitis.*
    - ***Epithelial casts*** – acute tubular necrosis

6. Crystals

They are represented by inorganic salts which are ***normally*** part of urine composition and can precipitate in an amorphous or crystallized form in renal tubules when the *urinary flow decreases* and the *pH is favorable*. Crystals have pathological significance if they are described in freshly passed urine, being physiologically present only in resting urine. Th pathological significance of an increased amount of the most frequently encountered crystals is as follows:

- **Ca^{2+} oxalate** crystals - ethylene glycol poisoning, urinary lithiasis.
- **Uric acid** crystals - gout, urinary lithiasis, ATN caused by tumor lysis syndrome in patients with cancer, renal failure.
- **Cystine** crystals - cystinuria, a rare hereditary cause of kidney stones.
- **Triple phosphate (struvite)** crystals - infection with bacteria that metabolise urea (Proteus)
- **Cholesterol crystals** - nephrotic syndrome

B. ADDIS-HAMBURGER URINARY SEDIMENT

It represents a more accurate method as it takes into account the urinary flow and expresses the result in cellular elements per minute.

- **Technique:** The urine is collected for three hours in the bladder, after the patient has urinated in the morning and has drunk 150ml of water. The sediment obtained from centrifuging 10ml of urine is examined under a microscope.
- **Normal values:**
  - red blood cells < 5000/minute
  - white blood cells < 2000/minute
- **Pathological changes:**
  - **Hematuria*:***
    - microscopic > 10.000 red blood cells/minute
    - macroscopic > 300.000 red blood cells/ minute
  - **Leukocyturia:**
    - 6.000 - 10.000 white blood cells/minute

V. URINE CULTURE

- **Indication**: *confirms the diagnosis of urinary tract infection.* It is performed when the urine sediment test raises the suspicion of urinary tract infection. It is expressed in number of colony forming units (CFU), the most frequently involved germs being: *E. coli,* then *Proteus, Klebsiella, Pseudomonas.*
- **Interpretation:**
  - sterile urine culture ⇒ *no germs in urine*
  - less than 1000 CFU/ml ⇒ *physiological bacteriuria*
  - between 1,000 and 10,000 CFU/ml ⇒ *contamination with urogenital flora*
  - between 10,000 and 100,000 CFU/ml ⇒ *borderline bacteriuria* with suspicion of urinary infection, urine culture should be repeated
  - **over 100,000 CFU/ml ⇒ *certain urinary infection***, it is mandatory to perform an antibiogram.

CHECKPOINT

***1. A proteinuria of 2.5 g/day together with hematuria and RBC casts is suggestive for:**
A. Nephritic syndrome
B. Nephrotic syndrome
C. Tubulo-interstitial nephropathy
D. Acute pyelonephritis
E. Diabetic nephropathy

2. Which of the following define chronic kidney disease with severe impairment of renal function?
A. Albuminuria > 300 mg/day
B. Urine albumin/creatinine ratio < 300 mg/g
C. Proteinuria 50 mg/24h
D. Polyuria with fixed isosthenuria
E. Oligo-anuria with fixed isosthenuria

3. Which of the following changes can be induced by the urinary tract infection with Proteus?
A. Leucocyturia
B. Green colored urine
C. Acetone odor
D. Alkaline urinary pH
E. Ammonia smell

4. Which of the following changes appear in acute glomerular nephropathy?
A. Proteinuria
B. Hematuria
C. Positive urine culture
D. The presence of RBC casts
E. The presence of leukocyte casts

***5. Which of the following is a characteristic of proteinuria in multiple myeloma?**
A. Glomerular proteinuria
B. Bence-Jones proteinuria
C. Selective glomerular proteinuria
D. Nonselective glomerular proteinuria
E. Severe proteinuria

6. Which of the following are specific for the nephrotic syndrome?
A. Proteinuria 1-3 g/day
B. Lipiduria
C. Leukocyte casts
D. Fatty casts
E. Hemoglobinuria

***7. In a lower urinary tract infection, the urine analysis shows:**
A. Leukocyte casts
B. Leucocyturia
C. Increased NGAL
D. Erythrocyte dysmorphism
E. Lipiduria

8. Which of the following urinary changes can occur in diabetic nephropathy?
A. Prerenal proteinuria
B. Bilirubinuria
C. Hypostenuria
D. Ketonuria
E. Glycosuria

***9. Which of the following is considered an early marker of glomerular disease used as a screening test for patients with diabetes mellitus or arterial hypertension:**
A. Hyperstenuria
B. Albuminuria
C. Microscopic hematuria
D. Decrease of pH
E. NGAL

10. Which of the following are true concerning the Addis-Hamburger sediment:
A. Takes into account the urinary flow and expresses the result in cellular elements/minute
B. Urine is collected in the bladder for 3 hours after the patient urinated in the morning and drank 150 ml of water
C. Urine is collected in the bladder for 24 hours after the patient urinated in the morning and drank 1500 ml of water
D. Normal values are: RBC <5000/min and WBC <2,000/min
E. Normal values are: RBC <10000/min and WBC <12000/min

CASE STUDIES

1. A 60-year-old patient is rushed to the emergency room for an altered general condition, fever, pain in the right costovertebral angle, dysuria.

Laboratory tests reveal:

Hb = 12 g/dl, MCV = 86 fl, MCH = 30 pg/E, MCHC = 34 g/dl
Urea = 140 mg/dl, Creatinine = 7 mg/dl, Uric acid = 5 mg/dl
K^+ = 6 mEq/l
Urinary osmolarity = 320 mOsm/l, $FeNa^+$ = 3%
Urine examination: pH 4,8, Nitrites +, Leukocyte esterase +, Proteins +
Microscopic urine examination: Leukocytes 57/HPF, Flat epithelial cells 23/HPF
Leukocyte casts 12/HPF

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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2. A 25-year-old patient has edema and oliguria that have suddenly appeared and the following laboratory results:

Urea = 220 mg/dl
Creatinine = 10.5 mg/dl
Proteinemia = 4 g/dl
Plasma Cholesterol = 300 mg/dl
Serum albumine = 1,8 mg/dl
ELFO serum proteins: Alb 40%, α2 globulines 19%, γ globulines 12%
Urine analysis: proteinuria (++++)
Microscopic urine examination: Leukocytes 1-2/HPF, Erythrocytes 52/HPF (with erythrocyte dysmorphism)
Fatty casts 12/HPF, Erythrocyte casts 72/HPF

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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NOTES

10. INVESTIGATION OF DIABETES MELLITUS AND HYPOGLYCEMIAS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Request and interpret the laboratory investigations that assess the impairment of glucose metabolism (hyper-/hypoglycemia).

2. Enumerate the diagnostic criteria for diabetes mellitus, gestational diabetes and prediabetes, respectively.

3. List the investigations required for the diagnosis of acute and chronic complications of diabetes mellitus.

4. Know the therapeutic targets in diabetes mellitus.

I. INSULIN - BRIEF PHYSIOLOGY OVERVIEW

The endocrine component of the pancreas is represented by the *Langerhans islets* (1-2% of the organ mass) that primarily consist of:

- A or α cells – 10-20%, which secrete glucagon
- B or β cells – 65-80%, which secrete insulin

– Insulin secretion in the β pancreatic cells occurs in 3 stages:

I. **Pre-pro-insulin** (109 amino acids) synthesis in the ribosomes

II. The cleavage of pre-pro-insulin into **proinsulin** (86 amino acids) in the endoplasmic reticulum

III. Proinsulin is split by proteases into **insulin** (51 AA) and the **C peptide** (33 AA) in the Golgi apparatus and both compounds are stored in the secretory granules (representing the *preformed insulin reserve*) of the pancreatic β cells.

The main physiological stimulus for insulin secretion is **glucose** which enters the β cells via the glucose transporter (GLUT) 2.

– **Glucose-stimulated** insulin secretion occurs in 2 steps:
  - **Early step (phase I)** with a duration of 3-7 minutes, in which *preformed insulin* is released
  - **Late step (phase II)**, which lasts for the entire duration of hyperglycemia (60-120 minutes in the case of the Oral Glucose Tolerance Test - OGTT) in which *newly formed insulin* is released.

– Insulin acts upon its specific receptors from the so-called "peripheral" tissues: liver, muscle and adipose tissue. Insulin is the main **anabolic hormone** in the body with effects on:
  - **Carbohydrate metabolism**:
    - Increases glucose uptake and glycolysis
    - Increases glycogenogenesis
    - Decreases glycogenolysis
    - Decreases neoglucogenesis
  - **Lipid metabolism**:
    - Increases the free fatty acids uptake and their beta-oxidation
    - Increases lipogenesis
    - Decreases lipolysis
  - **Protein metabolism**:
    - Increases amino acid uptake and protein synthesis
    - Decreases proteolysis

The main counter-regulatory hormones of insulin are: *glucagon, catecholamines, cortisol, growth hormone, and thyroid hormone*.

II. DIABETES MELLITUS

A. DEFINITION

Diabetes mellitus (DM) is defined as a heterogenous pathological state with respect to the etiopathogenesis, clinical manifestations and therapeutic approaches, whose essential manifestation is **hyperglycemia**. Hyperglycemia is due to either a deficit in insulin secretion or a deficit in insulin action, condition referred to as *insulin resistance*.

Insulin resistance (IR) is defined as a decrease in the insulin capacity to facilitate the uptake of glucose, fatty acids and amino acids by peripheral tissues or a decrease in the response of peripheral tissues (liver, fat, muscles) to insulin. Insulin deficiency or resistance leads to the impairment of protein and lipid metabolisms and fluid and electrolyte imbalance.

B. CLASSIFICATION

According to ADA (American Diabetic Association) and the WHO (World Health Organization) the following types of diabetes mellitus (DM) are defined:

- **Type 1 DM** (Insulin-dependent DM) - ***absolute*** insulin deficiency due to β cell destruction
- **Type 2 DM** (Insulin-independent DM) – ***relative*** insulin deficiency and insulin resistance
- **Other specific types of diabetes** (ex. chronic pancreatitis, endocrine pathologies)
- **Gestational diabetes**
- **Prediabetes**:
  - Impaired fasting plasma glucose (IFG)
  - Impaired glucose tolerance (IGT)
  - IFG + IGT

C. POSITIVE DIABETES DIAGNOSIS

1. CLINICAL diagnosis:

- *Polyuria* – due to the osmotic diuresis produced by glycosuria (the renal threshold from which glycosuria occurs corresponds to a value of glycemia = 160-180 mg/dL).
- *Polydipsia* - due to the increase of plasma osmolality and the associated intracellular dehydration.
- *Weight loss* – due to the increase in protein and lipid catabolism and the dehydration induced through osmotic diuresis, respectively.
- *Asthenia, fatigue*
- *Polyphagia*
- *Signs of infectious complications* – skin, genital and urinary tract
- *Symptoms and signs of acute metabolic complications* - ketoacidosis, lactic acidosis, non-ketotic hyperosmolar coma
- *Symptoms and signs of chronic degenerative complications* - vision impairment, intermittent claudication, paresthesia, etc.

2. LABORATORY diagnosis:

The laboratory parameters are mandatory for diagnosis confirmation and include the following investigations:

a) Fasting plasma glucose (FPG, measured at least 8 hours after the last meal):

- Blood glucose: **70 - 110 mg/dL** = *Normal*
- Blood glucose: **110 - 125mg/dL** = *Impaired fasting plasma glucose (IFG)*
- Blood glucose: **≥ 126 mg/dL** = *DM*

b) Random plasma glucose (determined at any time of day, regardless the time elapsed from the last meal):

- Blood glucose **≥ 200 mg/dl** = DM (in the presence of clinical symptoms of DM)

c) Oral glucose tolerance test (OGTT)

OGTT is indicated when/for:

- FPG is between 110-125 mg/dL
- People at high risk of developing DM: heredity, obesity, women who gave birth to children with macrosomia (weighing more than 4000 g)
- Diagnosis of gestational diabetes

Remember!

OGTT is NOT indicated if FPG is ≥ 126 mg/dL (in this case the diagnosis of DM is certain)

- **Principle:** after the measurement of the baseline glucose level (FPG) the patient is asked to drink 75 g of glucose powder dissolved in 300 ml of water followed by the assessment of blood glucose level at 2 hours.
- **Interpretation of blood glucose at 2 hours:**

- Blood glucose: **< 140 mg/dL** = *Normal*
- Blood glucose: **140-199 mg/dL** = *Impaired glucose tolerance (IGT)*
- Blood glucose: **≥ 200 mg/dL** = *DM*

d) Assessement of Glycated Hb (HbA1c)

Glycated hemoglobin (glycohemoglobin or HbA1c) is used to evaluate primarily the average of three-months blood glucose levels. HbA1c is formed by the non-enzymatic glycation of Hb when erythrocytes are exposed to chronic hyperglycemia for the past 3 months (the lifespan of erythrocytes = 120 days):

- HbA1c **< 5.7%** = *normal*
- HbA1c: **5.7 - 6.4%** = *prediabetes*
- HbA1c **≥ 6.5%** = *DM*

e) Glycosuria

- **Normal:** glucose is absent from the urine at qualitative evaluations and records values < 0.5g/day (1-15mg/dl) at quantitative assessements.
- **Pathological:** Glycosuria usually occurs when blood glucose exceeds 160-180 mg/dL (the renal threshold for the elimination of glucose) and indicates the *metabolic decompensation of DM*. The osmotic diuresis associated with glycosuria is responsible for the water-electrolyte imbalances.

f) The Glycemic Profile

It is performed in patients **diagnosed with DM** in order to *establish the doses of hypoglycemic therapy*. It consists in the repeated measurement of blood glucose, as follows:

- At 7.00 o'clock („a jeun", fasting)
- 2 hours after each of the 5 meals
- At midnight
- At 3.00 o'clock in the morning (minimal value of blood glucose)

g) Assessment of Serum Insulin

The evaluation of baseline insulinemia requires a fasting period of at least 8 hours and also the interruption of antidiabetic medication, including insulin administration 8 hours prior to the investigation.

- **Normal values**: 6-26 µU/ml
- **Clinical significance**:

- *Low values*: DM
- *High values*:
  - Insulinoma
  - Insulin resistance stage with compensatory hyperinsulinemia in type 2 DM
  - Insulin resistance due to obesity/metabolic syndrome, acromegaly, Cushing syndrome
  - Severe hepatic diseases

h) Assessment of Serum C-peptide

- **Principle:** the serum levels of insulin and C-peptide are strongly correlated because they are released in equimolar concentrations. Unlike insulin, which is predominantly metabolized by the liver, C-peptide is mostly metabolized by the kidney and is partialy eliminated in urine.
- **Indication:** the differential diagnosis of *true hypoglycemia* due to insulinoma (increased release of endogenous insulin) and *false hypoglycemia* due to administration of exogenous insulin.
- **Clinical significance:**

- *High values*: insulinoma, renal failure
- *Low values*: insulin overdose

i) Assessment of Other Hormones

Glucagon, cortisol and ***thyroid*** and ***somatotropic hormones*** can be assessed through radioimmunoassay methods for the diagnosis of secondary DM due to endocrinopathies.

j) HLA typing

Immunogenetic studies of people with confirmed DM and those at high risk have shown a clear association between type 1 DM and *HLA B8, B15, DR3 and DR4*.

D. DIAGNOSTIC CRITERIA FOR DM AND PREDIABETES

1. Diagnostic Criteria for DM

- **FPG ≥ 126 mg/dL**

or

- **Symptoms of DM + random plasma glucose ≥ 200 mg/dL**

or

- **Blood glucose at 2h during OGTT ≥ 200 mg/dL**

or

- **HbA1c ≥ 6.5%**

Remember!

In the absence of clinical symptoms or an unequivocal hyperglycemia, the tests must be repeated under the same conditions on another day to exclude a laboratory error.

2. Diagnostic criteria for GESTATIONAL DIABETES

OGTT is performed between week 24-28 of pregnancy. Fasting plasma glucose, blood glucose at 1 hour and 2 hours after the administration of 75 g of glucose are assessed. The diagnosis of gestational diabetes is positive if one of the following values exceeds the normal range:

- **FPG ≥ 92 mg/dl**

or

- **Blood glucose after 1 hour ≥ 180 mg/dl**

or

- **Blood glucose after 2 hours ≥ 153 mg/dl**

3. Diagnostic criteria for PREDIABETES

- **FPG: 110-125 mg/dL = IFG**
- **Blood glucose at 2h during OGTT: 140 - 199 mg/dL = IGT**
- **FPG = 110-125 mg/dL and blood glucose at 2h during OGTT: 140 - 199 mg/dl = IFG + IGT**
- **HbA1c: 5.7 - 6.4% = IFG and/or IGT**

4. Characteristics of Type 1 and Type 2 DM (Table 10.1)

Characteristic	Type 1 DM	Type 2 DM	Observations
Age of onset	Usually < 30 years	Usually > 40 years	There are obese children with Type 2 DM and also elderly patients with Type 1 DM
Tendency towards ketosis	High	Rare, but possible	Type 2 DM patients can also have ketoacidosis (in case of infections, acute coronary syndrome, pancreatitis, etc.)
Clinical characteristics	Constant, evident clinical signs	Variable	
Weight	Normo- or underweight	Frequently obese (80%)	The presence of obesity does not exclude the diagnosis of type 1 DM
Serum insulin levels	Severe (absolute) deficit	Variable (hypo-, normo-, hyper-insulinemia)	Serum insulin or C-peptide may be assessed
Association with other autoimmune diseases	Yes (Biermer anemia, Hashimoto thyroiditis, autoimmune hypoPTH, celiac disease, adrenal failure)	No	
Treatment with insulin	Mandatory	Sometimes required (insulin-requiring DM)	

E. Specific tests for the diagnosis of DM COMPLICATIONS

a) Diagnosis of ACUTE Complications

1. Diabetic Ketoacidosis (DKA)

It is the most common acute metabolic complication of **type 1 DM** which occurs due to a ***severe deficit of insulin*** that induces a severe hypercatabolic state (glycogenolysis, gluconeo-genesis, lipolysis, protein catabolism).

DKA is characterized by the following triad:

- **Hyperglycemia** (> 250 mg/dL)
- **Ketosis** (increased production/concentration of ketone bodies in the blood & urine)
- **Metabolic acidosis**

DKA is divided into 3 stages of severity based on acid-base balance parameters (Table 10.2):

Table 10.2. DKA stages of severity

Stage	Serum pH	Serum HCO_3^- (mmol/L)	Base deficit (mmol/L)
Ketosis	> 7.31	16-26	2-10
Precoma	7.21-7.30	11-15	11-15
Coma	≤ 7.2	≤10	≥ 16

- **Required investigations**:

- **Blood glucose** – increased (> 200 mg/dL)
- **Acid-base parameters** (pH, HCO_3^-, pCO_2, anionic gap) → metabolic acidosis with increased anionic gap due to increased ketone bodies
- **Ketonemia** – present (> 3mmol/L)
- **Urine ketone bodies** – present (> 2+ in rapid tests)
  - the presence of ketone bodies indicates metabolic decompensation – metabolic acidosis with ketoacidosis is typical for type 1 DM
- **Serum ions:**
  - **K^+** (normal 3.5 - 5 mmol/L) → often ***hypokalemia*** (loss of K^+ due to osmotic diuresis). ***Hyperkalemia*** may also occur due to metabolic acidosis.
  - **Na^+** (135-145 mmol/L) → ***hyponatremia*** (dilutional) or ***hypernatremia*** (loss of water due to osmotic diuresis)
- **Plasma osmolarity** - is increased due to hypernatremia (dehydration) and hyperglycemia
  - **Normal value: 275-295 mOsm/L**

Osmolarity (mOsm/l) =
$2x[Na^+ + K^+]$+Glucose (mg/dL)/18+Urea (mg/dL)/6

- **Urea, creatinine** – may be increased due to dehydration (pre-renal azotemia)

– **ECG** - changes due to the electrolyte imbalances, especially hypokalemia

Remember!
For every 100 mg/dl glucose in the extracellular space in addition to the normal value (~ 100 mg/dL), the amount of Na^+ is reduced by 1,6 mEq/l because of its dilution in an increased extracellular volume (hyperglycemia raises the osmolality of plasma which draws out water from cells). This is why serum sodium levels should be interpreted *only after their correction for the glycemia values* using the formula:

Na^+ corrected = Na^+ + 1.6 x (glycemia-100)/100

After insulin administration for the correction of hyperglycemia, most often *hypernatremia* due to dehydration becomes evident.

2. Hyperglycemic Hyperosmolar Coma (HHOC)

HHOC is an acute metabolic complication of **type 2 DM** characterized by **plasma hyperosmolarity but <u>without</u> ketoacidosis.**

• **Required investigations:**
- **Blood glucose** > 600 mg/dL
- **Plasma osmolarity** > 330 mOsm/L
- **Serum ketone bodies –** normal values (< 3mmol/L)
- **Urine ketone bodies –** absent (or 1+ in rapid tests)
- **Acid-base parameters –** normal
- **Serum ions:** electrolyte loss due to osmotic diuresis → **hyponatremia, hypokalemia**
- **Hematocrit:** increased by **hemoconcentration**
- **Urea, creatinine** → can be increased by dehydration (pre-renal azotemia)

b) Diagnosis of CHRONIC Complications

1. Diabetic MICROANGIOPATHY

i) Diabetic Kidney Disease (Nephropathy)
- **Albumin Excretion Rate (AER) > 300 mg/day** (severe albuminuria or proteinuria with clinical signs) regardless the value of glomerular filtration rate (eGFR)

or
- **Decrease of eGFR < 60 ml/min/1.73m²** regardless the value of AER

or
- Presence of an **AER = 30 - 300 mg/day** (moderate albuminuria) associated with **diabetic retinopathy**

Remember!
The **definitive diagnosis of diabetic nephropathy** requires the demonstration of glomerulosclerosis lesions by **renal biopsy**.

Table 10.3. Stages of CKD according to albuminuria

Description	Normal to mild increase	Moderate increase	Severe increase
Category	A1	A2	A3
AER (mg/24h)	<30	30-300	>300
ACR (mg/g)	<30	30-300	>300

AER = Albumin Excretion Rate
ACR = urinary albumin/creatinine ratio (more commonly used in clinical practice)

ii) Diabetic Retinopathy (DR) - is detected by eye fundus examination (fundus photography):
- **Non-proliferative Retinopathy (R1)**
  – Dot haemorrhages (capillary microaneurysms)
  – Blot haemorrhages (leakage of blood into deeper retinal layers)
  – Hard exudates (exudation of plasma rich in lipids and protein)
- **Pre-proliferative Retinopathy (R2)**
  – Venous beading/loops
  – Intraretinal microvascular abnormalities
  – Multiple deep, round haemorrhages
- **Proliferative Retinopathy (R3)**
  – New blood-vessel formation/neovascularization
  – Preretinal or subhyaloid haemorrhage
- **Advanced retinopathy**
  – Retinal fibrosis
  – Retinal traction detachment

2. Diabetic MACROANGIOPATHY

- ***Coronary Artery Disease (CAD)***
  – Silent myocardial ischemia
    - Holter monitoring
    - Angiography
  – Stable angina
    - ECG
    - Echocardiography
  – Acute myocardial infarction
    - ECG
    - Myocardial necrosis biomarkers
- ***Peripheral Artery Disease (PAD)***
  – Pulse palpation of peripheral arteries (posterior tibial and dorsalis pedis artery)

- Claudication index (the walking distance until the onset of pain or cramping in the calf)
- Doppler ultrasonography
- Ankle/Brachial (ABI) Index (Normal: 0.9-1.4)
  - **< 0.9** means PAD with variable atherosclerotic (ATS) obstruction
  - **> 1.4** means the presence of rigid, calcified, uncompressible arteries.
- Angiography

- ***Cerebrovascular disease***

- Carotid Dopper ultrasound with intima-media thickness (IMT) measurement
  - Normal < 0.9 mm
  - Mild to moderate ATS = 0.9-1.5 mm
  - Severe ATS > 1.5 mm
- AngioCT

3. Diabetic NEUROPATHY

- ***Diabetic distal symmetric polyneuropathy –*** subdivided into ***2 forms:***

o ***Predominantly sensory***

- Assessment of **tactile sensitivity** using the Semmes-Weinstein 10g monofilament. It is a simple, effective and inexpensive screening device for identifying diabetic patients at risk for foot ulceration. If patients fail to sense the monofilament after it bends, it is an indication of sensory loss!
- Assessment of **pain sensitivity** - using a needle
- Assessment of **thermal sensitivity** – through hot/cold exposure
- Assessment of **vibration sensitivity** – using the 128 Hz tuning fork

o ***Motor:*** evaluated by assessing the nerve conduction velocity

- ***Autonomic (vegetative) neuropathy***

Cardiovascular:

- Decreased/lost respiratory variability of the heart rate (highlighted during deep breathing on ECG)
- Resting sinus tachycardia
- Orthostatic hypotension (normal decrease in BP <10 mmHg, pathological > 30 mmHg)

Gastrointestinal

- Diabetic gastroparesis
- Diabetic enteropathy

Urogenital

- Neurogenic bladder (diabetic cystopathy)
- Erectile dysfunction

F. Therapeutic TARGETS in DM (according to ADA 2018)

- **Fasting plasma glucose: 80-130 mg/dL** (in women with gestational diabetes **≤ 95 mg/dL**)
- **Postprandial blood glucose levels < 180 mg/dL** (in women with gestational diabetes ≤ 140 mg/dL at 1 hour and ≤ 120 mg/dL 2 hours after meals)
- **HbA1c < 7%** (in women with DM who become pregnant **< 6%**), with 2 observations:
  - **HbA1c** should be **< 6,5%** in young patients with a short time of diabetes evolution, no complications and no important comorbidities, who have a low risk of hypoglycemia and are motivated to obtain the best glycemic control
  - **HbA1c** should be **< 8%** in elderly patients with a long term evolving disease, with chronic complications, several comorbidities, low life-expectancy and high risk of hypoglycemia.

III. HYPOGLICEMIA

- **Definition:** glucose level **< 70 mg/dL** with symptoms and signs caused by *neuroglycopenia and the physiological regulatory responses* caused by the release of counterregulatory hormones.
- **Classification**

- ***Exogenous*** – induced by insulin, some oral antidiabetic drugs, alcohol
- ***Endogenous***
  - *Organic* – insulinoma
  - *Functional* – idiopathic, gastric/intestinal resection

- **Physiological responses to hypoglycemia**

- ***Mild forms***: fatigue, dizziness, fainting sensation, cold sweats, chills, tremors, palpitations, hunger (preference for sweets)
- ***Moderate forms***: localized or generalized seizures with spasms, speech impairment, aggression, hallucinations, confusion;
- ***Severe forms***: heavy sweating, shortness of breath, irregular heart rate, coma

- **Laboratory diagnosis**

a. **Blood glucose** - at any time of the day

b. **Insulinemia/Fasting plasma glucose ratio**

$$\frac{I\ (\mu U/ml)\ x100}{G\ (mg/dl)-30}$$

- Interpretation:
  - **Normal** < 30

- **Functional hypoglycemia**: 50 – 200
- **Organic hypoglycemia**: > 200 (suspected insulinoma)

c. **Extended OGTT**

Performed with 75 g of glucose over a period of 5 hours, with glycemic evaluation at 20 minutes intervals in the first 2 hours, then every hour. A decrease of blood glucose **< 70 mg/dL** 2 hours after glucose administration is suggestive for a ***late functional hypoglycemia***, present in **obesity and metabolic syndrome** (hyperinsulinemia stage).

CHECKPOINT

***1.** Male patient, 57 years old, with Ist degree abdominal obesity, with a family history of diabetes melitus, has an OGTT performed that showed the following results:

- Fasting plasma glucose: 107 mg/dL
- Glycemia after 120 min: 180 mg/dL

How do you interpret these results?

A. The test is normal
B. Impaired fasting plasma glucose
C. Impaired glucose tolerance
D. Diabetes melitus
E. The results are inconclusive and the test should be repeated

***2.** Male patient, 65 years old, with type 2 DM, is brought to the emergency room due to simptoms consistent with the flu, with altered consciousness and with the following laboratory investigations:

- Blood glucose = 700 mg/dL
- Glycosuria: +++
- Ketonuria: absent
- Corrected Na^+ = 140 mmol/L
- K^+ = 4 mmol/L
- pH = 7.37
- Urea = 120 mg/dL

What is the most likely diagnosis?

A. Ketoacidotic coma
B. Hyperglycemic hyperosmolar coma
C. Diabetic coma with lactic acidosis
D. Hypoglycemic coma
E. Ketoacidotic precoma

3. Male patient, 55 years old, diagnosed with type 1 DM when he was 30 years old, presents for his routine check-up. The patient feels well, free of any symptoms.

Which of the following investigations would you indicate?

A. Fasting plasma glucose
B. Glycated hemoglobin
C. Albumin excretion rate
D. Fundus (eye) examination
E. OGTT

4. Male patient, 48 years old, diagnosed with type 2 DM presents for a routine check-up. He has:

- Fasting plasma glucose = 120 mg/dL
- HbA1c = 6.5%

Which of following statements are true?

A. His diabetes is well controlled
B. The HbA1c value is increased
C. The HbA1c value is in the target values
D. Patient had persistent hyperglycemia over the past 3 months
E. Urine examination will reveal ketone bodies

5. Which of the following represent diagnostic criteria of diabetic kidney disease:

A. Albumin excretion rate > 300 mg/day regardless the eGFR value
B. Decrease of eGFR < 60 ml/min/1.73m^2 regardless the AER
C. The presence of an AER between 30 and 300 mg/day associated with diabetic retinopathy
D. Albumin excretion rate > 300 mg/day + eGFR < 60 ml/min/1.73m^2
E. The presence of an AER between 30 and 300 mg/day + lack of retinopathy

6. What Investigations are needed for the diagnosis of diabetic ketoacidosis?

A. Ketone bodies in blood and urine
B. Acid-base parameters
C. Blood glucose
D. Glucose tolerance test
E. C peptide

7. The diagnosis of diabetic distal predominantly sensory symmetric polyneuropathy implies:

A. Assessement of tactile sensitivity with the help of the Semmes Weinstein 10g monofilament
B. Assessement of thermal sensitivity through exposure to hot/cold
C. Assessement of vibration sensitivity with the help of the 128 Hz tuning fork
D. Calculating the Claudication Index
E. Palpation of the pulse of the peripheral arteries (posterior tibial artery, dorsalis pedis artery)

8. Which of the following are present in the diabetic hyperosmolar coma:

A. Blood glucose above 600 mg/dL
B. Increased plasma osmolarity > 330 mOsm/L
C. Very high serum ketones
D. Ketonuria
E. Parameters of acid-base balance within normal limits

CASE STUDIES

1. A 22-year-old female patient, diagnosed with autoimmune thyroiditis accuses altered general condition, polyuria, polydipsia. She recalls repeated urinary infections and a 10 kg weight loss in the last 3 months.

Laboratory investigations:

- Blood glucose = 357 mg/dl
- Plasma ionogram:
  - Na^+ = 139 mmol/l
  - K^+ = 3 mmol/l
- Creatinine = 1,1 mg/dl
- Urea = 45 mg/dl
- ALT = 20 U/l
- AST = 16 U/l
- Urinary ketone bodies= ++
- Plasmatic ketone bodies= ↑↑

Blood gas analysis:
pH = 7,28
pCO_2 = 30 mmHg
HCO_3 = 15 mmol/l

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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2. A 59-year-old male patient, diagnosed with IIIrd degree hypertension 5 years ago (treated with beta-blockers, ACE inhibitors and diuretics), with 1st degree abdominal obesity (BMI= 32kg/m^2) has the following **laboratory investigations**:

- Fasting plasma glucose= 117 mg/dl
- Creatinine= 1,25 mg/dl (eGFR= 59 ml/min/1,73m^2; MDRD)
  ➔ Repeated after 2 days: 0,86 mg/dl (eGFR= 91 ml/min/1,73m^2; MDRD)
- Urea = 43 mg/dl
- Uric acid = 6 mg/dl
- Na^+ = 140mmol/L
- K^+ = 3,5mmol/L
- OGTT: Baseline = 121 mg/dl
  2h = 235 mg/dl
- HbA1c= 7.1%

What diagnosis do you suspect? What additional investigations would be justified? Provide arguments for your answer.

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NOTES

11. INVESTIGATION OF DYS/HYPERLIPIDEMIAS

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. Describe the structure and function of the main lipoprotein classes

2. Enumerate the types of primary and secondary hyperlipidemias

3. Specify the laboratory investigations used for the assessment of lipid metabolism

4. Interpret the changes of these laboratory investigations in the diagnosis of dys/hyperlipidemias

5. Know the concept of cardiovascular risk and the „therapeutic targets" in the management of hyper- and dyslipidemias

I. LIPOPROTEIN CLASSES – PHYSIOLOGICAL AND BIOCHEMICAL OVERVIEW

- **Definition**: Lipoproteins (LP) are the main plasma carriers of the insoluble lipid molecules (cholesterol and triglycerides).
- **Structure**: LP are macromolecules that consist of:
  - a **central nucleus** that contains triglycerides and cholesterol esters (CE) with hydrophobic properties
  - an **external layer** that contains: phospholipids, non esterified cholesterol and apoproteins that serve a solubilisation purpose in the aqueous environment of the plasma
- The main types of lipoproteins can be separated by:
  - **Ultracentrifugation**, according to the molecular density that allows classification in the 5 major types: chylomicrons, VLDL, IDL, LDL, HDL (Table 11.1.).
  - **Electrophoresis**, according to their electric charge that allows the separation of different fractions: α, β, pre-β.
- **Classess of LP:**
  - **Chylomicrons (CHY)**
    - Represent the largest class of LP, synthesized in the small bowel and mainly containing **exogenous triglycerides (TG)**. They transport **TG and cholesterol found in the ingested food** from the intestine to the liver.
  - **VLDL (Very Low Density Lipoproteins)**
    - VLDL particles are large, produced by the liver and transport mainly **endogenous TG**.
  - **IDL (Intermediate Density Lipoproteins)**
    - They are also called **„remnants"** and represent LP fractions that result from the VLDL catabolism. They are small particles, rich in cholesterol that contain a low amount of TG as well.
  - **LDL (Low Density Lipoproteins)**
    - They result from the partial hydrolysis of VLDL and represent the main atherogenous LP because they transport 60-70% of the total body cholesterol. LDL particles are partially uptaken and metabolized by the liver, whilst the remaining LDL is internalized by peripheral cells, including coronary and peripheral artery cells, therefore the process of atherosclerosis is strongly linked to plasma LDL level.
  - **HDL (High Density Lipoproteins)**
    - Are produced by the liver and the intestine and contain approximately 20-30% of the circulating cholesterol. HDL particles transport cholesterol from the peripheral tissues to the liver - a process known as cholesterol *retrograde transport*, which explains why HDL particles have a protective, anti-atherosclerotic role.

Table 11.1. The lipoprotein classes and ELFO.

LP class	Composition	Electrophoresis
Chylomicrons	Exogenous TG	No migration
VLDL	Endogenous TG	Pre-beta
IDL	CE, TG	Broad-pre-beta
LDL	CE	Beta
HDL	CE	Alfa

II. HYPERLIPOPROTEINEMIAS (HLP)

- **Definitions:**

a) Hyperlipoproteinemias = conditions associated with an increased level of lipoprotein fractions:

- **Primary HLP** - genetic predisposition
- **Secondary HLP** - as a consequence of other pathological conditions

b) Hyperlipidemia = increased plasma levels of total cholesterol (TC) > 200mg/dL and/or TG > 150mg/dl

Hyperlipoproteinemia = Hyperlipidemia

c) Dyslipidemia = combined modification of serum lipid levels:

- hyperlipidemia + HDLc < 35 mg/dL or
- isolated decrease of HDLc < 35 mg/dL

d) Atherogenous dyslipidemia = decreased HDLc + borderline hyperCT (200-239mg/dL) + hyperTG > 150 mg/dL

- **PRIMARY HLP - Classification:**

- *Old classification* (Fredrickson) – 5 phenotypes marked I, IIa, IIb, III, IV and V (Table 11.2)
- *New classification* – more relevant from the clinical point of view (Table 11.3)

Table 11.2. Primary Hyperlipoproteinemias – The Fredrickson Classification (old)

Type	Name	LP ↑	TG	TC
I	**Familial HyperCHY**	CHY	↑↑↑↑	N,↑
IIa	**Familial HyperCT**	LDL	N	↑↑
IIb	**Familial combined hyperlipidemia**	VLDL LDL	↑↑	↑↑
III	**Familial Dysbetahyperlipoproteinemia**	IDL CHY remnants	↑↑↑	↑↑
IV	**Familial HyperTG**	VLDL	↑↑	N,↑
V	**Familial Mixed HyperTG**	VLDL CHY	↑↑↑↑	↑

Table 11.3. Primary Hyperlipoproteinemias – NEW Classification

Name	Defect	LP ↑	TG	TC
Familial HyperCT (Type IIa HLP)	↓ No./Absence of LDLr	LDL	N	↑↑
Hereditary deficiency of Apo-B100	Normal no. of LDLr	LDL	N	↑↑
Polygenic HyperCT	↑ cholesterol synthesis ↓ No. of LDLr	LDL	↑	↑↑
Familial HyperTG (Type IV HLP)	↑ VLDL production LPL deficit Apo-CII deficit	VLDL	↑↑	N,↑
Familial deficiency of LPL (Type I HLP)	LPL is absent	CHY	↑↑↑↑	N,↑
Familial deficiency of Apo-CII (Type I HLP + Type V HLP)	LPL deficit	CHY VLDL	↑↑↑↑	↑
Familial combined hyperlipidemia (Type IIb HLP)	↑ VLDL production ↓ No. of LDLr	VLDL LDL	↑↑	↑↑
Familial dysbetalypoproteinemia (Type III HLP)	Apo-E deficit	IDL CHY remn.	↑↑↑	↑↑

LPL - lipoproteinlipase, LDLr – LDL receptors

Table 11.4. HYPERLIPOPROTEINEMIAS classification and the assessment of the SEVERITY DEGREE

Primary HLP	Secondary HLP	Severity degree	TC (mg/dL)	TG (mg/dL)
HYPERCHOLESTEROLEMIAS				
- Familial HyperCT - Hereditary deficit of ApoB100 - Polygenic HyperCT	Hypothyroidism Cholestasis (liver cirrhosis, obstructive jaundice, cholestatic hepatitis)	- borderline - moderate - severe	200-239 240-299 ≥ 300	< 200 < 200 < 200
HYPERTRIGLYCERIDEMIAS				
- Familial HyperTG - Familial deficiency of LPL - Familial deficiency of Apo-CII	Decompensated diabetes mellitus Chronic alcoholism Estrogen therapy	- borderline - moderate - severe	< 200 < 200 < 200	150-199 200-499 ≥ 500
MIXED HYPERLIPIDEMIAS				
- Combined familial hyperlipidemia - Familial dysbetalypoproteinemia	Nephrotic syndrome Chronic kidney disease Obesity	- moderate - severe	200-299 ≥ 300	200-499 ≥ 500

III. USUAL INVESTIGATIONS PERFORMED FOR LIPID METABOLISM ASSESSEMENT

Assessment of the **plasma lipid levels and profile** is one of the routine investigations, view the crucial role of lipids in the quantification of cardiovascular risk. In order to determine the lipid profile, patients must follow a normal diet for 2 weeks, without any medication that could influence lipid metabolism. The values will then be correlated with the age and gender of the patients.

1. Plasma appearance: at 4^0C, 24h after blood collection:

- a **clear** plasma indicates normal values or **hypercholesterolemia**
- a turbid or **lactescent** plasma indicates **hypertriglyceridemia**
- the presence of a **"floating" creamy layer** at the top of the sample indicates the presence of **chylomicrons**

2. Total lipids:

- **Normal value**: 400-800 mg/dL
- **Pathological findings:**
  Increased values:
  - Hyperlipoproteinemias
  - Hypothyroidism
  - Cholestatic syndrome
  - Diabetes mellitus
  - Chronic alcoholism
  - Estrogen therapy
  - Nephrotic syndrome
  - Chronic kidney disease
  - Obesity

  Decreased values:
  - Hepatic failure
  - Hyperthyroidism
  - Malabsorbtion syndrome

3. Total cholesterol (CT)

- **Normal value**: 140 - 200 mg/dL.
- **Interpretation**:
  - TC = 200 – 239 mg/dL indicates **borderline hypercholesterolemia**
  - TC = 240 – 299 mg/dL indicates **moderate hypercholesterolemia**
  - TC ≥ 300 mg/dl indicates **severe hypercholesterolemia**
- **Pathological findings:**
  Increased values:
  - Diet rich in cholesterol and saturated fats
  - Primary and secondary HLP (Table 11.4)

  Decreased values:
  - Hepatic failure
  - Hyperthyroidism
  - Malabsorbtion syndrome

4. Triglycerides (TG):

- **Normal value**: 50 - 150 mg/dL.
- **Interpretation**:

- TG = 150 – 199 mg/dL indicates **borderline HyperTG**
- TG = 200-499 mg/dl indicates **moderate HyperTG**
- TG ≥ 500 mg/dL indicates **severe HyperTG**

- **Pathological findings:**
  Increased values:
- Diet rich in **carbohydrates and alchohol**
- Primary and secondary HLP (Table 11.4)
  Decreased values:
- Hepatic failure
- Hyperthyroidism
- Malabsorbtion syndrome

Remember!
TG values over 200 mg/dL are asociated with an increase in the small and dense particles of LDLc with atherogenic potential.
TG values over 500-600 mg/dL are asociated with a high risk of acute pancreatitis.

5. HDL-cholesterol (HDLc)

- **Normal values:**
  - Men > 40 mg/dL
  - Women > 50 mg/dL
- **Clinical value:** decreased levels are asociated with coronary artery disease in both genders.

6. LDL-cholesterol (LDLc)

- **Assessement:** LDLc is obtained using the values of total cholesterol, TG, and HDLc (Friedewald's formula):

LDLc = TC – HDLc – TG/5 (mg/dL)

This formula can be used only when TG levels are < 400 mg/dL.

- **Normal** < 130 mg/dL
- **Clinical value:**
  - LDLc = 130-159 mg/dL indicates a **borderline coronary artery disease risk**
  - LDLc > 160 mg/dL indicates an **increased coronary artery disease risk**

7. Non-HDL cholesterol (Non-HDLc)

- **Significance**: estimates the number of atherogenic particles in the plasma (cholesterol from VLDL, IDL and LDL) and it correlates well with the Apo-B100 levels.

Non-HDLc (mg/dL) = TC – HDLc

- **Non-HLDc values**: 30mg/dl higher than LDLc values
- **Clinical value:** *secondary* therapeutic target for assessing the effects of lipid-lowering treatment in patients with cardiovascular risk when the goal to lower LDLc has been achieved.

8. Total cholesterol / HDL cholesterol (TC/HDLc)

- **Indication**: the ratio is used in order to evaluate the coronary artery disease risk in patients with a total cholesterol of **200-250 mg/dL**.
- **Clinical value**: levels **> 5 in men** and **> 4,5 in women** indicate an *increased coronary artery disease risk.*

9. Apolipoprotein assessement

a) Apo-B100 assessement

- ApoB is a major apolipoprotein of the structure of atherogenic lipoproteins (LDL, VLDL, IDL) and provides a good estimate of the number of these particles in the plasma, especially in the presence of high concentrations of LDL-C.
- **Clinical value:**
  - ApoB is equivalent from the point of view of cardiovascular risk prediction with LDLc and non-HDLc
  - It is used as a *secondary* therapeutic target when the target to lower LDLc has been met.

b) Apo AI assessement

- ApoAI is the major apoprotein of HDLc and provides an estimate of the serum concentration of HDLc.
- **Clinical value:**
  - Serum level below 150mg/dL corresponds to a low level of HDLc
  - Increased levels of Apo AI (and HDLc) decrease the cardiovascular risk

10. Lipoprotein (a) – Lp(a)

- Lp(a) is a modified LDL that contains apolipoprotein(a) with a structure similar to plasminogen. In high concentrations it will penetrate the arterial walls and cause a decrease in fibrinolysis with a pro-thrombotic effect and an increase in cholesterol accumulation with accelerated atherogenesis.
- **Indications**: its level should be evaluated in:
  - familial hyperCT
  - family history of cardiovascular disease
  - increased risk of cardiovascular disease
- **Normal**: < 20 mg/dL
- **Clinical value**: levels over **50 mg/dL** are an independent risk factor for the development of coronary artery disease.

11. Lipoprotein electrophoresis

- **Clinical value**: it shows lipoprotein gel migration, which is necessary in order to diagnose primary HLP (Table 11.1).

IV. ASSESSMENT OF CARDIOVASCULAR RISK

- **Cardiovascular (CV) risk** is defined as the probability of death due to a *CV cause* (lethal CV disease) over a defined period of time. Atherosclerotic CV disease is, most commonly, the product of a number of risk factors and its prevention in a particular person should be adapted to the total CV risk (the higher the risk, the more intense the action to lower it).
- Risk factors screening, including the lipid profile, is indicated in **men over 40 years of age** and **women over 50 years of age** or **postmenopausal**.
- Estimation of cardiovascular risk (introduced by the European Society of Cardiology in 2003) is recommended in clinical practice for **asymptomatic adults (apparently healthy)** with no evidence of cardiovascular disease and is performed using the **SCORE chart** (Systemic Coronary Risk Estimation) based on 5 major risk factors: **gender, age, smoking, BP values and total cholesterol** - Fig. 11.1
- The SCORE chart **should NOT be used** for people with:
  - Documented manifest CV disease
  - Familial hypercholesterolemia
  - Type 1 or 2 diabetes mellitus
  - Chronic kidney disease

because these patients are automatically at an *increased or very increased level of total cardiovascular risk*. For these patients models of risk estimation are not needed, but they require the *active management of all risk factors*.

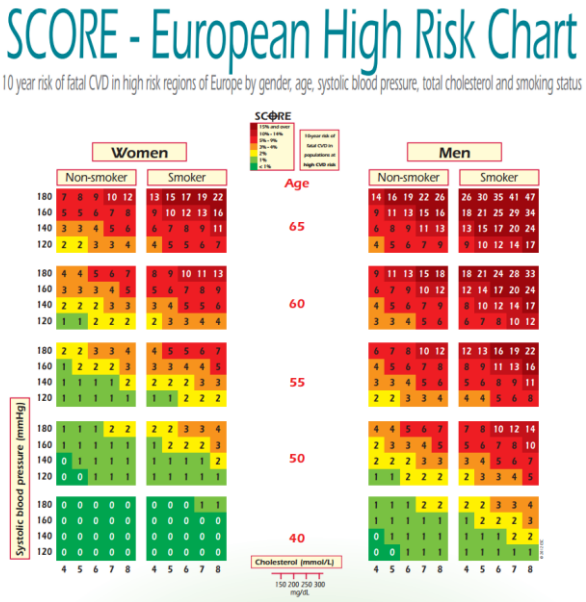

Figure 11.1. The SCORE diagram that evaluates the risk for **fatal cardiovascular disease over 10 years** in high risk European countries (ex. Romania), according to **age, gender, smoking, systolic BP and total cholesterol** (according to the Dyslipidemia Management Guide 2016)

- **Very high cardiovascular risk:**
  - Cardiovascular disease diagnosed by invasive or non-invasive methods (myocardial infarction, acute coronary syndrome, coronary revascularization, ischemic stroke, peripheral artery disease).
  - Type 2 DM or Type 1 DM with target organ damage (e.g., proteinuria) or with a major risk factor such as smoking, HT or dyslipidemia
  - Severe chronic kidney disease (eGFR < 30 ml/min/1.73m^2)
  - ***SCORE ≥10%***
- **High cardiovascular risk:**
  - a single very increased risk factor is present (severe hypercholesterolemia ≥ 300mg/dl, severe arterial hypertension ≥ 180/110mmHg)
  - DM that does not fit the criteria for very high risk
  - Moderate chronic kidney disease (eGFR 30-59ml/min/1.73m^2)
  - ***SCORE between 5% and 10%***
- **Moderate cardiovascular risk:**

– ***SCORE between 1% and 5%***

Observation!
A new marker used in CV risk assessment in moderate-risk patients was proposed, namely the coronary calcium score determined by high resolution tomography. Increased values of this score in these patients aggravates the individual CV risk and suggests an aggressive therapy.

– ***SCORE < 1%***

Observation!
The associated presence of factors such as obesity, physical inactivity, mental illness, atrial fibrillation, inflammatory diseases, sleep apnea aggravates the classification into an individual CV risk category, even if according to the main parameters the risk was moderate or low.

- **Low cardiovascular risk**

V. THERAPEUTIC TARGETS IN HYPERLIPIDEMIAS

1. LDL cholesterol

- **LDLc is the main therapeutic target** in most treatment strategies for dyslipidemias. LDLc target values **depend upon the estimated cardiovascular risk according to the SCORE diagram.**
- **Recommendations:**

a. Very high cardiovascular risk
– Target LDLc **< 70 mg/dL** or decrease **by ≥ 50%** when the target level cannot be obtained

b. High cardiovascular risk
– Target LDLc **< 100 mg/dL**

c. Moderate cardiovascular risk
– Target LDLc **< 115 mg/dL**

2. Non-HDLc

- **Recommendations:**

a. Very high cardiovascular risk
– Non-HDLc **< 100 mg/dL**

b. High cardiovascular risk
– Non-HDLc **< 130 mg/dL**

3. Triglycerides

The target value for TG is **< 150 mg/dL.**

4. Apo B100

- **Recommendations:** if Apo-B100 is available, the target values are:
  – ApoB100 **< 80 mg/dL** in patients with very high cardiovascular risk
  – ApoB100 **< 100 mg/dL** in patients with high cardiovascular risk

CHECKPOINT

***1. Which of the following is a cause of hypercholesterolemia?**
A. Apo-CII deficit
B. Hypothyroidism
C. Diabetes mellitus
D. Chronic alcoholism
E. Estrogen therapy

***2. Which of the following is a cause of isolated hypertriglyceridemia?**
A. Apo-B100 deficit
B. Hypothyroidism
C. Diabetes mellitus
D. Nephrotic syndrome
E. Liver cirrhosis

3. Atherogenous dyslipidemia is defined by:
A. Decreased HDL < 35 mg/dL in both genders
B. Hypercholesterolemia < 200 mg/dL
C. Hypertriglyceridemia > 200 mg/dL
D. Borderline hypercholesterolemia (200-239 mg/dL)
E. Hypertriglyceridemia > 150 mg/dL

4. Which of the following shows a high risk for developing coronary artery disease?
A. Total cholesterol = 180 mg/dl
B. LDL cholesterol = 180 mg/dl
C. HDL cholesterol = 25 mg/dl
D. Triglycerides = 80 mg/dl
E. Total cholesterol/HDL cholesterol ratio = 4

5. Which of the following represent the therapeutic target in hyperlipidemias diagnosed in patients with very high cardiovascular risk?
A. LDLc < 70 mg/dL
B. LDLc < 100 mg/dL
C. Non-HDLc < 130 mg/dL
D. Apo-B100 < 80 mg/dL
E. TG < 200 mg/dL

6. Non-HDL cholesterol:
A. Estimates the number of atherogenic particles in plasma and correlates well with the Apo-B level
B. Is calculated using the formula: TC - HDLc - TG/5(mg/dL)
C. Values are 30 mg/dL higher than LDLc values
D. Is used as a secondary therapeutic target in lipid-lowering therapy in patients with cardiovascular risk when the goal of LDLc reduction is achieved
E. Low values below the normal ones represent a coronary risk factor for both genders.

7. Which of the following are true regarding ApoB?
A. It is a major apolipoprotein in the structure of atherogenic lipoproteins (LDL, VLDL, IDL)
B. It is equivalent from the point of view of predicting the risk of cardiovascular events with LDLc and non-HDLc
C. It is the major apoprotein in HDLc and provides an estimate of serum HDLc
D. Low serum levels below 150mg/dL correspond to a low level of HDLc
E. The increased level reduces cardiovascular risk

*8. A patient aged 58 years, smoker, with IInd degree HT and type 2 diabetes mellitus is assessed in terms of cardiovascular (coronary) risk to establish the "therapeutic target" for dyslipidemia. Investigations:
TC = 250 mg/dL, LDLc = 170 mg/dL, TG = 140 mg/dL.
Which of the following is the therapeutic "target" for the lipid profile of this patient?
A. LDLc < 70 mg/dL or LDLc reduction by 50%
B. LDLc between 100 and 115 mg/dL
C. LDLc between 115 and 130 mg/dL
D. Total cholesterol < 150 mg/dL
E. Triglycerides < 70 mg/dL

*9. A 43 year old male is diagnosed with systemic atherosclerosis (atheroma plaques at coronary, carotid, peripheral level) and diabetes mellitus (HbA1c = 6.8%). His 48 year-old brother has recently died due to an acute myocardial infarction.
TC = 405 mg/dL, LDLc = 210mg/dL, HDLc = 30 mg/dL, TG = 140 mg/dL.
What is the most probable diagnosis?
A. Hypertriglyceridemia secondary to diabetes mellitus
B. Hypercholesterolemia secondary to diabetes mellitus
C. Familial hypertriglyceridemia
D. Mixed dyslipidemia
E. Familial hypercholesterolemia

CASE STUDIES

1. A 39 year-old male patient, currently asymptomatic and with no family history of cardiovascular disease, but with a personal history of type 1 diabetes treated with insulin (HbA1c = 6,1%), has the following plasma lipid values:

- Total cholesterol = 190 mg/dL
- LDL cholesterol = 129 mg/dL
- HDL cholesterol = 49 mg/dL
- Triglycerides = 728 mg/dL
- Lactescent plasma

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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2. A 55 year-old male patient, smoker, comes to the doctor for headaches. BP determination shows: Systolic pressure: 180 mmHg, Dyastolic pressure: 100 mmHg. His 58 year-old brother has recently died due to an acute myocardial infarction. Plasma lipid profile shows the following values:

- Total cholesterol = 315 mg/dL
- LDL cholesterol = 251 mg/dL
- HDL cholesterol = 30 mg/dL
- Triglycerides = 120 mg/dL

What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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NOTES

12. INVESTIGATION OF PROTEIN METABOLISM ABNORMALITIES

LEARNING OBJECTIVES

At the end of this chapter, students must be able to:

1. List the normal values for plasma proteins and proteinuria along with the main causes for their pathological changes

2. Define dysproteinemias and enumerate their main characteristics and causes

3. Define hyperuricemia and list its consequences.

I. TOTAL PLASMA PROTEINS

The euproteinemic state defines the balance between plasma protein synthesis, use and elimination or degradation.

• Normal values:

- **Plasma proteins**: 6,7 - 8,4 g/dL from which:
  - Albumins = 3,5 - 5,5 g/dL (50-60%)
  - Globulins = 2,0 - 3,5 g/dL (40-50%)
  - Albumin/globulin ratio = 1,2 - 1,5
- **Urine proteins**:
  - At rest < 150mg/day
  - After intense physical exercise < 250mg/day

• Pathological changes:

1. In the plasma:

a) Hyperproteinemia:

- ***Relative*** *(pseudo-hyperproteinemia):*
  - hemoconcentration due to severe dehydration (severe diarrhea, vomiting, excessive diuresis, sweating);
  - analysis errors: evaporation of the serum sample in the laboratory (rare).
- ***Absolute*** *(real):*
  - hyperimmunoglobulinemias due to polyclonal/monoclonal gammapathies and autoimmune disorders.

b) Hypoproteinemia:

- ***Relative*** *(pseudo-hypoproteinemia):*
  - hemodilution due to hypervolemic states (fluid hydration therapy, 3rd trimester pregnancy, congestive heart failure);
- ***Absolute*** *(real):*
  - protein loss: renal (nephrotic syndrome), digestive (enteropathies), cutaneous (burns), repeated paracentesis;
  - low synthesis: chronic liver disorders, malnutrition/malabsorbtion;
  - increased catabolism: severe infections, malignant tumors.

2. In the urine:

Normally, standard assays (by means of dipsticks) are negative for albumin. Early detection of moderate albuminuria can be suggestive for a nephropathy.

Proteinuria may be:

a) Physiological (functional, benign):

- after an intense physical exercise, exposure to cold, orthostatic, pregnancy.

b) Pathological:

- ***extrarenal proteinuria***: congestive heart failure, hypertension, diabetes mellitus.
- ***renal proteinuria***: may be due to *glomerular* causes (glomerulonephritis, nephritis associated to systemic lupus erythematosus, amyloidosis) or *tubular* causes (pyelonephritis, drug-induced tubulo-interstitial nephropaties).
- ***elimination of Ig light chains***
  - any disorder that implies an intense catabolism (nephrotoxicity of certain drugs, nephrotic syndrome, systemic lupus erythematosus) and leads to increased light immunoglobulin chains' urinary excretion.
  - the presence of monoclonal light chains in urine occurs in malignant disorders (multiple myeloma, Waldenstrom macroglobulinemia).

Bence-Jones proteinuria is defined as the elimination of Ig light chains and is mainly characterized by **thermolability** (formation of a precipitate after heating the urine to 60^0C, that dissapears when the temperature approaches the boiling point and reappears after the urine has been cooled down).

II. PROTEIN ELECTROPHORESIS

Plasma protein electrophoresis (ELFO) has the following normal values for the main protein fractions:

Albumins: 50 - 60% (3,5 - 5,5 g/dL)
Globulins: 40 - 50% (2,0 - 3,5 g/dL)

- **Alpha 1-globulins:** 3 - 6% (0,2-0,4 g/dL)
- **Alpha 2-globulins:** 7 - 10% (0,5-0,9 g/dL)
- **Beta-globulins:** 11 - 14% (0,5 - 1,1 g/dL)
- **Gamma-globulins:** 15 - 23% (0,7 - 1,7 g/dL)

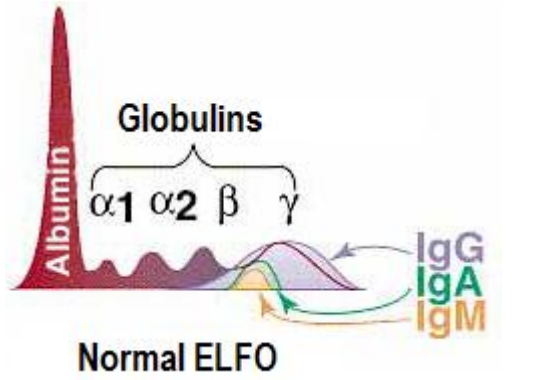


Figure 12.1. Normal electrophoresis

a) Albumins

Albumin represents the major plasma protein fraction.

• Pathological changes:

- ***Hyperalbuminemia****:*
  There is no REAL hyperalbuminemia. Any disorder that leads to a decreased plasma volume (for instance dehydration) can cause a major increase in the concentration of all plasma proteins, including albumin (pseudo-hyperalbuminemia).
- ***Hypoalbuminemia*** may be due to:
  - ***acute and chronic inflammation***: rheumatic disorders, most bacterial infections, tissue necrosis (especially in malignant tumors), viral infections that associate tissue destructions, burns, vasculitis;
  - ***decreased hepatic synthesis (late)***: severe hepatic disorders, malnutrition, tumors
  - ***increased loss***: renal loss (glomerular or tubular proteinuria), traumatic lesions, burns, gastrointestinal and lymphatic fistula, protein loss enteropathies, repeated evacuation of ascites fluid;
  - ***increased catabolism***: fever, malignant tumors, hormone-induced hypermetabolic states (thyrotoxicosis, Cushing disease);
  - ***increased plasma volume (pseudo-hypoalbuminemia due to hyper-volemia)***: pregnancy, congestive heart failure, exogenous estrogen administration.

b) Alpha-1 globulins

The α1-globulin fraction contains mainly α1-antitrypsin and α1-antichymotrypsin.

• Pathological changes:

- *Increased values*: acute and chronic inflammations (that follow an acute inflammation that was not healed)
- *Low values*: hereditary deficit of α1-antitrypsin.

c) Alpha-2 globulins

The α2-globulin fraction contains mainly α2-macroglobulin, haptoglobin, ceruloplasmin and apoprotein B100 (present in the VLDL and LDL classes of lipoproteins).

• Pathological changes:

- *Increased values*: acute inflammations and some chronic ones, nephrotic syndrome (associated with hypoalbuminemia) and in alcoholic liver disease (chronic hepatitis, cirrhosis - alcohol inhibits beta-oxidation of free fatty acids and induces increased TG synthesis under the form of VLDL).

d) Beta globulins

The β-globulin fraction contains transferrin, hemopexin, the complement components.

• Pathological changes:

- *Increased values*: primary billiary cholangitis, iron-defficiency anemia.

e) Gamma globulins

The γ-globulin fraction contains immunoglobulins (Ig G, Ig M, Ig A and E).

IgG is the main serum immunoglobulin that represents approximately 70% of the total plasma Ig amount. It has a major role in long-term antibacterial defense, being the immunoglobulin of the secondary immune response. It is the only fraction that can pass through the placenta, with a very important role in the newborn's immunity.

There are 4 subtypes, marked IgG1-IgG4. Can activate complement via the classical pathway.

• **Pathological changes:**

- *Increased values*: chronic hepatic disorders (hepatitis and cirrhosis), chronic/recurrent infections, autoimmune disorders, multiple myeloma, lymphoma, chronic lymphocytic leukemia.
- *Decreased values*: primary and secondary humoral immune deficiencies (AIDS), nephrotic syndrome, pregnancy (dilutional decrease), increased catabolism in rheumatoid arthritis (in the presence of the rheumatoid factor – an IgM anti-IgG antibody), immunosuppressive therapy.

IgM represents the main immunoglobulin of the primary immune response. Can activate complement via the classical pathway.

• **Pathological changes:**

- *Increased values*: an isolated increase in the adult may be suggestive for a viral infection (viral hepatitis) or early response to a bacterial or parasytic infection, rheumatoid arthritis (in the presence of the rheumatoid factor – an IgM anti-IgG antibody), autoimmune disorders, malignant disorders, primary billiary cholangitis (anti-mitochondrial antibodies belong to the IgM class), Waldenstrom macroglobulinemia. Increased levels may lead to increased blood viscosity, due to the high molecular weight of this Ig (pentamer), causing brain/peripheral hypoperfusion.

IgA represents the major immunoglobulin in secretions. Secretory IgA plays an important role in the respiratory, genitourinary and gastrointestinal defense system. The IgA present in colostrum and breast milk provides the newborn's defense against gastrointestinal infections. There are 2 subtypes, marked IgA1-IgA2. Can activate complement via the alternative pathway.

• **Pathological changes:**

- *Increased values*: alcoholic hepatitis and cirrhosis; chronic respiratory and gastrointestinal infections; multiple myeloma; lymphomas.
- *Decreased values*: isolated IgA deficiency, nephrotic syndrome.

IgE represents the main immunoglobulin involved in atopic/allergic reactions (**type I hypersensitivity**) and the only immunoglobulin capable of binding to mast cells. It is present in the respiratory and gastrointestinal tract mucosa (IgE is often increased in patients with IgA deficiency).

• **Pathological changes:**

- *Increased values*: allergic disorders (extrinsic asthma, allergic rhynitis, atopic dermatitis), parasitic infections, anaphylactic reactions.

III. DYSPROTEINEMIAS

Are **pathological changes that affect the ratio between plasma protein fractions**, with normal or abnormal values of proteinemia (depending on the primary disorder that led to this disturbance.

a) Dysproteinemia due to acute inflammation ('acute phase reaction')

Is characterized by:

- decreased serum albumins;
- **increased alpha-1 and alpha-2 globulins** (acute phase reactants): C reactive protein, fibrinogen, serum amyloid A, haptoglobin, protease inhibitors (α1-antitrypsin, α1-anti-chimotripsin, α2-macroglobulin), cerulo-plasmin.

From a clinical point of view, the most important are C reactive protein and serum amyloid A which together with fibrinogen (beta-globulin) are considered the *markers of the acute phase reaction*.

• **Causes:** bacterial infections (pneumonias, tuberculosis, pyelonephritis); burns; myocardial infarction; multiple lesions (including multiple fractures) or after surgery; acute episodes of chronic disorders (rheumatoid arthritis, Crohn disease), malignant tumors.

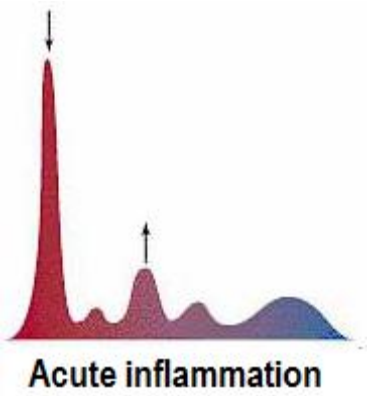


Figure 12.2. ELFO of plasma proteins in acute inflammation

b) Dysproteinemia due to chronic inflammation

Is characterized by:

- decreased serum albumins;
- **increased α-1, α-2 and mainly, γ-globulins**.

• **Causes:** chronic infections, collagen disorders, malignant tumors, multiple myeloma.

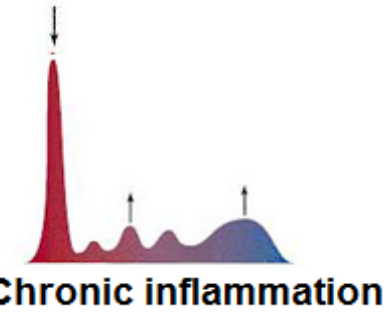


Figure 12.3. ELFO of plasma proteins in chronic inflammation

In *multiple myeloma*, plasma protein electrophoresis has a typical sharp spike appearance in the gamma domain. If only light Ig chains are produced, they will be excreted in the urine (Bence-Jones proteinuria). *Hyperproteinemia* is associated.

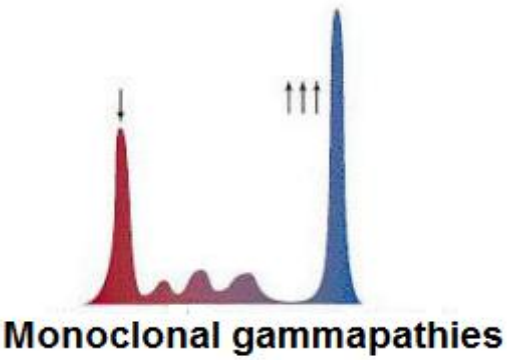


Figure 12.4. ELFO of plasma proteins in monoclonal gammapathies

c) Dysproteinemia due to chronic liver disease

Is characterized by:

- decreased serum albumins;
- **increased β and γ-globulins** (IgG, IgM and IgA) with the merge of the β and γ fractions on the ELFO and the occurrence of the „cirrhotic dome".

• **Causes:** chronic active hepatitis, cirrhosis.

Associates *hypoproteinemia* in severe/chronic forms.

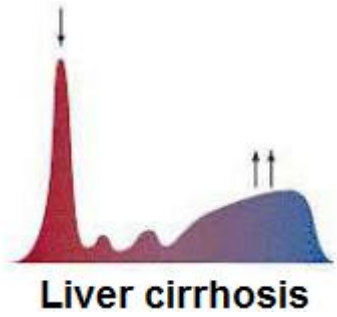


Figure 12.5. ELFO of plasma proteins in liver cirrhosis

d) Dysproteinemia due to nephrotic syndrome:

Is characterised by:

- decreased serum albumins and γ-globulins;
- **increased α-2 and β-globulins**

• **Causes:** increased permeability of the glomerular capillaries determines glomerular proteinuria (loss of albumins) with a compensatory increase in the globulin synthesis.

Usually is associated with *hypoproteinemia.*

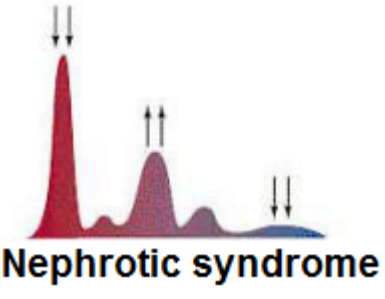


Figure 12.6. ELFO of plasma proteins in nephrotic syndrome

IV. ABNORMALITIES OF PURINE METABOLISM

Nucleic acids contain two types of nitrogen bases, pyrimidine and purine. The catabolism of purines (adenine and guanine) leads to the production of uric acid. In physiological concentrations of hydrogen ions, uric acid will mostly be ionized and is present in the plasma as sodium urate. Uric acid and urate are relatively insoluble molecules that can precipitate in aqueous solutions such as urine or synovial fluid. The formation of urate crystals is favored by low tissue pH and temperature (eg peripheral joints). The level of uric acid depends on the synthesis and degradation of purines, the ingestion of purines and the renal (66%) and intestinal (33%) elimination of urate.

a) HYPERURICEMIA

• **Definition**: increased serum levels of uric acid.

• **Normal values**:
  - Men: 2-7 mg/dL
  - Women: 2-5,7 mg/dL

• **Etiopathogenesis**: an increased level of sodium urate is caused by:
- increased uric acid synthesis due to excessive degradation of endogenous or exogenous (dietary) purines – ***metabolic hyperuricemia***
- decreased urate excretion – ***renal hyperuricemia***

b) GOUT

• **Definition: inflammatory arthritis** induced by hyperuricemia regardless of its cause (metabolic or renal), affecting men 5 times more frequently than women.

• **Causes:**
- **decreased uric acid excretion (90%)**
- **increased uric acid synthesis (10%)**

• **Clinical forms**:

***i) Acute* monoarticular inflammatory arthritis –** gout attack (followed by an asymptomatic phase between the attacks)

ii) Chronic gout - the persistence of a low-grade inflammation over which acute attacks overlap, with the possible appearance of joint damage.

iii) Chronic polyarticular tophaceous gout - rare (occurs in people with persistent hyperuricemia), characterized by chronic pain, structural joint damage and limited activity, with frequent exacerbations. Sodium urate deposits (tophi) form around the joints, Achilles tendon and in the skin and can ulcerate.

• **Positive diagnosis:**

1. Clinical examination

2. Serum uric acid (if at a first assessment it's level is not elevated it should be checked again a few weeks after the gout attack as serum levels may drop immediately after an acute episode)

3. Joint aspiration (arthrocentesis) and synovial fluid analysis - the presence of the sodium urate crystals CONFIRMS the diagnosis

4. Urine sediment examination

5. Serum urea, creatinine and glomerular filtration rate - assessment of the possible presence of renal impairment

6. Plain X-ray - bone erosions adjacent to tophaceous deposits, important for CHRONIC gout evaluation

• **Pathogenesis of the GOUT ATTACKS:**
- ***Triggering factors:***
  - hyperproteic diet (red meat, organs – liver, kidney)
  - dehydration (eg., overdose of thiazide and loop diuretics) $\Rightarrow \downarrow$ uric acid excretion ($\downarrow$ GFR + $\uparrow$ tubular reabsorption)
  - alcohol ingestion ($\uparrow$ lactic acid synthesis - competition at the level of the renal transporter)
  - aspirin $\Rightarrow \downarrow$ uric acid excretion ($\downarrow$ tubular secretion)
- ***Clinical manifestations:***
  - nocturnal acute pain with abrupt onset (usually after exposure to a triggering factor) that lasts around 7 days
  - local inflammatory signs (usually present in the first metatarsophalangeal joint): intense pain, redness, swelling, increased local temperature with lymphangitis and subsequent desquamation of the overlying tegument
  - systemic inflammatory signs: leukocytosis, fever, increased ESR.

c) URATE NEPHROPATHY

• **Characteristics:**
- renal stones formation that occurs in the presence of hyperuricemia, hyperuricosuria and low fluid intake, which leads to chronic nephropathy.
- concentrated urine (low fluid intake) + low urine pH (protein-rich diet) lead to urate precipitation in the collecting ducts

- calculi size varies between a grain of sand to coral-like calculi
- calculi composition: monosodic urate (± calcium oxalate, calcium phosphate).

d) ACUTE URIC ACID NEPHROPATHY

• Characteristics:

- clinical form of acute tubular lesion associated, usually, with secondary hyperuricemia in leukemia and lymphoma
- renal tubular obstruction caused by urate (nephrotoxic endogenous agents) causes the increase in tubular pressure with the important decrease of renal blood flow and GFR.

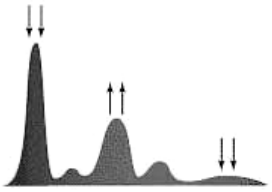
Observation!

The main classes of drugs administered in gout are:

- **Antiinflammatory drugs that alleviate gout attacks and may be profilactically administered in between gout attacks**:
  - NSAIDs (NOT aspirin!) ⇒ decrease prostaglandin synthesis
  - colchicin ⇒ inhibits neutrophilic migration and phagocytosis
  - glucocorticoids (administered locally in the joint) ⇒ decrease the phagocytic cell activation
- **Drugs that reduce the levels of uric acid**:
  - xantin-oxidase inhibitors (allopurinol - Milurit, febuxostat- Adenuric) ⇒ prevent uric acid synthesis
  - uricosuric agents (probenecid - Benemid) ⇒ inhibit urate tubular reabsorbtion
  - urate-oxidase medication obtained by genetic recombination (rasburicase-Elitex) which causes the metabolization of uric acid to allantoin (the enzyme does not exist in humans naturally)

CHECKPOINT

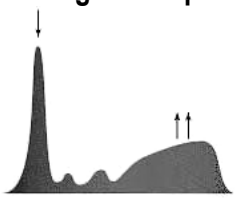
***1. The following electrophoresis:**



Is suggestive for which diagnosis:

A. Acute inflammation
B. Chronic inflammation
C. Nephrotic syndrome
D. Liver cirrhosis
E. Multiple myeloma

***2. The following electrophoresis**



Is suggestive for which diagnosis:

A. Acute inflammation
B. Chronic inflammation
C. Nephrotic syndrome
D. Liver cirrhosis
E. Multiple myeloma

3. A 52-year-old patient is admitted into hospital for hematemesis, melena and increased body weight. The diagnosis was established as alcoholic liver cirrhosis.
Which of the following investigations belong to the patient in question?

A. Serum proteins = 4,5 g/dl
B. Beta-globulins = 19%
C. Albumins = 65%
D. Gamma-globulins = 13%
E. Alpha 1-globulins = 10%

4. Which of the following are true about IgE?

A. Is the major immunoglobulin in secretions
B. Is present mainly in collostrum and breast milk
C. Is increased in allergic asthma
D. Is increased in parasytic infections
E. It is the only Ig that passes through the placenta

5. Choose the correct answers regarding gout attacks:

A. Can be induced by a high protein diet
B. Can be due to dehydration
C. Involves the presence of local inflammatory signs
D. Involves only systemic inflammatory manifestations
E. NSAID administration is counterindicated

6. The recommended drugs for gout treatment are the following:

A. Drugs that increase tubular reabsorbtion of urate
B. Drugs that reduce urate tubular reabsorbtion
C. Xantin-oxidase inhibitors
D. Thiazide diuretics
E. Loop diuretics

7. Dysproteinemia in chronic liver disease involves the following:

A. Decreased plasma albumin levels
B. Increased beta and gamma globulins
C. Decreased IgG, IgM and IgA immunoglobulins
D. The appearance of a typical "spike" in the gamma region
E. Merging of α1 and α2 peaks on ELFO with the appearance of the "cirrhotic dome"

8. Select the correct statements regarding acute uric acid nephropathy:

A. It is a clinical form of acute tubular lesion
B. It is usually associated with secondary hyperuricemia in leukemia and lymphoma
C. It is associated with hyperuricaemia and reduced fluid intake
D. It causes the decrease in renal blood flow
E. It only occurs in metabolic hyperuricemia

CASE STUDIES

1. A 50-year-old patient arrives to the hospital accusing severe pain in the left toe, with nocturnal onset and exacerbated by contact with the bed linen. The patient is feverish.
What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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2. A 45-year-old patient presented with acute pain in the lumbar and dorsal region, altered general condition and weight loss (3 kg in 3 months). Bone marrow biopsy indicated the presence of numerous plasma cells and serum ELFO revealed a band of paraproteins. The urinalysis shows monoclonal Ig light chains.
What diagnosis do you suspect? What additional investigations would be justified?
Provide arguments for your answer.

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